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Sadashiva *et al.,* p. no. 278
(Tomato hybrids for processing
Left : Arka Apeksha; Right : Arka Vishesh)

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JOURNAL OF HORTICULTURAL SCIENCES

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Short Communications

In this issue…

As per the assessment of World Bank, horticulture accounts for 30% of India's agricultural GDP from 8.5% of cropped area. This clearly indicates the commercial potential of horticultural produce. Journal of Horticultural Sciences tries to contribute by assimilating the knowledge generated in different disciplines for the growth of horticulture.

Crop improvement helps in having better varieties and hybrids. Rootstock breeding is one of the important strategies. Mango rootstock having dwarfness is a desirable trait. ***Perveen et al.*** *developed mutants of polyembryonic mango genotype Nekkare to identify the dwarf rootstocks.* ***Pooja et al****. evaluated the tamarind genotypes and found that genotype K-9 was superior because of pod size, pod weight, pulp weight and pod yield per tree. Breeding tomatoes suitable for processing is the need of the hour.* ***Sadashiva et al.*** *developed tomato hybrids Arka Apeksha and Arka Vishesh suitable for processing along with triple disease resistance to tomato leaf curl disease, bacterial wilt and early blight.* ***Merin et al.*** *screened yard long bean and identified the lines resistant to anthracnose. Inheritance of quantitative and qualitative fruit traits in brinjal was studied by* ***Sidhu et al*** *who report that epistatic interactions significantly influenced most of the traits. Phenotypic trait association in brinjal upon drought stress was studied by* ***Faizan et al.*** *and they identified the traits associated with low moisture stress tolerance.* ***Sankar and Singh*** *identified Passiflora quadrangularis L. genotypes in Arunachal Pradesh and report that it is a vegetable with nutritive value suitable for that region.* ***Rajiv et al****. identified accessions of Nerium that displayed profuse blooming and suitable for commercial cultivation and landscaping.*

Agronomical practices when given due attention, many production constraints can be addressed properly. By adjusting the time of different horticultural operations, we can enhance the production in fruit crops. ***Chander et al.*** *report that the fruiting in custard apple cultivar 'Arka Sahan' could be advanced by 8-9 weeks to June from the normal season of August-September with comparable or better fruit quality by pruning 75% of the last season's growth during October.* ***Manjunath et al.*** *report that wetting soil volume up to 70% recorded higher mean fruit yield of 34.8 kg/plant (9.68 t/ ha) in mango.* ***Hiremata et al****. report that growth and yield characters may be considered in selection criteria for the improvement of yield in arecanut based on the correlation and path analysis.* ***Kiran et al****. report that with suitable NPK combination along with green manure and sewage sludge, good yield of carrot could be obtained in Pakistan.* ***Mahala and Sharma*** *studied the effect of different growth media on biometric parameter of brinjal and chilli seedlings under shade net house and report that vermiculite + perlite + vermi-compost (1:1:2) as growth media was found more productive.New formulations of micronutrients are being tested for their efficacy.* ***Srivastava et al.*** *record that nano formulation of ferrous sulfite could enhance the growth, flowering and oil yield in calendula.* ***Singh et al****. report that the amount of nitrogen can be reduced to 1/3rd to grow cut chrysanthemums planted at twice the row spacing for longer cut stems of appreciable vase life.* ***Smitha et al.*** *standardized the production package for potted China Asters and report that growing in 6" plastic pots using the substrate combination of soil +sand +FYM (1:1:1 v/v/v) along with the weekly application of nutrient solution of 96:18:108 ppm NPK/plant can give excellent results.*

Post-harvest handling of produce in appropriate way can reduce the loss happening between the harvest and arrival of produce at the end-user's door steps. In this issue, few reports on post-harvest management of horticultural produce are published. ***Mshora et al.*** *studied the effect of chitosan coating on physico-chemical properties and enzyme activities in mango cv. Dashehari and found that chitosan coating at 1.0% was effective in enhancing the storability and quality of*

mango fruits at cool storage temperatures. ***Pandidurai et al****. optimized the freeze-drying parameters for moringa (Moringa oleifera) flower powder using response surface methodology. The optimum conditions for osmotic dehydration of aonla in salt medium has been identified by* ***Sujayasree et al.***

Assessment of yield loss helps the policy makers. Yield loss due to tomato late blight by Phytophthora infestans has been reported by ***Sandeepkumar et al****. where they report how to mitigate the yield loss due to this disease and the benefit of growing late blight resistant tomato hybrid 'Arka Abhed'.* ***Praful et al.*** *report the yield loss due to ChiVMV in chilli and the transmission by aphids in Karnataka, India.* ***Skinner et al.****attempted to control downy mildew in cucumber with ultraviolet (UV-C) energy and mulch. They observed that UV-C was not effective but use of reflective mulch delayed the disease onset relative to black mulch in fields.* ***Palanna et al.*** *report the morphological and molecular diversity of Ganoderma spp. causing basal stem rot of coconut in Southern dry tracts of Karnataka. First report of Lasiodiplodia theobromae has been made on the Flacourtia montana, a wild edible fruit tree of Western Ghats, India by* ***Rasmi et al.*** *IPM practice for major pests and diseases in French bean was developed by* ***Chandrashekara et al.*** *suitable for the North Western India that takes care of major pests and diseases of French bean.*

Basic research helps to understand many biological processes in plants. ***Kanade et al.*** *studied the variability inleaf volatile and phenolic acid profiles of polyembryonic mango genotypes and found that they are suitable as biochemical marker to identify the polyembryonic seedlings.Pollen morphology of Giant Himalayan Lilly was studied by* ***Lal et al.*** *and they report the ideal conditions for the pollen germination and pollen tube growth.*

For mass multiplication of planting material for medicinal crops also tissue culture can be used. An effective and rapid in vitro regeneration protocol of Kalmegh (Andrographis paniculata) was established by ***Monika et al. Ganji et al.*** *observe that estimation of content of phenolic compounds in plums can be used as a biochemical marker in graft incompatibility.Graft compatibility is an important trait in many fruit crops especially in plums. Circular RNAs (CircRNAs) are covalently closed non-coding RNAs that play an important role in a variety of biological processes.* ***Bhavya et al.*** *demonstrated the importance of CircRNAs in ToLCBaVD resistance and suggest that CircRNAs could be key regulators of gene expression during disease resistance in tomato in response to tomato leaf curl.* ***El-Hadidy et al.*** *provides us the taxonomic revision of the cultivated species of Mimusops (Sapotaceae) in Egypt, with new records.*

Taking the horticultural technologies to end users fulfills the purpose of research work in different disciplines. FPOs are contributing immensely currently in horticulture also in reaching out to the farmers. ***Mukherjee et al****. studied the performance of three FPOs in Maharashtra and give the comparative analysis.* ***Das Gupta*** *studied the growth and potential of horticulture in North Eastern India and has reports that it is imperative to develop infrastructure, modernize farming and establish seamless value chains with greater market integration.*

On behalf of the Editorial Team, I extend our sincere thanks to all the authors, readers, reviewers and executive committee members of SPH for their continuous support and faith in the team. Hope the coming new year 2023 will bring cheers to all stakeholders of Horticulture Domain. Wish you all a very Happy New Year 2023.

S. Sriram
Editor-in-Chief

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Original Research Paper

Characterization and evaluation of putative mutant populations of polyembryonic mango genotype Nekkare for dwarfing rootstock traits

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ABSTRACT

Availability of dwarfing rootstocks is an important pre-requisite for improving productivity of mango orchards in India as it facilitates high density planting as well as impart uniformity within an orchard. An attempt was made to induce variability in polyembryonic mango genotype Nekkare for dwarfness by treating kernels with different doses of gamma radiation ranging from 15 to 35 Gy. Irradiation created significant variation in plant height, stem girth, number of nodes, inter-nodal length, number of leaves, leaf blade length and leaf blade width. The highest reduction in seedling height along with highest variation was observed at 35 Gy where the seedling height ranged from 11.50 to 33 cm with a mean of 23.12 cm as compared to mean plant height of 44.55 cm in control ranging from 33.50 to 56 cm. Further, the effect of irradiation on stomatal parameters was also investigated and the highest stomatal length and width was recorded at 15 Gy (63.39 µm) and 20 Gy (63.12 µm) respectively while 30 Gy treatment produced maximum stomatal density (13.85 per µm^2). Furthermore, the concentration of ABA was found to be highest (429.1 ng/gm) in morphologically dwarf (putative mutant) progenies of Nekkare. The results suggest effectiveness of induced mutation for developing dwarfing rootstocks in mango to be used in high density planting.

Keywords: Gamma irradiation, mango, mutation, Nekkare and stomatal parameters

INTRODUCTION

Mango (*Mangifera indica*) enthroned as the king of fruits in India enjoys a eminent position in the horticultural bounty of the country. Indian mango industry has a global presence in production as well as export of fresh fruits and processed products. India is the largest producer of mango in the world accounting for more than 55% of total world's production with an area of 2,293,000 ha and production of 20,798,000 MT (NHB, 2019). However, the productivity (per hectare yield) of mango in the country remains as low as 8 t/ha. Low planting densities, presence of old and senile orchards, propagation on seedling rootstocks of unknown origin, irregular bearing habit and lack of genetically uniform dwarfing rootstocks are considered as some of the major reasons for low orchard efficiency of mangoes in India. In the purview of global climate change and need for intensive cropping to meet the domestic as well as export demand, potential of polyembryonic genotypes to be used as rootstock needs to be properly investigated. Meanwhile, the lack of genetic variability in these genotypes owing to the presence of true to type nucellar seedlings limits their use in the mango rootstock breeding programme. Mutation breeding has been extensively used in several crops for improvement of yield or yield attributing traits as well as for enrichment of existing germplasm (Ahloowalia and Maluszynski, 2001). In heterozygous crops like mango also, induced mutations have been widely used for creation of variability for different traits (Karsinah *et al.,* 2012; Rime *et al.* 2019). In mutation breeding, gamma rays and ethyl methane sulfonate (EMS) are the most commonly used physical and chemical mutagens respectively. Induced mutation has been known to create variations in plant height, growth pattern, leaf characteristics and various biological and

physiological traits including stomatal size and number (Kovacs and Keresztes, 2002). Stomatal structure (including size and density) is the major plant cellular component contributing towards growth and development (owing to its role in gaseous exchange and transpiration thus directly affecting photosynthesis) of the plants besides playing a crucial role in stress tolerance (Yasmeen *et al.*, 2020). Hence, any change in stomatal parameters resulting from mutagenesis could be highly significant. Here, an attempt was made to create variability in a vigorous polyembryonic mango genotype Nekkare, known for its tolerance to salinity stress (Pandey *et al.*, 2014; Laxmi *et al.*, 2021) for plant stature employing induced mutation approach using gamma irradiation. The objective of this study was to determine the variability created among the selected putative mutants generated through different doses of gamma irradiation ranging from 15 Gy to 35 Gy, for various morphological traits as well as to investigate the structural changes in stomatal parameters followed by hormonal profiling of selected putative mutants to understand the effectiveness of induced mutation for developing dwarfing rootstocks in mango to be used in high density planting.

MATERIALS AND METHODS

Site of experiment and plant material

This research was conducted in ICAR-Indian Institute of Horticultural Research, Bengaluru. Six month old putative mutant seedlings of polyembryonic mango variety Nekkare used in this study were generated by treating seed kernels of Nekkare with five different doses of gamma irradiation viz., 15Gy, 20Gy, 25Gy, 30Gy and 35Gy (280 kernels per treatment) (unpublished data). From the putative mutant population thus generated, a total of 100 putative mutant seedlings (20 from each treatment) along with control (untreated nucellar seedlings) of short, medium and tall stature (based on plant height) were selected for taking further observations.

Morphological characterization

Morphological observations on plant height (cm), stem girth (mm), number of internodes, inter-nodal length (cm), number of leaves, leaf blade length and leaf blade width for 100 selected putative mutant progenies of Nekkare along with control were recorded based on mango descriptor (IPGRI 2006). Volatile profiling was used to ascertain the nucellar origin of control seedlings (unpublished data).

Stomatal parameters

Again, for these 100 putative mutants and control seedlings, stomatal size and number was determined using the procedure as described by Hamil *et al.* (1992). For measuring number of stomata, fully matured and expanded leaves were selected and plucked between 12-01 PM. Freshly plucked leaves were kept in butter paper, labelled properly and brought to laboratory for further analysis. For determining the number of stomata, abaxial surface of fully matured and expanded leaves plucked between 12 noon to 1 PM coated with transparent nail polish and left for 30 minutes to dry. Glass slides were prepared by peeling off the imprinted layer from the abaxial surface of the leaf sample. The slides were then examined under the microscope (OLYMPUS; Light microscope digital camera; DP-22/DP-27) at a magnification of 10x and stomata numbers were recorded and expressed as number of stomata per mm^2. For measuring stomatal size, slides were examined at a magnification of 40x and length and width of stomata was recorded and expressed in µm.

Phytohormone profiling

Further, morphologically distinct dwarf and tall statured putative mutant seedlings along with control plants were selected for phytohormone profiling using Liquid chromatography-mass spectrometry (LC-MS).

Extraction procedure

Phyto-hormones were extracted using the method described by Pan *et al.* (2008). For extraction, 3g leaf sample was completely homogenized using 1-propanol/H_2O/concentrated HCl (2:1:0.002, v/v/v) followed by sonication for 30 minutes at 4°C keep and kept overnight. Next day dichloromethane was added to the homogenate and sonicated for 30 min followed by centrifugation at 12,000 rpm for 10 minutes. After centrifugation, the bottom layer was transferred to a conical flask containing sodium sulphate to remove any traces of water and then evaporated using flash evaporator. Once the sample was completely dried, it was dissolved in 80% methanol and loaded through C18 solid-phase extraction (SPE) cartridges.

The SPE process included: (a) Pre-conditioning: It included washing the column with 5 mL methanol and 5 mL 80% methanol. (b) Adsorption: After the extract

was loaded to the column, all matrices were adsorbed by the cartridge and (c) Elution: In this step, the residual plant hormones were eluted with another 5 mL 80% methanol.

The obtained elute (5 mL) was then evaporated using flash evaporator at 35°C and finally the dried sample was dissolved in 500 µL methanol–0.05% formic acid (1:1, v/v). The solution was filtered using a nylon filter paper and injected into LCMS for further analysis.

LC and MS-MS conditions

The initial gradient was composed of 85% solvent A and 15% of solvent B with a hold time of 1 min. At 12 minutes, the gradient was changed to 15% of solvent A and 85% of solvent B, with a hold time for 1 minute and at 14 minutes, linear gradient was followed by 85% of solvent A and 15% of solvent B with a hold time of 0.5 minute. The system was then returned to the initial conditions at 15 mins and equilibrated for 1 minute before the next injection. The flow rate was 0.2 mL/minute. The analytical column was 2.1 x 50 mm UPLC BEH-C18 column (Waters, USA) with 1.7µm particles, protected by a vanguard 2.1 X 5 mm BEH C-18 with 1.7µm. Guard column (Waters, USA) was used with column temperature maintained at 25°C. Identification and quantification of the elute hormones was done using a TQDMS/ MS (Waters, USA) system, optimized for the hormone analysis.

Mobile phase used were

(i) Solvent - A: water/Acetonitrile/acetic acid (95/5/ 0.05, v/v/v) and (ii) Solvent - B: Acetonitrile/water/ acetic acid (95/5/0.05, v/v/v)

Statistical analysis

Descriptive statistics was calculated for morphological and stomatal parameters using standard procedure.

RESULTS AND DISCUSSION

Effect of gamma irradiation on morphological traits

Gamma rays are the most widely used physical mutagen employed in mutation breeding of crop plants and are well known for bringing about morphogenetic and endomorphic changes in plants (Ali *et al.*, 2016; Yasmeen *et al.*, 2020). In the present study considerable variation was created for different morphological traits in the putative mutant population of polyembryonic mango genotype Nekkare generated by gamma irradiation (Table 1a and 1b; Fig 1a-f). Plant height was found to be the most affected trait by irradiation (Fig. 2 and 3). A reduction in plant height (in comparison to control) with increasing dosage of irradiation was recorded with highest mean plant height in control being 44.55 cm while among the treatments, mean plant height ranged from 28 cm (15 Gy) to 21.35 (25 Gy). Further, although the mean plant height of population generated through 35 Gy treatment was 23.12 cm, the progenies it produced were as short as 11.50 cm and the maximum plant height recorded in this treatment was 33 cm in contrast to control where the plant height ranged from 33.50 to 56 cm. The use of gamma irradiation for developing dwarf statured mango varieties to be used for high density planting has been attempted for a very long time (Sharma and Majumder, 1985, 88; Sharma, 1987).

A decrease in stem girth with increasing doses of irradiation was also observed and the highest mean stem girth was recorded in control (9.07 cm) which declined to 6.53 cm in 25 Gy treatment. However, the progeny with largest stem girth (11.32 cm) was produced by 35 Gy treatment. Mutation affects the process of cell division and cell elongation along with disruption of protein synthesis, hormonal and enzymatic balance and water and gaseous exchange ability of plants thus leading to reduction in plant height as well as overall growth of plants (Ali *et al.*, 2016; Asare *et al.*, 2017). The results of the present study are in confirmation with earlier findings reporting the reduction in plant height with increasing doses of irradiation in mango (Rime *et al.* 2019), guava (Zamir *et al.*, 2003), grapes (Surakshitha *et al.*, 2017) and apple (Atay *et al.*, 19).

Further, the variation for number of nodes was highest in control along with highest mean number of nodes being 7.2 while the lowest mean number of nodes was recorded in 15 Gy being 5.76. Mean inter-nodal length also declined with increasing doses of irradiation. The number of leaves in all the treated populations was less than control where the mean number of leaves was 24.60 ranging from 17 to 35. Among the treated populations, the lowermost treatment (15 Gy) produced highest mean number of leaves ranging from 9 to 35 while the lowest mean number of leaves (15.93). Reduction in number of leaves with increasing doses of gamma irradiation has been reported in Arumanis mango of Indonesia (Karsinah *et al.,* 2012) and EMS derived population of mango hybrid Arka Puneet (Rime *et al.,* 2019).

Gamma irradiation resulted in the production of smaller and narrower leaves exhibiting a reduction in leaf blade length with increasing dosage of irradiation (Fig. 4). The highest mean blade length was recorded in control (18.15 cm) which decreased to 11.62 cm in 35 Gy treatment while the lowest leaf blade length (11.21 cm) with highest CV (17.13) was observed in 25 Gy treatment. Following the same trend, the mean leaf width was found to be highest in control being 5.56 cm which decreased in the entire gamma irradiated population exhibiting highly profound reduction in the higher dosage of irradiation and the lowest leaf blade width was recorded for 35 Gy treatment being 3.65 cm along with highest CV (14.23). In contrary to our results, Rime *et al.,* (2019) reported production of longer and wider leaves in the EMS derived putative mutant population of mango hybrid Arka Puneet while it is in congruence with the findings of Surakshitha *et al*. (2017) where they observed a gradual reduction in leaf length and leaf width with increased dosage of gamma rays in grape cultivars Red Globe and Muscat.

1a : Nekkare Control

1b : Treatment 1 (15 Gy)

1c : Treatment 2 (20 Gy)

1d : Treatment 3 (25 Gy)

1e : Treatment 4 (30 Gy)

1f : Treatment 5 (35 Gy)

Fig. 1 : Seedlings of Nekkere plants raised from gamma irradiated seeds

Fig. 2 : Dwarf putative mutant of Nekkare

Fig. 3 : Dwarf putative mutant of Nekkare

Fig. 4 : Narrow leaf shape in Nekkare mutant

Table 1a : Morphological variability induced in polyembryonic mango variety Nekkare through different doses of gamma irradiation.

Treatment	Plant height (cm)				Stem girth (cm)				Number of nodes				Inter-nodal length (cm)			
	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.
Control	44.55	33.5	56	16.41	9.07	8.22	10.02	7.5	7.2	5	12	29.86	6.1	3	9	28.08
15 Gy	28	14	41.5	30.86	7.64	4.15	10.94	26.97	5.76	4	10	26.4	4.5	2	8	32.87
20 Gy	24.47	16	36	21.49	7.41	4.87	10.22	16.88	6.19	4	8	20.63	3.75	2	6	27.54
25 Gy	21.35	12.5	36.5	26.66	6.53	3.6	9.13	22.21	6.47	5	9	14.16	3.27	2	6	32.95
30 Gy	22.87	16	35.5	21.62	7.18	5.06	10.89	22.57	6.42	4	10	25.6	3.58	2.5	5	22.9
35 Gy	23.12	11.5	33	32.75	7.34	5.22	11.32	24.71	5.85	4	8	18.27	3.62	2.5	6.5	29.96

Table 1b : Morphological variability induced in polyembryonic mango variety Nekkare through different doses of gamma irradiation

Treatment	Number of leaves				Leaf blade length (cm)				Leaf blade width (cm)			
	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.	Mean	Min	Max	C.V.
Control	24.6	17	35	23.17	18.15	13.54	21.16	13.01	5.56	4.6	6.76	11.77
15 Gy	18.76	9	35	39.23	13.43	7.42	15.04	13.07	4.08	3.28	5.1	11.87
20 Gy	17.94	9	31	28.89	11.63	9.06	14.8	15.08	3.88	3.18	4.64	10.11
25 Gy	15.93	9	29	43.06	11.21	8	14	17.13	4.03	3.36	4.74	10.8
30 Gy	18.21	9	31	33.58	11.94	9.8	16.3	15.13	3.73	3	4.54	8.84
35 Gy	18.92	9	30	34.82	11.62	8.6	15	16.93	3.65	2.66	4.56	14.23

Stomatal parameters

In our study we observed high variability for different stomatal parameters like stomatal density, length and width of stomata (Table 2; Fig. 5a-d). Highest mean stomatal length (65.07 µm) was recorded in control (64.55 to 65.43 µm) with a CV of 0.65. Among the putative mutant populations, the highest (63.39 µm) and lowest (61.9 µm) mean stomatal length was recorded in 15 Gy and 35 Gy irradiation treatments respectively (Table 2).

Variations in stomatal width was also recorded in the putative mutant populations and the mean stomatal width of all the treatments except the putative mutant population generated through 25 Gy irradiation was found to be higher than control plants which showed less variation for this trait having a CV of 0.99 and mean stomatal width of 60.83 µm varying between 60.15 to 61.57 µm. Among the putative mutant populations, the mean stomatal width increased at lower doses (15 and 20 Gy) while it declined at higher doses (30 and 35 Gy) while remaining higher than control. The highest (63.12 µm) and lowest (60.92 µm) mean stomatal width was recorded in population generated through 20 Gy and 25 Gy respectively. An increase in stomatal length and width was recorded in sugarcane at lower doses of gamma irradiation (10 and 20 Gy) which declined further with increased doses (30 and 40 Gy) (Yasmeen *et al.* 2020). Similarly, a significant reduction in stomata length and number at higher doses of gamma irradiation was observed in Kinnow (Mallick *et al.,* 2016), grapes (Kok, 2011) and mango (Rime *et al.*, 2019).

Stomatal density was found to decrease with increasing dosage of irradiation and a profound variation was observed among the populations developed by different treatments as revealed by the values of coefficient of variability. The mean stomatal density for control plants was recorded to be 14.33 (per 20000 μm^2) with a very low coefficient of variability being 4.94. Among the treatments, mean stomatal density decreased in lower doses viz., 15 Gy and 20 Gy of being 12.64 (per 20000 μm^2) and 12.43 (per 20000 μm^2) respectively while it increased at 25 Gy (13.3 per 20000 μm^2) and 30 Gy (13.85 per 20000 μm^2). Our results are in contrary to the findings of Yasmeen *et al.* (2020) who reported an increase in stomatal density at lower doses of irradiation. However, the highest dose of irradiation (35 Gy) resulted in marked reduction in the stomatal density being 11.82 (per 20000 μm^2). A lower stomatal density is indicative reduced plant vigour and stature (Majumder *et al.*, 1981) and this result is also confirmed by the production of highly reduced plant height at 35 Gy treatment in this study. Dwarfing pear variety, '601D' is reported to have shorter intermodal length, thick and broad leaves, lower stomatal density and larger stomatal size as compared to it vigorous mutant '601T' (Liu *et al.*, 2022). The decline in stomatal number at higher doses of irradiation has also been reported in citrus (Mallick *et al.*, 2016), mango (Rime *et al.*, 2019) and sugarcane (Yasmeen *et al.*, 2020).

Correlation analysis

Correlation analysis of physical mutagenesis with the stomatal and plant morphological parameters gave interesting insights into the data (Table 3). In general, the overall correlation between doses of gamma irradiation and stomatal (except stomatal width) and plant morphological parameters seemed to be negative and traits like plant height, stem girth, leaf blade length, leaf blade width and stomatal length showed highly strong negative correlation with irradiation doses (-0.899, -0.815, -0.887, -0.914 and -0.919 respectively). Contrarily, a strong positive correlation was observed between stomatal length and morphological traits viz., plant height, stem girth, number of leaves, leaf blade length and leaf blade width (0.919, 0.902, 0.843, 0.907 and 0.875 respectively). Further, a positive correlation between stomatal length and stomatal density was seen (0.611) while stomatal width was negatively correlated with stomatal density (-0.600). Different morphological traits like plant height, stem girth, number of leaves, leaf blade length, leaf blade width divulged positive association among each other and significant positive correlations were observed between plant height and stem girth (0.961), number of leaves (0.957), leaf blade length (0.994), leaf blade width (0.964). A positive correlation between stomatal density and tree height has been reported previously (Camargo and Marenco, 2011, Yasmeen *et al.,* 2020) and increased stomatal density has been found associated with reduced tree height (Barrientos-Pérez and Sánchez-Colín, 1982).

Table 2 : Variability in stomatal density, stomatal length and width induced in polyembryonic mango variety Nekkare through different doses of gamma irradiation

Treatment	Stomatal density (per 20000 µm area)					Stomatal length (µm)					Stomatal width (µm)				
	Mean	St. Err.	Min	Max	CV	Mean	St. Err.	Min	Max	CV	Mean	St. Err.	Min	Max	CV
Control	14.33	0.53	12.67	17.33	4.94	65.07	0.19	64.55	65.43	0.65	60.83	0.27	60.15	61.57	0.99
15 Gy	12.64	0.48	9	18	18.98	63.39	0.42	59.09	68.03	3.34	62.87	0.47	58.41	69.7	3.73
20 Gy	12.43	0.58	7.33	18	22.52	63.34	0.7	55.39	69.95	5.28	63.12	0.51	57.05	68.65	3.86
25 Gy	13.3	0.51	9	17.67	16.69	62.13	0.63	56.86	66.11	4.42	60.92	0.52	55.61	63.57	3.72
30 Gy	13.85	0.36	8.67	15	12.84	62.82	0.73	57.62	70.76	5.06	61.43	0.55	56.02	65.1	3.92
35 Gy	11.82	0.67	10	17.67	18.77	61.9	0.74	56.86	66.11	4.48	61.59	0.58	55.61	64.96	3.53

5a : Nekkare Mother Plant

5b : Nekkare Control plant

5c Putative mutant seedling N 63

5d Putative mutant seedling N 91

Fig. 5 : Stomata character of Nekkare plants subjected to gamma irradiation.

Table 3 : Correlation analysis of doses of gamma radiation, stomatal parameters, morphological traits

Variables	Gamma irradiation doses	PH	SG	NN	NL	LBL	LBW	SD	SL	SW
Gamma irradiation doses	1									
PH	-0.899	1								
SG	-0.815	0.961	1							
NN	-0.620	0.696	0.552	1						
NL	-0.748	0.957	0.982	0.618	1					
LBL	-0.887	0.994	0.949	0.690	0.951	1				
LBW	-0.914	0.964	0.857	0.808	0.865	0.958	1			
SD	-0.563	0.579	0.431	0.872	0.483	0.619	0.677	1		
SL	-0.941	0.919	0.902	0.651	0.843	0.907	0.875	0.611	1	
SW	0.047	-0.281	-0.118	-0.665	-0.286	-0.317	-0.414	-0.600	-0.013	1

Values in bold are different from 0 with a significance level alpha=0.05
PH-Plant height; SG-Stem girth; NN-Number of nodes; NL-Number of leaves; LBL-Leaf blade length; LBW-Leaf blade width; SD-Stomatal density; SL-Stomatal length; SW-Stomatal width

Plant hormones

Cell elongation and proliferation are the key processes determining height of a plant and plant hormones regulate these processes in a species dependent manner (Yue *et al.*, 2016). Among the putative mutant population created by different dosage of gamma radiation 10 morphologically distinct dwarf and tall seedlings were selected to determine the levels of two growth promoting (IAA and GA7) and one growth inhibiting (ABA) phytohormones. IAA has been found to be associated with cell division, expansion and differentiation thus regulating the plant height (Liu *et al.*, 2022). GA is another important phytohormone that regulates plant height and most of the dwarf phenotypes are results of disruption in GA biosynthesis or signal transduction (Magome *et al.*, 2010). Nekkare is a vigorous genotype and the concentration of IAA was recorded to be highest (4.16 ng/gm) in control samples and tall (putative mutant) progenies (1.56 ng/gm) while dwarf (putative mutant) progenies were found to contain the lowest (0.78 ng/gm) amount of IAA. Similar to IAA, the highest (0.26 ng/gm) concentration of GA_7 was recorded in control followed by tall (putative mutant) progenies while the lowest amount of GA_7 occurred in dwarf (putative mutant) progenies (Table 4). The interaction between auxin and gibberellin has been known to have implications on plant growth where auxins act as a messenger compound that links the apical bud with biosynthesis of active GAs in the expanding inter-nodes (Ross and O'Neill, 2001). This suggests the possibility that the main growth-regulating function of endogenous IAA could be to maintain the levels of GA in the internode (Ross *et al.*, 2001). This relationship between IAA and GA was also observed in our study where the high levels of IAA in tall (putative mutant progenies) corresponded to the increased levels of GA_7 in the same. Great variation for the concentration of ABA was also recorded in the studied samples which was found to occur in concentration as high as 429.1 ng/gm in dwarf (putative mutant) progenies to as low as 47.61 ng/gm in control plants. Dwarfing apple and citrus trees are reported to contain more bark ABA concentration than the vigorous trees and exogenous application of ABA has resulted in shortened inter-nodes and decreased growth in apple (Noda *et al.* 2000; Tworkoski and Fazio 2011). Dwarf stature of pear variety '601D' is also considered to be a result of over accumulation of ABA (Liu *et al.*, 2022). The highest concentration of ABA in dwarf (putative mutant) progenies in our study further endorses the role of ABA in regulating plant height.

Table 4 : Concentration of plant hormones in control, tall and dwarf plants of polyembryonic mango variety Nekkare

Phytohomone (ng/gm)	Control	Tall	Dwarf
IAA	4.16	1.56	0.78
ABA	47.61	305.93	429.10
GA7	0.26	0.13	0.09

CONCLUSION

The results indicate that gamma irradiation is a potential tool for creating variability for morphological and endomorphic traits. Considerable variation for plant height and other morphological traits governing overall plant stature like inter-nodal length, number of leaves, leaf size etc. were recorded along with alteration in stomatal length, width and density. Reduction in plant height, shorter inter-nodes along with production of smaller and narrower leaves at higher dose of gamma irradiation (35 Gy) corresponded with occurrence of smaller and broader stomata with reduced stomatal density. Furthermore, the concentration of ABA was found to be highest in morphologically dwarf (putative mutant) progenies of Nekkare. The results confirm the effectiveness of higher dosage of gamma irradiation (35 Gy) for developing dwarfing rootstocks in mango to be used in high density planting.

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Original Research Paper

Characterization and evaluation of morphological and yield traits of tamarind genotypes

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ABSTRACT

The evaluation of morphological and yield traits of tamarind genotypes was carried out during 2017-18 at Forest Research Station, Govinkovi, Honnali taluk, Davangere district. The experiment was laid out in randomized complete block design with 16 genotypes and three replications. Trees were 14-years-old and of grafted origin. All the morphological and yield traits showed significant difference among the selected genotypes indicating the presence of adequate variations. The genotypes recorded morphological variation in terms of tree shape (semi-circle to irregular shape), foliage arrangement (dense to sparse), flowering time (early, mid and late), stem colour (dark brown, brown and light brown), bud colour (greenish white, pink, dark pink), petal colour (yellow and pale yellow), pod colour (greyish brown, brown, light brown and dark brown), pulp colour (light brown, brown and reddish brown), pod shape (straight, slightly curved, curved and deeply curved) and pod size (very big, big, medium and small). The analysis of variance revealed significant difference with respect to tree height, stem girth, pod traits, pod yield per tree (K-9 : 12.80 kg), number of pods per tree (NTI-52 : 989.07) and pulp per cent (K-9 : 48.87). Among the 16 genotypes, the genotype K-9 was found superior with respect to pod size, pod weight, pulp weight and pod yield per tree. Genotype K-9 was found promising and due to perennial in nature further evaluation is required for stability.

Keywords: Pod traits, tamarind and vegetative traits.

INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a multipurpose tree belonging to the family Leguminosae (Fabaceae). It is a tropical fruit tree used primarily for its fruits, either eaten fresh or processed. In India, it is commonly grown in Karnataka, Madhya Pradesh, Bihar, Chattisgarh, Andhra Pradesh and Tamil Nadu. It is a robust tree which grows well even under different climatic conditions *viz*., tropical, subtropical and arid. In olden days it was grown from self sown seeds or by sowing seeds of unknown parentage. Hence, they exhibit a wide range of variation for morphological and yield traits. This variation may be due to effect of genetic or environmental or both. Therefore, it may be worthwhile focusing only on the very best trees in relation to neighboring ones and trees may be selected within the ecological zones. Before formulating any selection programme, it is necessary to understand the extent of variation among the genotypes and apply them for an increase in the pod and pulp production (Nicodemus *et al.*, 1997).

Many tamarind trees have been identified which were of seedling origin and they were multiplied vegetatively and maintained in the gene bank. Different genotypes which are propagated by vegetative means are being cultivated in different parts of the country. They have to be evaluated for morphological and yield attributing traits. Further, the elite lines may be useful in selection programme for the development of new cultivars.

MATERIALS AND METHODS

This study was carried out during 2017-18 at Forest Research Station, Govinkovi, Honnali taluk, Davangere district which is situated in the Southern

Transitional Zone of Karnataka at a latitude of 14.165367 and longitude of 75.6680832. The experiment was laid out in randomized complete block design with three replications and 16 genotypes *viz.,* K-9, NTI-52, K-11, S-7, S-8, S-14, S-3, N-6, D-2, C-4, D-9, NTI-89, D-19, S-6, K-10 and K-12. The morphological traits recorded were tree height, stem girth, tree shape, foliage arrangement, flowering time, stem colour, bud colour, petal colour, pod colour, pulp colour, pod shape, pod size and shell detachability along with that yield traits were also recorded. The height of the tree was measured from base to tip of the tree by using a pole and measuring tape, it was expressed in terms of meters. The girth of the tree trunk was measured by using a measuring tape and the reading was expressed in terms of meters. Tree shape, foliage arrangement, stem colour, bud colour, petal colour and pod shape were characterized based on visual observations. Tree shape was categorized as dome, cone, oval, round, semi-circle and irregular shapes. Foliage arrangement was categorized as dense and sparse arrangement. Based on the time of flower initiation, flowering time was categorized as early, mid and late flowering. Stem colour was categorized as light brown, brown and dark brown. Bud colour was categorized as greenish white, pink and dark pink. Petal colour was categorized as pale yellow and yellow. Pod and pulp colour was classified by using colour chart of Royal Horticultural Society (R.H.S), London. Pod shape was categorized as deeply curved, curved, slightly curved and straight. Based on the length, width and curvature of the pod, pod size was classified as very big, big, medium and small. Based on the ease of separation of shell from the pod, shell detachability was classified as easy, slightly hard and hard. Yield traits such as pod yield per tree was recorded at different intervals of harvest and expressed in kilograms. The number of pods per tree was recorded from each harvest and total yield was computed. Pulp per cent was calculated by dividing weight of pulp by weight of pod and multiplied by 100. Shell per cent was calculated by dividing weight of shell by weight of pod and multiplied by 100. Fibre per cent was calculated by dividing the weight of fibre over weight of pod and multiplied by 100. The number of fibres per pod was counted after separating fibres from the pulp and was expressed in numbers. The beak length of each pod was measured with the help of a thread and expressed in terms of centimetres. The experimental data recorded on various traits during the investigation were analyzed statistically using the method of analysis of variance (ANOVA) for randomized complete block design (RCBD) as given by Gomez and Gomez (1984). Whenever 'F' test was found significant for comparing the means of two treatments, the critical difference (C.D. at 5%) was worked out.

RESULTS AND DISCUSSION

In the present study the morphological traits showed significant variation (Table 1) among the selected genotypes indicating the presence of adequate variations. Two different shapes of tree were observed *viz.,* semicircle (K-9, S-7, N-6, D-2, D-19, K-10 and K-12) and irregular (NTI-52, K-11, S-8, S-14, S-3, C-4, D-9, NTI-89 and S-6). The foliage arrangements observed were dense (K-9, NTI-52, S-14, N-6, D-2, C-4, D-9, NTI-89, D-19, S-6, K-10 and K-12) to sparse(K-11, S-7, S-8 and S-3). Early (K-9, NTI-52, K-11, N-6, NTI-89, K-10 and K-12), mid (S-7, S-8, S-14, S-3, C-4 and S-6) and late flowering (D-2, D-9 and D-19) was recorded among the genotypes and pod beak was present in all the 16 genotypes. The variations with respect to the above characters are due to the effect of genotypic character and environmental conditions. Variation in tamarind genotypes were also earlier reported by Algabal *et al.* (2012) and Bhogave *et al.* (2018).

The colour traits among the genotypes showed wide variations (Table 2), in which the stem colour ranged from dark brown, brown and light brown. Bud colour ranged from greenish white, pink and dark pink. The different petal colours recorded were yellow and pale yellow. Variation with respect to petal and bud colour is due to genotypic effect. These wide range of colouration in reproductive organs that can serve as an immense breeding value and can be used not only as a morphological marker in progeny testing programme but can also enhance the fruit set by enhancing the pollinators. While, the different pod colour recorded were greyish brown, light brown, brown and dark brown. The colour of the pulp varied from light brown, brown to reddish brown. The variation with respect to the colour traits is due to the distinct feature of different tamarind genotypes and supported by Bhogave *et al.* (2018).

Table 1 : Variation in morphological traits and flowering time of tamarind genotypes

Trait	Category	Genotypes
Tree shape	Semi circle	K-9, S-7, N-6, D-2, D-19, K-10 and K-12
	Irregular	NTI-52, K-11, S-8, S-14, S-3, C-4, D-9, NTI-89 and S-6
Foliage arrangement	Dense	K-9, NTI-52, S-14, N-6, D-2, C-4, D-9, NTI-89, D-19, S-6, K-10 and K-12
	Sparse	K-11, S-7, S-8 and S-3
Flowering time	Early	K-9, NTI-52, K-11, N-6, NTI-89, K-10 and K-12
	Mid	S-7, S-8, S-14, S-3, C-4 and S-6
	Late	D-2, D-9 and D-19

Table 2 : Variation in colour of the selected tamarind genotypes

Genotype	Stem colour	Bud colour	Petal colour	Pod colour	Pulp colour
K-9	Dark brown	Dark pink	Yellow	Brown	Reddish brown
NTI-52	Brown	Dark pink	Pale yellow	Brown	Brown
K-11	Brown	Dark pink	Yellow	Grayish brown	Brown
S-7	Light brown	Pink	Pale yellow	Grayish brown	Light brown
S-8	Light brown	Pink	Yellow	Brown	Reddish brown
S-14	Brown	Dark pink	Pale yellow	Dark brown	Reddish brown
S-3	Brown	Dark pink	Yellow	Light brown	Brown
N-6	Brown	Dark pink	Yellow	Light brown	Light brown
D-2	Brown	Pink	Pale yellow	Brown	Brown
C-4	Brown	Greenish white	Pale yellow	Brown	Brown
D-9	Light brown	Pink	Pale yellow	Brown	Brown
NTI-89	Brown	Greenish white	Pale yellow	Brown	Reddish brown
D-19	Brown	Dark pink	Pale yellow	Grayish brown	Reddish brown
S-6	Brown	Greenish white	Pale yellow	Dark brown	Reddish brown
K-10	Light brown	Pink	Pale yellow	Grayish brown	Brown
K-12	Light brown	Pink	Pale yellow	Grayish brown	Brown

With respect to the shape of the pod *viz.,* straight, slightly curved, curved and deeply curved were recorded. Based on the length, breadth and curvature of the pod, pod size is classified as very big, big, medium and small sized pods. The variation with respect to the above traits (Table 3) is due to the effect of genotypic difference among the genotypes. Based on the ease of separation of shell from the pod, shell detachability was classified as easy, hard and very hard. This variation (Table 3) is due to the compactness or attachment of seeds to the pulp or shell to the pulp. Apart from this, it also depends on shell thickness. The results are also supported by Sharma *et al.* (2015).

Among 16 genotypes studied, the longest tree height was recorded in K-9 (5.08 m) while, the shortest was recorded in K-10 (3.00 m) and the maximum stem girth was recorded in K-9 (1.01 m) whereas, the minimum was recorded in S-7 (0.48 m). The variation with respect to tree height and stem girth (Table 4) is due to the effect of genotypic difference among the genotypes and also due to the differential utilization of resources from the soil. Such factors are known to cause morphological and genetic evolutionary

Table 3 : Variation in pod shape, pod size and shell detachability of tamarind genotypes.

Genotype	Pod shape	Pod size	Shell detachability
K-9	Deeply curved	Very big	Very hard
NTI-52	Slightly curved	Medium	Easy
K-11	Slightly curved	Small	Hard
S-7	Deeply curved	Big	Hard
S-8	Slightly curved	Medium	Easy
S-14	Slightly curved	Medium	Easy
S-3	Slightly curved	Medium	Easy
N-6	Curved	Big	Easy
D-2	Slightly curved	Medium	Easy
C-4	Slightly curved	Medium	Easy
D-9	Curved	Medium	Hard
NTI-89	Slightly curved	Big	Easy
D-19	Curved	Medium	Easy
S-6	Curved	Medium	Easy
K-10	Straight	Medium	Very hard
K-12	Slightly curved	Medium	Very hard

Table 4 : Variation in quantitative traits of tamarind genotypes

Genotype	Tree height (m)	Stem girth (m)	Pod yield per tree (kg)	Pulp per cent per pod	Shell per cent per pod	Fibre per cent per pod	Number of fibres per pod
K-9	5.08	1.01	12.80	48.87	21.47	6.17	4.03
NTI-52	4.13	0.85	10.79	40.78	27.06	2.84	4.27
K-11	3.17	0.58	4.77	41.18	23.83	2.16	3.07
S-7	3.67	0.48	5.97	42.10	22.39	3.17	5.00
S-8	3.67	0.53	5.03	46.38	22.07	5.13	3.97
S-14	3.33	0.58	4.55	44.45	15.78	3.24	3.80
S-3	4.23	0.62	3.96	52.03	28.13	3.45	2.90
N-6	4.00	0.77	9.07	42.03	22.21	6.94	7.33
D-2	4.50	0.93	6.88	38.45	25.97	2.96	5.37
C-4	4.17	0.65	4.53	44.50	27.60	3.10	2.97
D-9	4.00	0.60	5.33	38.07	26.15	3.16	4.93
NTI-89	4.13	0.57	8.72	42.13	26.03	3.51	4.03
D-19	3.87	0.60	6.84	44.52	17.18	3.94	4.93
S-6	4.10	0.49	4.44	42.15	16.53	4.13	4.37
K-10	3.00	0.60	8.21	40.52	27.53	4.92	7.23
K-12	3.20	0.70	7.38	35.09	28.87	5.48	5.87
S. Em ±	0.35	0.06	0.45	1.24	0.75	0.31	0.30
C. D @5%	1.01	0.17	1.30	3.58	2.17	0.90	0.86

divergences among the population. The supporting results have also been reported by Rao and Subramanyam (2010) and Divakara and Rathakrishnan (2011).

Significant difference in pod traits (Table 4) indicates the scope of genetic improvement. The genotype K-9 recorded significantly higher pod yield (12.80 kg/tree) and pulp per cent (48.87 %). The variation is attributed due to the difference in pod length, width, circumference, thickness and difference in the rate of development of vascular tissues (Pooja *et al.,* 2018). The highest number of pods per tree was recorded in NTI-52 (989.07) and the lowest number of pods per tree was recorded in S-8 (206.48) (Fig. 1). The minimum pod yield per tree was recorded in S-3 (3.96 kg/tree). The variation with respect to number of pods per tree is due to the higher number of primary and secondary branches and also inherent genetic makeup of each genotype. Apart from this, it also depends on environmental conditions. The highest beak length was recorded in D-19 (0.05 cm) and the lowest was observed in S-7, S-14, N-6, D-9, K-10 and K-12 (0.01 cm) (Fig. 2). The maximum number of fibres per pod was recorded in N-6 (7.33) and the minimum was recorded in S-3 (2.90). The difference in fibre number

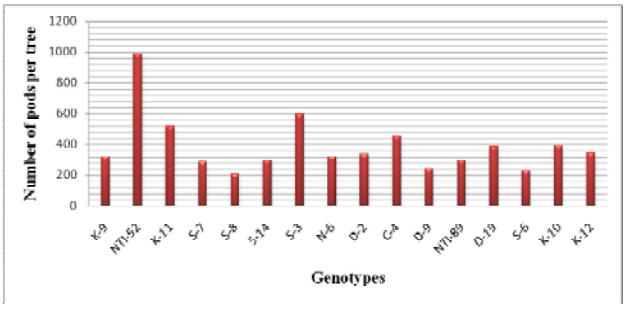

Fig. 1 : Variation in number of pods per tree of tamarind genotypes

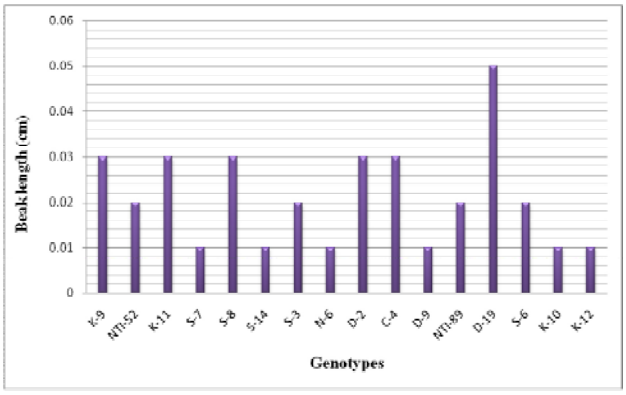

Fig. 2 : Variation in the beak length of tamarind genotypes

is attributed to the genetic makeup of each genotype. These findings are in line with the views reported by Fandohan *et al.* (2011) and Singh and Nandini (2014).

The highest pulp per cent was recorded in S-3 (52.03 %) which was on par with K-9 (48.87 %) and the lowest was observed in K-12 (35.09 %). The difference in pulp per cent per pod (Table 4) is clearly attributed due to the length, width, thickness and pulp content of the pod and also distinct feature of different genotypes. Similar results have also been reported by Prabhushankar *et al*. (2004) in tamarind and Usha *et al.* (2018) in macadamia nut. A significant difference was observed among the genotypes in respect of shell per cent per pod. The maximum shell per cent was recorded in K-12 (28.87 %) which was on par with S-3 (28.13 %) and C-4 (27.60 %) and the lowest was recorded in S-14 (15.78 %) which was significantly lower than all other genotypes. The variation in shell per cent is due to the difference in pod size, shell thickness and shell weight. Apart from this, it is inherent genetic makeup of each genotype. Similar variations with respect to shell per cent was also observed in tamarind by Sivakumar (2000) and Kotecha and Kadam (2002). The highest fibre per cent per pod was recorded in N-6 (6.94 %) which was on par with K-9 (6.17 %) and the lowest was observed in K-11 (2.16 %). The variation with respect to fibre per cent is due to the difference in the rate of development of vascular tissues in the pod, fibre weight per pod and also distinct feature of the different genotypes. Similar variations with respect to fibre per cent were also reported by Hanamashetti and Sulikeri (1997), Mastan *et al*. (1997) and Divakara (2008) in tamarind.

CONCLUSION

The study revealed existence of considerable variations among the genotypes for all the traits studied. The genotype K-9 was found superior compared to all other genotypes with respect to pod size, pod weight, pulp weight and pod yield per tree. Therefore, genotype K-9 found promising and subjected for further evaluation to ensure consistent results for utilization.

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Original Research Paper

Breeding tomatoes suitable for processing with triple disease resistance to tomato leaf curl disease, bacterial wilt and early blight

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ABSTRACT

India is the second largest producer of tomato with 11 per cent global share and cultivated on an estimated area of 0.76 million hectares with productivity of 24 tonnes per hectare. Less than 1% of the produce is processed when compared to 26% in other major producing countries. Of the estimated more than 41 million tonnes of tomato processed globally, only 130,000 tonnes were processed in India and domestic demand for processed tomato products is expanding at an estimated 30% annually. At present traditional fresh market tomato cultivars are being processed though such cultivars are unsuitable for processing. Processors in India are looking for high yielding tomato cultivars with high total soluble solids (5-6 º Brix), acidity not less than 0.4%, pH less than 4.5 and uniform red colour with a/b colour value of at least 2. In addition, firm fruited tomato cultivars with joint less pedicel (j2) which facilitate mechanical harvesting or rapid hand picking. ICAR-Indian Institute of Horticultural Research has recently developed two high yielding F_1 hybrids in tomato *viz:* Arka Apeksha and Arka Vishesh suitable for processing. On evaluation for three years, both the hybrids recorded good level of total soluble solids (4.5-5º Brix) and colour value of 2. Further, both the hybrids had high yield potential (80-90 tonnes / hectare) with triple disease resistance to tomato leaf curl disease, bacterial wilt and early blight. Arka Apeksha and Arka Vishesh were also bred with jointless pedicel making them suitable for mechanical harvesting. Our experimental studies on vine storability revealed that all the fruits were intact on plants even 110 days after transplanting in the main field facilitating once over harvest.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops cultivated in the world. It is used as salads and also cooked vegetable in the preparation of curries. Processed items such as tomato puree, ketchup, pickle, chutney, whole peeled tomatoes, and tomato powder are also consumed considerably. Tomatoes are also an important source of vitamins and minerals. They are an excellent source of phosphorus, iron and vitamin A, B and C (Cobley and Steele, 1976). They also contain small amounts of the B complex vitamins; thiamin, niacin and riboflavin (Naika *et al*., 2005). They are loaded in minerals, vitamins, essential amino acids, sugars and dietary fibers. Most importantly, tomatoes are rich in carotenoids, especially lycopene (Beecher, 1998). Lycopene and other flavonoids in tomato serve as good source of antioxidants (Agarwal and Rao, 2000). Tomato occupies 5.05 million hectares with a productivity of 37 t/ha in the world. In India it is cultivated in an estimated area of 0.81 million hectares with productivity of 25.3 t/ha (FAO STAT 2020). One of the major reasons for low productivity in India is due to prevalence of various biotic and abiotic stresses. White fly (*B. tabaci*) transmitted tomato leaf curl disease, bacterial wilt (*R. solanacearum*) and early blight (*A. solani*) cause economic yield losses in the major tomato growing areas of the country and elsewhere in the world (Lukyanenko, 1991). Though India is the second largest producer

of tomato with 11 % global share, less than 1% of the produce is processed when compared to 26% in other major producing countries Of the estimated more than 41 million tonnes of tomato processed globally, only 130,000 tonnes are processed in India and domestic demand for processed tomato products is expanding at an estimated 30% annually (Subramaniam, 2016). At present traditional fresh market tomato cultivars are being processed though such cultivars are unsuitable for processing. Processors in India are looking for high yielding tomato cultivars with high total soluble solids (5-6º Brix), acidity not less than 0.4%, pH less than 4.5 and uniform red colour with a/b colour value of at least 2 (Stevens and Rudich, 1978). In addition, firm fruited tomato cultivars with joint less pedicel (j2) which facilitate mechanical harvesting or rapid hand picking. Tomato breeding programme at ICAR-Indian Institute of Horticultural Research, Bengaluru has resulted in the development of high yielding dual purpose F_1 hybrids with triple disease resistance to tomato leaf curl disease (ToLCD), bacterial wilt (BW) and early blight (EB) suitable for both fresh market and processing.

MATERIALS AND METHODS

Development of triple disease resistant lines and F_1 hybrids

Back cross breeding method was adopted during 2005 to pool genes carrying resistance to ToLCD, BW and EB. An advanced breeding line IIHR-2202 (CLN-2123-Dc1F1-111-17-21-2-12) with combined resistance to ToLCD +BW received from The World Vegetable Center (WVC) was crossed with EB resistant line IIHR-1816 (NCEBR1). The resultant F_1 was backcrossed to IIHR-1816 and further advanced up to BC1F7 to develop seven advanced breeding lines with triple disease resistance to ToLCD+BW+EB (Fig. 1).

All the seven advanced breeding lines were resistant to ToLCD (*Ty 2*), BW and EB had high potential with good fruit quality attributes like deep red and firm fruits. All the seven lines were crossed with eight advanced breeding lines received from The World Vegetable Center, Taiwan in a line x tester design to develop 56 hybrids with triple disease resistance. Two hybrid combinations *viz.,* IIHR-2834 (TLBER-12-21-43-1) x IIHR-2833 (CLN-2498D) later named as Arka Rakshak and IIHR-2835 (TLBER-38-7-4-27) x IIHR-2832 (CLN-2498E) later named as Arka Samrat were resistant to ToLCD+BW+EB with high yield potential & excellent fruit quality attributes. Both Arka Rakshak and Arka Samrat were identified at Institute level for commercial cultivation during 2010 (Fig. 2).

IIHR-2202 (Combined resistant to ToLCV + BW) x IIHR-1816 (Moderately resistant to EB)
↓
F1 x IIHR-1816
↓
BC1F1
(Triple disease resistant recombinants selected underartificial conditions and advanced)
↓
BC1F7
(Seven advanced breeding lines *viz;* TLBER-7-12-15-28, 7-12-15-29, 7-4-11-29, 7-4-11-34, 38-7-4-27, 38-7-41-43 and 12-21-43-1 with triple resistance were selected)

Fig. 1 : Flow chart detailing the development of triple disease resistant tomato lines

RESULTS AND DISCUSSION

Development of dual purpose F_1 hybrids in tomato with triple disease resistance

Breeding for dual purpose tomato was initiated during 2016. Our aim was to develop high yielding triple disease resistant F_1 hybrids suitable for both fresh market and processing for year-round cultivation under open. Several hybrid combinations were attempted involving the triple disease resistant parent IIHR-2834 which had jointless (j2) pedicel which facilitates mechanical harvesting. IIHR-2834 was crossed with two advanced breeding lines *viz.,* IIHR-2918 (ToLCVRES4-F3-21-9-1) and IIHR-2917 (ToLCVRES4-F3-188-1-1) which were resistant to ToLCD (*Ty 3*) and BW and later named as Arka Apeksha (H-385) and Arka Vishesh (H-391) respectively (Fig. 3).

Performance of dual purpose F_1 hybrids: Arka Apeksha (H-385) and Arka Vishesh (H-391)

A total of eighteen F_1 hybrids including two hybrids *viz.,* H-385 (IIHR-2834 x IIHR-2918) and H-391 (IIHR-2834 x IIHR-2917) were evaluated for two years *viz.,* 2017 (rainy season), 2017-18 (winter

IIHR-2834

IIHR-2835

IIHR-2833

IIHR-2832

Arka Rakshak

Arka Samrat

Fig. 2 : Triple disease resistant F_1 hybrids developed at ICAR-IIHR

Arka Apeksha (H-385)

Arka Vishesh (H-391)

Fig. 3 : Arka Apeksha and Arka Vishesh dual purpose tomato F_1 hybrids

season) and 2018 (rainy season) and 2018-19 (winter season) respectively under open field conditions. Five commercial F_1 hybrids *viz.,* Arka Rakshak, Arka Samrat, Abhinava, Lakshmi and Shivam were also included as checks. During rainy season (2017), H-397 was the highest yielder followed by H-391 (94 t/ha), H-387 (84 t /ha) & H-385 (83 t /ha) (Table 1).

During winter season (2017-18), H-391 (44 t/ha) was the top yielder followed by H-387 (34 t/ha) and H-397 (33t/ha) (Table 2). During rainy season (2018), H-397 (51.88T/ha), H-387 (46t/ha), H-391 (28 t/ha) and H-385 (25 t/ha) (Table 3) were the top yielders among the processing type. During winter season (2018-19) both H-391 (31 t/ha) and H-385 (30t/ha) were also high yielders (Table 4).

Mean yield over all the seasons revealed that H-385 (46 t/ha) &H-391 (43 /ha) (Table 5) expressed high yield potential over the commercial dual purpose hybrid Abhinava (40 t/ha). Both these two hybrids also recorded average fruit weight of 70g-90g with high TSS (5^0Brix) and deep red firm fruits (8 kg/cm^2) which meet present day market demand. During rainy season (2018), both the hybrids *viz.,* H-385 and H-391 were triple disease resistant to ToLCD+BW+EB, whereas commercial hybrids expressed moderate resistance and susceptible reaction to ToLCD and BW (Table 6). Four season's data revealed that H-385 and H-391 had high yield potential & commercially acceptable fruit quality attributes with triple disease resistance to ToLCD, BW and EB. Pooled analysis for yield per hectare over three years confirmed yield stability of Arka Apeksha and Arka Vishesh (Table 7)

Table1 : Performance of tomato F_1 hybrids at ICAR-IIHR, Bengaluru (Rainy season, 2017)

Hybrid	Estima-ted yield (t/ha)	Average Fruit weight	No of fruit/ kg (g)	Fruit length (cm)	Fruit width (cm)	Pericarp thickness (cm)	TSS (^{0}Brix)	Fruit firmness (kg/cm^2)	No. of locules	Shelf Life (Days)	Fruit colour	Fruit shape
H-385	83.00	81.23	12.31	6.25	4.50	0.10	5.00	8.25	2.00	-	-	-
H-387	84.00	92.85	10.77	5.55	5.00	0.50	5.10	9.75	3.00	18	R	Sq. Round
H-391(Arka Vishesh)	93.17	101.11	9.89	5.90	5.00	0.75	5.50	7.00	3.00	19	R	Sq. Round
H-397	112.83	91.66	10.91	4.90	6.00	0.70	5.00	8.00	6.00	12	R	Obl-rd
H-423	52.50	95.24	10.50	6.10	5.50	0.55	4.70	8.25	3.00			
H-501	79.50	93.98	10.64	5.15	6.00	0.70	5.00	7.75	4.00	14	R	Obl-rd
H-502	89.50	80.45	12.43	4.35	5.80	0.65	4.00	8.25	4.00	-	-	-
H-504	72.25	95.15	10.51	5.10	5.90	0.50	4.80	6.75	6.00	-	-	-
H-505	74.67	101.42	9.86	5.10	6.00	0.60	5.00	9.50	4.00	-	-	-
H-506	98.33	113.38	8.82	5.15	6.60	0.90	4.10	6.75	5.00	13	R	Obl-rd
PH-1021	88.83	134.41	7.44	5.35	6.00	0.60	4.70	8.75	6.00	8	R	Obl-rd
PH-1025	89.00	127.23	7.86	5.75	6.00	0.70	5.10	7.25	6.00	9	R	Obl-rd
PH-6321	92.23	120.05	8.33	6.00	7.30	0.65	4.15	7.50	5.00	9	R	Obl-rd
Arka Rakshak	90.17	72.94	13.71	5.90	5.00	0.55	4.40	8.75	3.00	18	DR	Oval
Arka Samrat	103.33	89.93	11.12	5.10	6.20	0.80	4.90	9.00	4.00	19	DR	Obl-rd
Lakshmi	102.33	63.69	15.70	5.25	4.80	0.55	4.25	6.25	4.00	13	DR	Oblate
Shivam	99.83	86.43	11.57	4.65	5.65	0.50	4.80	6.75	5.00	13	DR	Oblate
Abhinav	88.33	90.91	11.00	5.50	4.60	0.60	4.35	8.25	2.00	13	DR	Oval
CD @5%	27.83	18.21	2.08	0.32	0.15	0.10	0.73	0.78	0.15			
CV (%)	19.17	8.21	11.07	2.30	0.95	5.25	5.47	3.70	2.26			

Table 2 : Performance of tomato F_1 hybrids at ICAR-IIHR, Bengaluru (Winter season, 2017-18)

Hybrid	Estimated yield (t/ha)	Average Fruit weight (g)	No of fruit/ kg	Fruit length (cm)	Fruit width (cm)	Pericarp thickness (cm)	TSS (^{0}Brix)	Fruit firmness (kg/cm^2)	No. of locules
H-385 (Arka Apeksha)	-	-	-	-	-	-	-	-	-
H-387	34.00	85.86	11.65	6.83	6.33	0.67	5.17	8.00	3.00
H-391 (Arka Vishesh)	43.67	89.56	11.17	7.17	6.17	0.90	4.83	7.17	2.00
H-397	33.33	105.30	9.50	6.33	7.17	0.77	5.17	7.17	4.00
H-423	-	-	-	-	-	-	-	-	-
H-501	16.08	125.00	8.00	6.50	7.67	0.97	5.20	7.00	6.00
H-502	24.83	109.01	9.17	6.67	7.33	0.70	4.83	7.50	5.00
H-504	-	-	-	-	-	-	-	-	-
H-505	34.33	97.64	10.24	5.17	6.17	0.67	5.00	9.00	3.00
H-506	34.83	105.30	9.50	5.17	6.33	0.50	5.00	6.00	4.00
PH-1021	29.17	120.37	8.31	6.00	6.67	0.63	5.17	8.33	4.00
PH-1025	32.33	132.28	7.56	7.00	7.50	0.70	4.83	8.00	4.00
PH-6321	38.17	112.04	8.93	6.67	6.77	0.73	5.17	8.00	3.00
Arka Rakshak	40.00	93.94	10.65	6.67	6.83	0.83	4.17	7.50	4.00
Arka Samrat	40.17	86.75	11.53	6.17	7.00	0.80	5.00	8.00	3.00
Lakshmi	39.83	88.38	11.31	6.83	6.17	0.83	5.00	8.00	2.00
Shivam	37.83	72.12	13.87	6.50	6.17	0.53	5.17	7.00	5.00
Abhinav	32.33	92.98	10.76	4.50	6.00	0.60	5.00	6.17	4.00
CD @5%	15.62	30.37	2.12	0.65	0.49	0.10	0.40	0.39	0.31
CV (%)	6.78	6.81	2.21	1.29	2.00	7.49	2.87	1.45	2.14

Table 3 : Performance of promising tomato hybrids during rainy season (2018-19)

Hybrid	Estimated yield (t/ha)	Average Fruit weight (g)	No of fruit/kg	Fruit length (cm)	Fruit width (cm)	Pericarp thickness (cm)	TSS (^{0}Brix)	Fruit firmness (kg/cm^2)	No. of locules
H-385 (Arka Apeksha)	24.67	66.67	15.67	5.63	5.27	0.70	5.00	8.20	3.33
H-387	46.09	100.00	10.00	5.03	4.90	0.70	4.33	8.17	3.00
H-391(Arka Vishesh)	28.07	68.70	14.67	5.33	5.60	0.97	4.83	9.17	3.00
H-397	51.88	81.20	12.33	4.93	6.30	0.97	4.17	6.63	6.00
H-423	22.67	83.33	12.00	6.20	5.30	0.80	5.17	7.37	3.00
H-501	21.04	120.37	8.33	5.33	6.03	0.87	5.00	6.50	5.00
H-502	2.81*	93.94	10.67	4.00	5.90	0.91	5.17	7.33	5.67
H-504	1.04*	91.41	11.00	4.03	4.90	0.47	5.73	6.50	5.00
H-505	9.79	96.97	10.33	4.50	4.80	0.50	4.77	8.33	4.33
H-506	22.19	100.67	10.00	5.23	6.23	0.77	4.23	6.17	6.00
PH-1021	27.60	120.37	8.33	5.03	6.20	0.57	5.37	7.50	5.00
PH-1025	17.81	120.37	8.33	5.23	5.90	0.30	4.60	8.17	5.00
PH-6321	40.63	116.67	8.67	5.17	6.03	0.60	5.10	10.33	5.00
Arka Rakshak	52.71	91.41	11.00	6.17	5.83	0.93	5.17	10.30	3.00
Arka Samrat	40.73	120.37	8.33	4.97	5.73	0.83	5.33	8.70	5.00
Lakshmi	4.38*	84.92	12.00	5.10	5.33	0.70	4.17	7.50	5.67
Shivam	25.52	81.20	12.33	3.93	6.00	0.53	5.23	7.67	6.00
Abhinav	25.83	103.70	9.67	6.10	4.87	0.90	4.50	8.50	2.00
CD@5%	18.50	15.12	2.17	0.31	0.45	0.18	0.79	0.48	0.42
CV (%)	9.30	0.96	2.45	0.46	3.12	2.82	0.65	1.99	1.26

Table 4 : Performance of tomato hybrids during winter season (2018-19)

Hybrid	Estimated yield (t/ha)	Fruit length (cm)	Fruit width (cm)	Pericarp thickness (cm)	TSS (^{0}B)	Fruit Firmness (kg/cm^2)	No of locules
H-385 (Arka Apeksha)	30.0	6.8	6.1	0.7	5.3	7.5	2.3
H-387	28.8	6.9	5.6	0.7	4.5	7.0	3
H-391 (Arka Vishesh)	31.4	6.5	5.6	0.7	4.7	6.6	2.7
H-423	28.9	7	5.6	0.7	4.4	7.3	3.3
H-501	24.8	5.5	7	0.4	4.8	6.0	5.3
H-502	28.1	4.5	6.7	0.6	5.4	5.7	5.7
H-504	28.6	4.2	5.8	0.5	5.5	6.1	5.0
H-505	26.1	5.8	6.8	0.7	5.3	6.9	5.7
H-506	28.0	5.3	6.5	0.8	4.6	6.9	5.3
PH-1021	28.3	5.4	5.8	0.5	5.4	7.7	5.7
PH-1025	25.3	6.6	7	0.5	5.2	5.9	6.3
PH-6321	32.3	5.7	5.7	0.6	5.5	6.0	6.3
Arka Abhed	28.9	5.4	6.1	0.7	5	6.7	6.0
Arka Rakshak	26.6	6.1	5.4	0.7	5.2	6.7	2.7
Arka Samrat	23.7	6.2	6.2	0.5	5.5	6.0	4.0
Lakshmi	22.9	4.4	5.8	0.5	5.8	5.8	4.0
Shivam	21.6	4.6	5.5	0.6	4.8	6.0	4.7
Abhinav	30.7	6.3	5.2	0.7	5	7.8	2.0
CD (P=0.05)	5.35	0.25	0.45	0.2	0.65	0.86	1.58
CV (%)	2.4	0.53	0.88	3.91	1.4	1.61	3.99

Table 5 : Mean Performance of selected tomato F_1 hybrids for yield and quality parameters

Hybrid	Estimated yield (t/ha)	AverageFruit wt (g)	TSS (^{0}Brix)	Fruit firmness (kg/cm^2)	% Increase in yield over Abhinav
H-385 (Arka Apeksha)	46.00	95.14	5.15	7.85	15
H-387	41.75	77.56	4.70	7.80	
H-391 (Arka Vishesh)	43.19	69.88	4.90	7.20	7.5
H-397	47.46	74.43	4.60	7.30	
H-423	31.20	82.96	4.40	6.90	
H-501	33.49	101.55	5.35	6.40	
H-502	33.83	83.76	5.10	6.90	
H-504	31.38	88.17	5.30	6.75	
H-505	33.80	94.95	4.75	7.90	
H-506	40.04	88.97	4.90	7.00	
PH-1021	36.92	108.80	5.15	7.05	
PH-1025	39.34	92.57	5.15	6.90	
PH-6321	42.96	88.12	4.90	7.65	
Arka Rakshak	43.78	72.07	4.90	7.80	
Arka Samrat	42.56	75.82	5.30	7.30	
Lakshmi	35.88	65.68	5.15	6.55	
Shivam	38.00	72.89	4.95	6.55	
Abhinav	39.77	72.88	4.80	7.70	

Table 6 : Reaction of tomato F_1 hybrids to ToLCBV, BW, EB and LB during rainy season (2018)

Hybrid	Reaction to ToLCBV Disease Severity Score		Disease Reaction	Bacterial wilt incidence (%)	Reaction to EB (PDI)	Reaction to LB (PDI)
	30dpi	60dpi				
H-385 (Arka Apeksha)	0.44±0.22	1.00±0.19	HR	2 (R)	05 (HR)	97 (HS)
H-387	0.67±0.19	0.89± 0.29	HR	2 (R)	10 (HR)	92 (HS)
H-391(Arka Vishesh)	0.33 ± 0.19	0.67± 0.00	HR	1 (R)	10 (HR)	100 (HS)
H-397	0.00± 0.00	0.44± 0.19	HR	0 (HR)	05 (HR)	0 (HR)
H-501	0.22 ± 0.11	0.44 ± 0.11	HR	0 (HR)	20 (R)	94 (HS)
H-506	0.22 ±0.11	0.67± 0.00	HR	2 (R)	15 (R)	92 (HS)
Arka Rakshak	1.78 ±0.11	2.44± 0.19	MR	6 (R)	15 (R)	100 (HS)
Arka Samrat	2.00 ±0.19	2.78± 0.19	MR	8 (R)	20 (R)	100 (HS)
Abhinava	1.11± 0.11	1.67 ± 0.19	MR	9 (R)	30 (MR)	98 (HS)
Lakshmi	0.67± 0.19	2.17 ± 0.11	MR	58 (HS)	30 (MR)	100 (HS)
Shivam	0.83± 0.19	3.00 ± 0.11	S	45 (S)	05 (HR)	93 (HS)
Punjab Chuhara	1.75± 0.09	4.00 ± 0.00	HS	-	-	-
CD@5%	0.652	0.650				14.11
CV %	36.32	22.32				6.89

Note: dpi= days of post inoculation, HR= Highly Resistant, MR= Moderately Resistant, R= Resistant, S= Susceptible and HS= Highly Susceptible

Table 7 : Pooled analysis for estimated yield per hectare

Hybrid	Yield (t/ha)		
	2017	2018	2019
H-385 (Arka Apeksha)	83.0	24.7	30.0
H-387	84.0	46.1	28.8
H-391 (Arka Vishesh)	93.2	28.1	31.4
H-397	112.8	51.9	28.9
H-423	52.5	22.7	28.9
H-501	79.5	21.0	24.8
H-502	89.5	2.8	28.1
H-504	72.3	1.0	28.6
H-505	74.7	9.8	26.1
H-506	98.3	22.2	28.0
H-1021	88.8	27.6	28.3
H-1025	89.0	17.8	25.3
H-6321	92.2	40.6	32.3
Arka Rakshak	90.2	52.7	26.6
Arka Samrat	103.3	40.7	23.7
Laxmi	102.3	4.4	22.9
Shivam	99.8	25.5	21.6
Abhinav	88.3	25.8	30.7
CD (P=0.05)		10.86	
CV (5%)		24.58	

Assessment of Arka Apeksha (H-385) and Arka Vishesh (H-391) for processing qualities

Processing qualities in fine pulp were estimated in Arka Apeksha and Arka Vishesh at ICAR-IIHR, Bengaluru. Both the hybrids exhibited higher values for TSS (>5^{0}Brix), lycopene (>12 mg/100g) and colour index (47) (Table 8). Processing qualities in tomato puree was also estimated in Arka Apeksha and Arka Vishesh. Arka Vishesh recorded the highest TSS (11.20^0 Brix) when compared to commercial puree marketed by popular processing Industries such as Dabur, Kisan and Morton (Table 9). Both the hybrids also exhibited higher values for lycopene (> 13 mg/100g). Higher values were also observed for TSS (>27^{0}Brix) & lycopene (>14 mg/100g) in tomato paste in both the hybrids (Arka Apeksha & Arka Vishesh) (Table 10). In order to assess the processing qualities and suitability of Arka Apeksha (H-385) and Arka Vishesh (H-391), fruit samples were supplied to four commercial processing industries located in the different states in the country *viz.,* Sahyadri Foods, Nashik, Maharashtra state, Sun-sip Foods, Srinivasapura, Karnataka State, Jadli Foods, Krishnagiri, Tamil Nadu State and Cremica Food Industries Ltd., Phillaur, Punjab State. Sahydri Foods analysed fruit samples of four entries *viz.,* Arka Apeksha, Arka Vishesh, Abhinav and Arka Ashish (a pure line selection from UC82B) for Hunter Lab colour value, lycopene, acidity, TSS, pH and total solids in the initial pulp and puree (Table 11). Colour value was more than 2 in the puree in all the samples. Arka Apeksha and Arka Vishesh recorded TSS 4^0 Brix and >12^0 Brix in the initial pulp and puree respectively which was slightly more than dual

Table 8 : Processing qualities attribute for fine pulp

Hybrid	TSS (°Brix)	Total Solids (%)	Juice Yield (%)	pH	Acidity (%)	Vit-C (mg/ 100g)	Lycop-ene (mg/ 100g)	Tomato Colour Index
Abhinav	5.68	7.54	74	4.2	0.38	27.84	10.26	44
H-397	5.66	6.86	67	4.1	0.33	17.52	12.19	45
Arka Vishesh (H-391)	5.40	6.60	70	4.3	0.54	15.83	12.97	47
Arka Apeksha (H-385)	5.33	6.88	74	4.2	0.54	20.15	13.41	47
H-387	5.00	7.26	74	4.1	0.52	13.85	11.95	47

Table 9 : Processing qualities- tomato puree

Hybrids	TSS (°Brix)	pH	Acidity (%)	Vit-C (mg/ 100g)	Lycopene (mg/ 100g)	Colour Value	Tomato Colour Index
Abhinav	10.2	4.1	0.66	36.56	11.90	0.99	42.18
H-397	10.80	4.1	0.67	22.26	13.91	1.20	45.65
Arka Vishesh (H-391)	11.20	4.1	0.83	28.75	13.03	1.36	47.21
Arka Apeksha (H-385)	8.80	4.1	0.81	27.54	13.41	1.28	47.99
H-387	10.33	4.0	0.72	24.06	13.38	1.34	50.46
Dabur old	9.8	3.9	0.59	13.42	14.02	1.21	45.22
Dabur new sample	10	3.8	0.60	14.66	13.92	-	-
Kisan	8.9	4.0	0.62	26.47	10.65	1.31	47.10
Morton	9.0	4.0	0.634	19.74	10.72	1.14	45.38

Table 10 : Processing qualities-tomato paste

Hybrid	TSS (°Brix)	Acidity (%)	Colour Value as per formula	Tomato Colour Index	Vitamin C (mg/ 100g)	Lycopene (mg/ 100g)	Tomato Colour Index
Abhinav	28.0	1.95	1.20	47.72	47.13	12.85	28.0
H-397	27.5	1.31	1.73	53.36	32.08	14.16	27.5
Arka Vishesh (H-391)	27.0	1.72	1.38	48.98	38.97	14.13	27.0
Arka Apeksha (H-385)	26.2	1.43	1.40	50.65	36.58	14.15	26.2
Indira	28.0	1.12	1.21	45.22	32.33	14.08	28.0

purpose commercial hybrid Abhinav and processing variety Aka Ashish. But Arka Ashish (476) recorded highest lycopene (C/2 scale) followed by Abhinav (473), Arka Apeksha (469) and Arka Vishesh (442). Acidity was less than 0.26 in Arka Ashish and Abhinav. pH was less than 4.3 in all the entries. Arka Apeksha recorded the highest total solids (86.35%) (Table 11) in the puree. Both Arka Apeksha and Arka Vishesh had acceptable processing qualities. Sun-sip foods analysed fruit samples in Arka Apeksha, Arka Vishesh and Abhinav for process time, Brix, acidity, pH, colour value and number. of pouches filled. All the parameters were on par with each other in the initial pulp and the final product, where as Arka Apeksha (2 hr 12 min) took less time compared to Arka Vishesh (2 h 25min) & Abhinav (2 h 27 min)

Table 11 : Processing quality parameters of selected hybrids

Parameter	H-391 (Arka Vishesh)				Abhinav				H-385 (Arka Apeksha)				Arka Ashish			
	Initial Pulp		12 brix puree		Initial Pulp		12 brix puree		Initial Pulp		12 brix puree		Initial Pulp		12 brix puree	
Hunter Lab colour value	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale	On C/2 Scale	On D65/2 Scale
L	32.92	31.27	23.74	23.1	32.38	31.15	24.3	23.5	32.75	30.54	23.65	22.77	32.77	31.12	23.17	22.37
a	34.36	31.45	27.89	26.47	34.04	31.85	30.86	29.18	33.26	30.05	29.48	28.16	34.09	31.91	30	28.4
b	16.75	15.3	13.55	12.18	15.7	14.19	14.16	12.96	17.25	15.33	13.64	12.6	17.03	15.37	13.7	12.49
a/b	2.052	2.055	2.058	2.173	2.169	2.244	2.179	2.251	1.928	1.96	2.162	2.234	2.002	2.076	2.189	2.273
Lycopene C/2	440	NA	442	NA	471	NA	473	NA	409	NA	469	NA	428	NA	476	NA
Brix	4	12.2	4	12	4	12.2	3.2	12.1								
Acidity	0.3	0.94	0.25	0.63	0.35	0.62	0.26	1.07								
pH	4.1	3.93	4.14	4.1	4.12	4.09	4.14	4.16								
Total Solids (%)	**95.22%**		**85.38%**		**94.76%**		**85.91%**		**95.15%**		**86.35%**		**96.13%**		**84.78%**	

Courtesy: Mr. Sachin, Sahyadri foods, Nashik, Maharashtra

Table 12 : Processing quality parameters of selected hybrids

Parameter	Arka Vishesh (H-391)		Arka Apeksha (H-385)		Abhinav	
Net fruit weight (kg)	6		5.85		6	
Process Time	2 Hrs 25 Min		2 Hrs 12 Min		2 Hrs 27 Min	
Readings	Initial	Final	Initial	Final	Initial	Final
Brix	4.05	12.12	4.28	12.21	4.41	12.31
Acidity %	0.28	0.89	0.28	0.85	0.31	0.89
pH	4.16	3.51	4.01	3.71	4.0	3.85
Colour-a/b	1.75	1.98	1.9	1.97	1.9	1.98
No of Pouches Filled	2 No's	2 No's	2 No's			

Courtesy: Mr. Hemanth, Sun-Sip foods, Srinivasapura, Karnataka

Table 13 : Physical, chemical, organoleptic analysis of fresh fruits

Parameter	Abhinav	Arka Vishesh (H-391)	Arka Apeksha (H-385)
T.S.S.	4.2°Brix	4.2°Brix	4.2°Brix
ACIDITY (% as C/A)	0.27%	0.33%	0.38%
SEEDS PERCENTAGE	VERY LESS	VERY LESS	VERY LESS
COLOUR	DEEP RED	DEEP RED	DEEP RED
TASTE	NATURAL & CHARACTERSTICS OF RIPE TOMATO	NATURAL & CHARACTERSTICS OF RIPE TOMATO	NATURAL & CHARACTERSTICS OF RIPE TOMATO
FLUSH	GOOD/DEEP RED	GOOD/DEEP RED	GOOD/DEEP RED
FLAVOR	TYPICAL RIPE TOMATO FLAVOR	TYPICAL RIPE TOMATO FLAVOR	TYPICAL RIPE TOMATO FLAVOR
APPEARANCE	SOUND & GOOD	SOUND & GOOD	SOUND & GOOD

Table 14 : Processing quality characteristics of Hybrids; Arka Vishesh (H-391) and Arka Apeksha (H-385)

Hybrid	T.S.S. (°Brix)	pH	Acidity (%)	Hunter color value on C2 illumination	Viscosity (30 sec.) BOSTWICK	Visual observation
Arka Vishesh(H-391)	4.05	4.39	0.36	L=32.56 a=35.37 b=16.67 ab=2.12	14.00	Less juicy, soft skin, less seed
	4.00	4.44	0.32	L=35.80 a=34.89 b=17.96 ab=1.94	14.00	
	3.94	4.42	0.35	L=30.19 a=34.45 b=16.16 ab=2.13	14.20	
Mean	4.00	4.41	0.34	a/b=2.06	14.00	
Arka Apeksha (H-385)	4.15	4.36	0.38	L=32.52 a=35.12 b=16.84 ab=2.09	12.00	Hard skin, less seed
	4.10	4.43	0.33	L=36.51 a=35.75 b=18.09 ab=1.98	12.50	
	4.05	4.42	0.35	L=32.94 a=35.87 b=17.43 ab=2.06	12.50	
Mean	4.1	4.4	0.35	a/b=2.04	12.33	

Courtesy: Cremica, Phillaur, Punjab

Table 15 : Processable characteristics for Arka Vishesh (H-391) and Aka Apeksha (H-385)

Parameter	H-391	H-385	Parameters desired by Processing Industry
TSS (degree ^{O}Brix)	4-4.6	4-4.7	4.2 or higher (Higher the better)
Colour value	1.98-2.12	1.96-2.09	> 1.95
Acidity (%)	0.32-0.36	0.34-0.38	<0.40
pH	4.21-4.41	4.12-4.40	< 4.40
Texture/ Firmness	4.09-5.41	4.05-4.30	> 4
Lycopene (mg/100g fresh weight)	8.5-10.5	11.12-11.42	>8.0
Lycopene in tomato paste (mg/100g fresh weight)	14.14	14.15	>14
Viscosity (Bostwick, cms/30 sec)	14-14.20	12-12.50	7-14

(Table 12). Jadli foods carried out physical, chemical and organoleptic analysis of fresh fruits. All the parameters in Arka Apeksha and Arka Vishesh were on par with commercial hybrid Abhinav (Table 13). Cremica analysed both Arka Apeksha and Arka Vishesh for TSS (4^{0}Brix), pH (4.4), acidity (0.35), colour value (>2) and viscosity (12-14) (Table 15).

CONCLUSION

Values obtained for all the parameters revealed that both the hybrids in Arka Apeksha and Arka Vishesh were suitable for processing. The processing qualities analysed by four commercial processing industries were in the acceptable range as desired by the processing industry in India. However, there is a need to breed tomato varieties / F_1 hybrids with higher TSS ($5.5\text{-}6^{0}$ Brix).

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Screening of yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt) genotypes for resistance to *Colletotrichum gloeosporoides*

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ABSTRACT

Anthracnose is one of the most destructive fungal diseases caused by *Colletotrichum gloeosporoides* in yard long bean, leading to complete crop loss at all stages and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. Few bush type cowpea cultivars have been earlier identified as reliable sources of resistance while trailing types are susceptible, but high yielding. Breeding resistant varieties is suggested as the only practical strategy, especially under hot and humid condition. Fifty-yard-long bean genotypes belonging to bush, semi erect and pole types were screened against anthracnose disease through artificial inoculation under pot culture. The present study identified the resistant varieties of vegetable cowpea through artificial inoculation followed by detached leaf assay. Among the 50 varieties of yard long bean observed, Kanakamony, dual purpose yard long bean was found highly resistant with disease severity of 3.67% followed by Arimbra local.

Keywords: Anthracnose, *Colletotrichum gloeosporoides* and yard long bean

INTRODUCTION

Yard long bean (*Vigna unguiculata* subsp. *sesquipedalis* (L.) Verdcourt), a trailing type of vegetable cowpea (2n= 24) is one of the most popular and remunerative vegetable crops traditionally grown in Kerala. Due to the favourable agro climatic conditions, the crop has gained much importance and has come to occupy a prime position among the vegetable crops raised in the state. But the production of vegetable cowpea is hindered by an array of diseases that cause growth suppression or death of plants, leading to reduction in yield and productivity. Sreeja (2014) conducted periodical survey in the potential cowpea growing areas of Thiruvananthapuram district and reported that among the six major fungal diseases, anthracnose was found to be the most predominant one (0-55%). Disease index ranged between 0 - 33.30 and considerable yield losses were attributable to the disease.

Anthracnose is a destructive fungal disease caused by *Colletotrichum* spp. In India, the incidence of anthracnose disease was first reported from Maharashtra (Rao, 1966). According to Emechebe and Lagoke (2002), all stages of the crop and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. Previous attempts to identify reliable sources of resistance have led to the identification of few bush type resistant cowpea (Kumar, 1999), as most of the trailing cultivars are susceptible to anthracnose, but high yielding compared to the bushy or semi-trailing types.

The disease is pan-tropical in distribution and is being widely recorded in regions where conditions are wet and humid. Once the infection is incited under favourable condition, its management using fungicides is difficult. Breeding resistant varieties is suggested as the only practical strategy, especially under hot and humid condition. The present study identified the resistant varieties of vegetable cowpea through artificial inoculation followed by detached leaf assay.

MATERIALS AND METHODS

Fifty yard-long bean genotypes belonging to bush, semi erect and pole types were screened against anthracnose disease through artificial inoculation under pot culture. Seeds of vegetable cowpea genotypes were collected from different parts of India, including the released varieties of SAUs and ICAR institutes and stored at refrigerated conditions for the

study. The most virulent isolate of *C. gloeosporoides* was used for artificial inoculation (Fig. 1 1). To isolate the fungal spores for artificial inoculation, infected plant parts were collected from field, washed in tap water, and surface sterilised using 0.1% mercuric chloride followed by washing thrice with autoclaved distilled water.

The fungus was cultured in potato dextrose agar medium and mycelial growth was observed through microscope. All Petri dishes were incubated at room temperature (25±2^{0}C). Petri dishes were examined for the growth of the pathogen and the morphological characteristics were observed. Inoculum was prepared from secondary culture of 7 day- old culture, by scrapping off the mycelium and spores and suspended in 100 ml sterile distilled water.

Seven seeds each of fifty genotypes were sown in two pots. After the germination, five healthy seedlings were retained and excess thinned out. Artificial inoculation was done on 20 days old seedlings kept in pots inside the greenhouse. Each plant was sprayed with 20 ml of spore suspension of virulent strain of *C. gloeosporoides* having a concentration of 10^6 spores ml^{-1} by following serial dilution method. The plants were covered with moistened polythene covers to maintain high humidity (Fig. 2). Inoculated plants were observed daily for disease incidence. Observations on disease incidence were taken on three, five, seven, nine, fifteen, twenty, twenty-five and thirty days. Final observation was taken when the disease was well expressed. Disease severity was assessed using the scale 0-5 reported by Latunde and Dada (1990).

0. No infection
0.5. Hypersensitive spots on main stem only
1: Trace of infection – Small anthracnose lesions on main stem, petioles of lower leaf only
2. Slight infection – Lesions on stem, petioles and branches
3. Moderate infection – Advanced anthracnose lesions on stem, petioles, branches, veins on the abaxial surface of leaves
4. Severe – Advanced anthracnose lesions on stem, petioles, branches, leaves, veins and peduncles
5. Very Severe – Advanced anthracnose lesions on stem, petioles, branches, leaf veins, spreading lesions on peduncle and pods

Based on the percentage of plant area infected, disease severity/ intensity was calculated using the following formula (Wheeler, 1969).

$$\text{Per cent disease severity} = \frac{\text{Sum of all numerical ratings}}{\text{Total no. of plants taken for observation}} \times \frac{100}{\text{Maximum Disease category}}$$

Per cent disease incidence was calculated by using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

Based on the per cent disease severity, the genotypes were grouped into 5 categories as adopted by Rajkumar *et al.* (1995).

Disease severity	Category
0	Immune
1 - 10	Highly resistant
10.1 - 25	Moderately resistant
25.1 - 50	Moderately susceptible
Above 50	Highly susceptible

Five plants of the resistant genotype identified through artificial inoculation were grown in the field and detached leaf assay was done just before flowering to confirm resistance.

RESULTS AND DISCUSSION

Observations on disease incidence were recorded at 10 days after infection. The infection was first observed in leaves, reddish brown streaks were very prominent on veins and veinlets. Prominent mildew symptoms were also observed on the leaf lamina (Fig. 3). Finally, leaves became chlorotic and detached from the infected plant. Reddish brown lesions were also developed on the stem. These individual lesions coalesced to form large sunken lesions and covered the whole stem causing drying up of the veins which is known as vine blackening. The pods were rottened, grey in colour, covered with black fruiting bodies of fungus. According to the visible symptoms, the plants were awarded disease scores. Among the 50 genotypes tested, Kanakamony, a dual purpose yard long bean variety was found to be highly resistant with disease severity of 3.67%, followed by Arimbra local with

Table 1 : Percent disease severity and disease reaction of yard long bean genotypes for anthracnose incidence

Genotype	Source Severity	Percent Disease	Disease Reaction
Anaswara	KAU	22.15	Moderately resistant
Bagyalakshmi	KAU	13.90	Moderately resistant
Kanakamony	KAU	3.67	Highly resistant
Arimbra local	KAU	9.58	Highly resistant
Vyjyanthi	KAU	56.84	Highly susceptible
Mithra	KAU	46.90	Moderately susceptible
Githika	KAU	56.70	Highly susceptible
Lola	KAU	54.90	Highly susceptible
Manjari	KAU	38.80	Moderately susceptible
Sharika	KAU	63.80	Highly susceptible
Vellayani Jyothika	KAU	55.90	Highly susceptible
KAU Deepika	KAU	50.90	Highly susceptible
CO6	TNAU	24.70	Moderately resistant
Pusa Beej	IARI	66.45	Highly susceptible
Kashi Kanchan	IIVR	47.64	Moderately resistant
Arka Garima	IIHR	23.90	Moderately resistant
Arka Mangala	IIHR	24.30	Moderately resistant
Arka Samradhi	IIHR	21.60	Moderately resistant
FH 31	Farm House, Trivandrum	56.50	Highly susceptible
FH 55	Farm House, Trivandrum	53.50	Highly susceptible
FH 7	Farm House, Trivandrum	70.78	Highly susceptible
VS 38	KAU	64.99	Highly susceptible
VS 53	KAU	50.12	Highly susceptible
VS 58	KAU	44.64	Moderately susceptible
VS 16	KAU	70.00	Highly susceptible
TCR 17	NBPGR	69.80	Highly susceptible
TCR 18	NBPGR	41.50	Moderately susceptible
TCR 19	NBPGR	56.00	Highly susceptible
TCR 50	NBPGR	47.25	Moderately susceptible
TCR 53	NBPGR	26.80	Moderately susceptible
TCR 54	NBPGR	36.00	Moderately susceptible
TCR 55	NBPGR	78.91	Highly susceptible
TCR 56	NBPGR	36.00	Moderately susceptible
TCR 60	NBPGR	59.49	Highly susceptible
TCR 61	NBPGR	54.00	Highly susceptible
TCR 63	NBPGR	59.45	Highly susceptible

TCR 68	NBPGR	31.70	Moderately susceptible
TCR 69	NBPGR	69.22	Highly susceptible
TCR 70	NBPGR	36.23	Moderately susceptible
TCR 74	NBPGR	26.89	Moderately susceptible
TCR 75	NBPGR	44.47	Moderately susceptible
TCR 77	NBPGR	35.50	Moderately susceptible
TCR 80	NBPGR	37.95	Moderately susceptible
TCR 84	NBPGR	54.25	Highly susceptible
TCR 86	NBPGR	33.63	Moderately susceptible
TCR 91	NBPGR	55.20	Highly susceptible
TCR 92	NBPGR	33.30	Moderately susceptible
TCR 93	NBPGR	65.33	Highly susceptible
TCR 96	NBPGR	39.82	Moderately susceptible
TCR 125	NBPGR	37.49	Moderately susceptible
SE (m)±	-	1.13	-
C.D. (0.05)	-	3.22	-

Fig. 1. *Colletotrichum gloeosporoides* culture

Fig. 2. Inoculation on host plant

Fig. 3. Symptoms on susceptible host

Fig. 4. Resistant line Kanakamony

9.58% disease severity. TCR 55(78.91 %) was found to be highly susceptible followed by FH-7 (70.78 %) (Table 1). Among the genotypes evaluated, eight showed moderately resistant reaction from 13.90 to 23.40%. In the eighteen moderately susceptible genotypes, much difference could not be noticed in disease severity values and most of them were pole types. In detached leaf assay, the leaves of Kanakamony was symptomless, and the resistance was confirmed in vitro (Fig. 4).

Anthracnose is one of the most destructive fungal diseases caused by *Colletotrichum gloeosporoides* in yard long bean, leading to complete crop loss in all stages and its parts like hypocotyls, stem, peduncle, flowers, leaves and pods were seriously affected. *C. gloeosporioides* has a wide host range including *Brassica campestris*, legumes, pigeon pea, soybean, brinjal, pumpkin, cucumber, tomato, spinach, mung bean, broad bean, cowpea, *etc.* (Sharma and Kulshrestha, 2015). Symptomatology studies on anthracnose of cowpea was conducted by Sreeja (2014). The initial symptoms appeared as minute, circular to irregular spots on the leaves which later increased in size and turned light to dark brown in colour and coalesced together to form large, necrotic spots on the leaves with shot holes. On the stem and vines, symptoms appeared as spindle shaped lesions with light grey centre and reddish-brown margin which enlarge upto 10-12 mm in length. In the later stages, small, irregular deep-seated reddish-brown spots

appear on the pods also. Isolation, characterization and identification of the pathogen revealed that *Colletotrichum gloeosporioides* was associated with anthracnose.

Adebitan and Olufajo (1998) reported that grain types exhibited better resistance to anthracnose. Artificial inoculation of fifty genotypes confirmed the resistance in Kanakamony, followed by Arimbra local, hence these varieties are valuable sources for anthracnose resistance breeding programme. Kanakamony is a dual-purpose cowpea (grain cum vegetable) belonging to the sub-species *cylindrica*. Shiny *et al*. (2015) reported that susceptible bush-type cultivar Pusa Komal and pole type cultivar Lola showed 100 and 68.80 % disease severity while the immune bush type cultivar Kanakamony was free from the symptoms and pole type Arimbra Local showed 8.80 % disease severity using SDS-PAGE.

CONCLUSION

These results support that Kanakamony is highly resistant to *C. gloeosporoides* causing anthracnose. Since fungicides and chemicals are not fully effective, the transfer of resistance from the cultivars to trailing types can only be a durable solution. Hence the results revealed that the highly resistant genotypes Kanakamony and Arimbra local could be recommended for crop improvement programmes in trailing type vegetable cowpea for enhancing the resistance to anthracnose.

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Original Research Paper

Inheritance studies on different quantitative and qualitative fruit traits in brinjal (*Solanum melongena* L.)

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ABSTRACT

Generation mean analysis of brinjal lines, GL 401 × BR 104 (CROSS I), GL 401 × W 230 (CROSS II) and W 230 × RMO 1142 (CROSS III) six generation of three crosses *viz.* highlighted the involvement of epistatic interactions (duplicate) for most of the qualitative traits. However, the number of fruits per plant in CROSS I & III and fruit girth, calyx length, and yield per plant in CROSS II confirmed the occurrence of complementary epistasis. Mainly, additive effect for fruit girth, non-additive effect for calyx length, calyx width, peduncle girth, fruit weight, and fruit length, and both types for peduncle length, number of fruits /cluster, number of fruits/ plant, and yield/ plant were experienced. Additive × dominance or dominance × dominance type of interactions were more prevalent than additive × additive type of interactions for different traits. Cluster bearing was monogenic dominant and green color of calyx as well as peduncle was dominant over purple with the duplicate type of epistasis. Fruit shape was digenic with incomplete dominance. Fruit color displayed digenic control in CROSS I & II and tri-genic ratio in CROSS III with incomplete dominance of purple and green pigmentations producing variable color intensity in homozygous or heterozygous conditions.

Keywords: Additive effect, brinjal, dominance, epistasis, fruit color, inheritance

INTRODUCTION

Brinjal (*Solanum melongena* L., 2n=24) is known for its high diversity of fruit traits. India, being the centre of diversity, has primitive types of green, very small, bitter fruits with hard pulp and thick peel. Domestication, natural inter-crossing and hybridization among different species and cultivars, natural mutations, incessant selection have created broad fruit diversity in brinjal (Frary *et al*., 2007). India has enormous diversity in brinjal color (light to dark purple and almost black, green, white, variegated), shape (round, long, oblong, oval, pear type), size (big, medium, and small), bearing habit (solitary or in clusters) and other fruit traits (Swarup, 1995). There are many region-wise set preferences among the consumers based on these traits. From the marketing point of view, purple, dark purple or black varieties suitable for '*Bhartha*' are generally preferred in North India, while, long and green types are preferred in Bihar and Southern Karnataka. In brinjal, fruiting behaviour, fruits per plant and fruit weight are highly correlated and important indicators of high yield potential (Mangi *et al*., 2016).

For the improvement of market-oriented traits, a suitable breeding method with set objectives can aid in the selection of desired genotypes. The breeder can only proceed for genetic improvement of such traits with a confirmed knowledge about the mode of their inheritance. In previous studies, inheritance of fruit colour in brinjal was reported as monogenic, digenic, or tri-genic based on the colour of parents involved in the crosses (Kamini *et al*., 2007; Liu *et al*., 2016), while the others have reported polygenic inheritance with the fitment of additive-dominance-epistasis (Pang *et al*., 2008; Patidar, 2015). Fruit shape was under the control of a single gene with partial dominance of elongated or long fruits over round fruits and role of maternal effect in the expression of the trait as reported by Aravindakshan (2003). However, the involvement of one basic gene in complementation to the other three genes was also shown for fruit shape (Kamini *et al*., 2007). In contrast to this Qiao *et al*. (2011) reported the quantitative nature of fruit shape by fitting additive major gene + additive-dominant polygene model (D-2 model). Patidar (2015) reported the dominance of purple calyx over the green.

Although a few studies have been reported on the inheritance of fruit shape, color, bearing habit, a combined approach describing the genetics of all these traits is lacking. Because of the importance of these traits in the improvement of brinjal, the present investigation was planned to study the inheritance of various quantitative as well as qualitative fruit traits in brinjal.

MATERIALS AND METHODS

The present investigation was carried out at Vegetable Research Farm of Department of Vegetable Science, Punjab Agricultural University Ludhiana, Punjab, India with six generations of each for three crosses *viz*; GL 401 × BR 104 (Cross I), GL 401 × W 230 (Cross II) and W 230 × RMO 1142 (Cross III) attempted among four diverse parents in 2016-17. Each cross was used to develop F_2, BC_1P_1, and BC_1P_2 generations in summer 2017. Six generations (P_1, P_2, F_1, F_2, BC_1P_1, and BC_1P_2) of each cross were evaluated in a tri-replicated trial in randomized block design. Each replication of each cross carried 10, 50, and 100 plants for each parent and hybrid, each backcross and F_2, respectively. The crop was raised following recommended cultural practices. Each plant was observed for various qualitative and quantitative fruit traits related to consumer preference and yield potential. Generation means analysis for six generations in each cross was done using the software BMM (Singh, 1993) for estimating the gene effects. The scaling test (A, B, C) was used for checking the presence of epistasis (Mather, 1949). A three-parameter model or additive dominance model (ADM) was applied to estimate m, [d], and [h] and test goodness of fit (χ^2) in the absence of epistasis (Cavalli, 1952), and six parameter model was used to determine epistatic interactions between the alleles when the goodness of fit (χ^2) for Additive dominance model was significant. The goodness of fit of the models was tested from expected and observed frequencies for the parameters (three or six) under investigation. The significance of the parameters in each model was used to explain the inheritance of quantitative traits. For analysis of qualitative traits, Mendelian ratios of F_2 and backcross generations were tested through the χ^2 test and the inheritance pattern of such traits was confirmed.

RESULTS AND DISCUSSION

Inheritance of quantitative traits

The generation mean analysis of three different crosses for various quantitative traits in brinjal revealed the adequacy of the simple Additive dominance model for calyx width in *GL 401 × W 230*. In all the other traits, the significance of A, B, C scales and inadequacy of the Additive dominance model highlighted the influence of epistatic interactions for their expression (Table 1). Six parameter model (Table 2) unveiled significant additive genetic effects with or without additive × additive interactions for fruit girth, fruit weight, peduncle length, and the number of fruits per cluster in *GL 401 × BR 104*, for fruit girth, calyx length, calyx width, peduncle length, peduncle girth, and the number of fruits per plant in *GL 401 × W 230*. These traits from these specific crosses can be fixed in homozygous conditions easily by the selection of individuals based on their phenotype and carrying forward the progeny of selected individuals only. Therefore, the expression of these traits can easily be improved through the pedigree method as suggested in earlier reports of Yadav *et al*. (2017) and Santhosha *et al*. (2017) in brinjal.

The genetics of fruit length, number of fruits per plant and yield per plant in *GL 401 × BR 104*, fruit length, average fruit weight, number of fruits per cluster and fruit yield in *GL 401 × W 230* and fruit length, fruit girth, calyx width, peduncle girth, number of fruits per plant in *W 230 × RMO 1142* displayed highly significant dominant genetic effects along with the significance of different types of epistatic interactions. Between both genetic effects, the magnitude of dominance was on the higher side for most of these traits. As both the components cannot be easily fixed, these traits in specific crosses can further be improved through the reciprocal recurrent selection that involves phenotypic selection and inter-crossing and later on can be used in heterosis breeding to exploit non-additive gene effects. The importance of both additive and non-additive effects in the present study was substantiated with the findings of Santhosha *et al*. (2017) and Yadav *et al*. (2017) in brinjal.

Dominant effects as well as dominance × dominance type of interactions were highly significant with higher magnitude for calyx length, calyx width, peduncle girth, and average fruit weight in *GL 401 × BR 104*, for peduncle girth in *GL 401 × W 230,* and calyx

Table 1 : Scaling and joint scaling test for fruit traits in brinjal

Trait	A	B	C	χ^2_{ADM}
Fruit length (cm)				
CROSS I	-1.50 ± 0.52**	0.89 ± 0.55	-2.91 ± 1.06**	18.66**
CROSS II	-1.71 ± 0.52**	2.11 ± 0.60**	-5.78 ± 0.84**	75.76**
CROSS III	-0.79 ± 0.27**	-1.79 ± 0.41**	-1.53 ± 0.53**	25.45**
Fruit girth (cm)				
CROSS I	0.34 ± 0.17*	-0.01 ± 0.20	0.76 ± 0.30*	9.15**
CROSS II	0.86 ± 0.16**	0.70 ± 0.19**	2.60 ± 0.30**	80.52**
CROSS III	0.30 ± 0.22	-1.72 ± 0.25**	-1.94 ± 0.36**	73.30**
Calyx length (mm)				
CROSS I	-3.91 ± 1.25**	-8.01 ± 1.53**	-12.55 ± 2.19**	41.20**
CROSS II	-2.53 ± 1.21*	-0.04 ± 1.36	-4.79 ± 2.02*	7.86*
CROSS III	-3.94 ± 0.92**	-10.23 ± 0.93**	-12.55 ± 1.60**	134.44**
Calyx width (mm)				
CROSS I	-3.29 ± 0.88**	-10.74 ± 1.14**	-12.84 ± 1.68**	95.10**
CROSS II	0.44 ± 0.61	0.40 ± 0.64	-2.09 ± 1.11	7.80
CROSS III	-1.88 ± 0.71**	-7.05 ± 0.85**	-9.12 ± 1.34**	72.12**
Peduncle length (mm)				
CROSS I	-3.89 ± 1.96*	-3.39 ± 1.99	-11.21 ± 3.05**	13.66**
CROSS II	-3.33 ± 1.40*	-0.29 ± 1.43	-8.40 ± 2.08**	18.11**
CROSS III	-13.15 ±1.01**	-13.52 ± 1.18**	-16.61 ± 1.79**	279.69**
Peduncle girth (mm)				
CROSS I	-0.75 ± 0.21**	-2.37 ± 0.32**	-1.84 ± 0.44**	61.54**
CROSS II	-0.29 ± 0.20	0.27 ± 0.23	1.20 ± 0.35**	19.49**
CROSS III	-0.24 ± 0.21	-0.95 ± 0.26**	-1.64 ± 0.39**	19.86**
Average fruit weight (g)				
CROSS I	-23.84 ± 9.53*	-5.78 ± 13.53	65.87 ± 18.99**	19.26**
CROSS II	-73.31 ± 7.34**	10.75 ± 7.20	-69.45 ± 9.26**	145.67**
CROSS III	-43.10 ± 5.91**	-123.47 ± 10.61**	-105.84 ± 11.22**	245.53**
Number of fruits per cluster				
CROSS I	-0.11 ± 0.05*	-0.02 ± 0.04	-0.35 ± 0.07**	37.63**
CROSS II	0.46 ± 0.09**	-0.17 ± 0.11	0.65 ± 0.16**	44.74**
CROSS III	-0.91 ± 0.10**	-0.33 ± 0.07**	-0.81 ± 0.15**	102.05**
Number of fruits per plant				
CROSS I	-2.01 ± 0.72**	4.91 ± 0.52**	4.36 ± 0.96**	109.66**
CROSS II	-0.84 ± 0.75	-2.54 ± 0.69**	-7.61 ± 1.12**	54.37**
CROSS III	-5.10 ± 1.09**	-3.07 ± 0.96**	-11.06 ± 1.53**	56.38**
Yield per plant (kg)				
CROSS I	-0.77 ± 0.17**	0.82 ± 0.23**	1.73 ± 0.27**	82.39**
CROSS II	-1.91 ± 0.17**	0.10 ± 0.15	-2.31 ± 0.21**	192.79**
CROSS III	-1.87 ± 0.13**	-2.42 ± 0.21**	-2.81 ± 0.28**	243.74**

*,** significant at 5% and 1% levels, respectively. ADM- Additive Dominance Model, CROSS I-GL 401 × BR 104, CROSS II -GL 401 × W 230 and CROSS III-W 230 × RMO 1142

Table 2 : Estimation of gene effects and inter-allelic interactions in the best fit model for fruit traits in brinjal

Trait	*M*	*d*	*h*	*I*	*j*	*L*	Type of epistasis
GL 401 × BR 104							
Fruit length (cm)	10.83 ± 1.16**	4.18 ± 0.11**	6.23 ± 2.76**	2.30 ± 1.15**	-2.40 ± 0.69**	-1.69 ± 1.69**	Duplicate
Fruit girth (cm)	7.05 ± 0.36**	-2.33 ± 0.04**	-1.65 ± 0.90	-0.44 ± 0.36	0.35 ± 0.25	0.11 ± 0.56	Duplicate
Calyx length (mm)	36.42 ± 2.03**	-0.14 ± 0.47	-8.95 ± 5.31	0.62 ± 1.98	4.10 ± 1.71*	11.30 ± 3.59**	Duplicate
Calyx width (mm)	22.79 ± 1.22**	-3.77 ± 0.37**	-17.11 ± 3.25**	-1.18 ± 1.16	7.45 ± 1.12**	15.21 ± 2.38**	Duplicate
Peduncle length(mm)	40.32 ± 2.59**	6.31 ± 0.58**	-1.00 ± 6.99	3.93 ± 2.53	-0.50 ± 2.27	3.34 ± 4.95	Duplicate
Peduncle girth (mm)	9.94 ± 0.43**	-2.11 ± 0.12**	-6.48 ± 1.08**	-1.30 ± 0.41**	1.61 ± 0.35**	4.43 ± 0.69**	Duplicate
Average fruit weight (g)	29.92 ± 24.55**	-44.51 ± 1.73**	-254.25 ± 60.98**	-95.44 ± 24.49**	-18.07 ± 16.44	125.03 ± 37.33**	Duplicate
Number of fruits/cluster	0.91 ± 0.05**	0.09 ± 0.02**	0.27 ± 0.15	0.22 ± 0.05**	-0.10 ± 0.06	-0.09 ± 0.11	Duplicate
Number of fruits/plant	20.37 ± 0.93**	4.10 ± 0.28**	-4.84 ± 2.41*	-1.47 ± 0.89	-6.92 ± 0.83**	-1.43 ± 1.56	Complementary
Fruit yield/plant (kg)	5.27 ± 0.35**	-0.02 ± 0.06	-4.56 ± 0.91**	-1.68 ± 0.34**	-1.59 ± 0.28**	1.63 ± 0.58**	Duplicate
GL 401 × W 230							
Fruit length (cm)	4.98 ± 0.98**	6.15 ± 0.11**	15.06 ± 2.52**	6.17 ± 0.97**	-3.82 ± 0.73**	-6.57 ± 1.63**	Duplicate
Fruit girth (cm)	5.38 ± 0.32**	-0.07 ± 0.05	-0.70 ± 0.80	-1.03 ± 0.32**	0.17 ± 0.23	-0.53 ± 0.51	Complementary
Calyx length (mm)	27.81 ± 1.85**	6.88 ± 0.34**	3.18 ± 4.87	2.21 ± 1.82	-2.49 ± 1.50	0.36 ± 3.36	Complementary
Calyx width (mm)	14.88 ± 0.16**	2.99 ± 0.16**	0.67 ± 0.34*	-	-	-	Incomplete dominance
Peduncle length(mm)	34.54 ± 2.39**	11.25 ± 0.52**	1.62 ± 6.16	4.78 ± 2.34*	-3.05 ± 1.94	-1.16 ± 3.88	Duplicate
Peduncle girth (mm)	6.82 ± 0.36**	0.85 ± 0.06**	-2.45 ± 0.91**	-1.15 ± 0.35**	-0.56 ± 0.27*	1.17 ± 0.59*	Duplicate
Average fruit weight (g)	97.02 ± 12.90**	51.07 ± 0.95**	-8.81 ± 33.95	6.89 ± 12.87	-84.06 ± 9.98**	55.68 ± 21.66**	Duplicate
Number of fruits/cluster	2.15 ± 0.19**	-0.57 ± 0.03**	-1.09 ± 0.47*	-0.36 ± 0.19	0.63 ± 0.14**	0.08 ± 0.30	Duplicate
Number of fruits/plant	21.47 ± 1.07**	-2.70 ± 0.26**	3.31 ± 2.79	4.23 ± 1.04**	1.71 ± 0.91	-0.85 ± 1.85	Duplicate
Fruit yield/plant (kg)	2.02 ± 0.26**	1.03 ± 0.04**	0.14 ± 0.70	0.51 ± 0.26**	-2.01 ± 0.22**	1.28 ± 0.45**	Complementary
W 230 × RMO 1142							
Fruit length (cm)	9.32 ± 0.61**	-3.27 ± 0.06**	-2.10 ± 1.58	-1.05 ± 0.61	0.99 ± 0.45*	3.63 ± 1.02**	Duplicate
Fruit girth (cm)	6.05 ± 0.39**	-2.10 ± 0.05**	-1.11 ± 0.99	0.47 ± 0.38	1.98 ± 0.29**	0.99 ± 0.66	Duplicate
Calyx length (mm)	31.71 ± 1.54**	-6.93 ± 0.28**	-16.24 ± 3.86**	-1.63 ± 1.52	6.29 ± 1.21**	15.80 ± 2.52**	Duplicate
Calyx width (mm)	16.60 ± 1.03**	-4.87 ± 0.31**	-7.73 ± 2.65**	0.19 ± 0.98	5.18 ± 0.90**	8.74 ± 1.86**	Duplicate
Peduncle length(mm)	44.26 ± 1.93**	-6.14 ± 0.40**	-42.83 ± 4.83**	-10.06 ± 1.88**	0.37 ± 1.46	36.73 ± 3.04**	Duplicate
Peduncle girth (mm)	5.91 ± 0.35**	-1.55 ± 0.11**	-0.34 ± 0.89	0.46 ± 0.34	0.71 ± 0.30*	0.73 ± 0.58	Duplicate
Average fruit weight (g)	209.74 ±15.83**	-96.17±0.82**	-303.60 ± 41.26**	-60.73 ±15.81**	80.36 ±11.89**	227.30 ±26.10**	Duplicate
Number of fruits/cluster	2.18 ± 0.16***	0.61 ± 0.03**	-2.08 ± 0.39**	-0.43 ± 0.16**	-0.59 ± 0.11**	1.67 ± 0.24**	Duplicate
Number of fruit/plant	18.21 ± 1.43**	7.30 ± 0.24**	6.33 ± 3.86	2.90 ± 1.41**	-2.03 ± 1.20	5.27 ± 2.69**	Complementary
Fruit yield/plant (kg)	3.92 ± 0.24**	-0.94 ± 0.05**	-5.71 ± 0.64**	-1.48 ± 0.24**	0.55 ± 0.20**	5.77 ± 0.45**	Duplicate

*,** significant at 5% and 1% levels, respectively.

length, peduncle length, average fruit weight, number of fruits per cluster and yield per plant in *W 230 × RMO 1142*. Therefore, these traits can be improved through the exploitation of heterosis breeding in these specific crosses as reported earlier by (Kumar and Arumugam, 2013 and Santhosha *et al*., 2017) in brinjal. In the present investigation, the negative sign of additive × additive interactions for fruit girth, calyx width, peduncle girth, average fruit weight, number of fruits per plant, and yield per plant in *GL 401 × BR 104*, for fruit girth, peduncle girth, and number of fruits per cluster in *GL 401 × W 230* and fruit length, calyx length, peduncle length, average fruit weight, number of fruits per cluster and yield per plant in *W 230 × RMO 1142* disclosed the presence of dissociated gene pairs, while the positive sign of [*i*] interaction in other traits in these crosses indicated the presence of associated gene pairs in parents. The associated gene pair from the parents will lead to faster improvement of the mentioned traits in particular crosses in brinjal. The negative values of the dominance for fruit girth, calyx length, calyx width, peduncle length, peduncle girth, average fruit weight, number of fruits per plant and yield per plant in *GL 401 × BR 104*, for peduncle girth, average fruit weight, and number of fruits per cluster in *GL 401 × W 230* and fruit length, fruit girth, calyx length, calyx width, peduncle length, peduncle girth, average fruit weight, number of fruits per cluster and yield per plant in *W 230 × RMO 1142* suggested the dominance of decreasing alleles and while the positive sign of [*h*] in other traits indicated the dominance of increaser alleles for the target traits. The dominance of increaser alleles will raise the expression of such traits in heterosis breeding.

Duplicate type of epistasis was noticed for most of the traits in different crosses suggesting selection should be mild in earlier generations and intense in later generations. However, the number of fruits per plant (*GL 401 × BR 104*), fruit girth, calyx length and yield per plant in *GL 401 × W 230*, and the number of fruits per plant in *W 230 × RMO 1142* highlighted the occurrence of a complementary type of epistasis. Duplicate and complementary types of epistasis were also following the statements of Dhameliya and Dobariya (2009) and Devmore (2016). In this study, non-significant interactions for peduncle length and fruit girth in *GL 401 × BR 104* and calyx length in *GL 401 × W 230* pointed toward the occurrence of higher-order interactions or the presence of linkages. Therefore, more generations were required to be evaluated for the elucidation of genetic in these traits.

Inheritance of qualitative traits

The present investigation also explained the inheritance of various qualitative traits like fruit color and shape as well as pigmentation of calyx and peduncle from three crosses **(**Table 3). Light purple fruits in *GL 401 × BR 104* (green × dark purple) were segregated in F_2 into three phenotypic classes with 108, 53, and 39 plants bearing dark purple, green, and light purple fruits, respectively, and this segregation pattern was in agreement with the 9:3:4 ratio. However, BC_1P_1 and BC_1P_2 were segregated into 1:1 ratios for light purple: green fruits and light purple: dark purple fruits, respectively. It indicated digenic inheritance of fruit colour with supplementary gene interaction. However, the intensity of green and purple fruits varied due to incomplete dominance. In *GL 401 × W 230*, light green F_1 was segregated into 9:3:4 (supplementary) ratio for light green (105 plants), dark green (36 plants), and white (59 plants) in F_2. BC_1P_1 resulted in light green and dark green fruits and BC_1P_2 segregated into light green and white fruits in 1:1 proportion again confirmed digenic inheritance in this cross. However, the reduction in the intensity of green fruits suggested the incomplete dominance of the green colour of fruits. In *W 230 × RMO 1142* (white× reddish-purple), F_1 with light purple fruits segregated in F_2 progeny into 24:12:12:12:4 ratio for light purple (69 plants), reddish-purple (39 plants), dark purple (40 plants), whitish-green (36 plants), and white (16 plants) that suggested tri-genic inheritance of fruit colour in brinjal. BC_1P_1 progeny was segregated into 1:2:1 for whitish green, light purple, and white fruits and BC_1P_2 resulted from 1:2:1 ratio for light purple, reddish-purple, and dark purple fruits. The reduction in the intensity of fruit colour again suggested the incomplete dominance of purple and green colours. In earlier studies, digenic tri-genic, and polygenic inheritance was reported by Liu *et al*. (2016); Kamini *et al*. (2007) and Patidar (2015), respectively. Overall, the inheritance of fruit colour was complex in brinjal, but segregation behaviour of three crosses highlighted trigenic (purple, green, and white) control with supplementary effects, where the genes responsible for purple and green colour display incomplete dominance that led to a reduction in the intensity of colour in fruits. Therefore the homozygous and heterozygous condition of three genes in various combinations along

Table 3 : Qualitative inheritance of different fruit traits in F_2, BC_1P_1, and BC_1P_2 progenies of variable crosses in brinjal

Trait	Cross	F_1	Progeny	Progeny size	Phenotypes	Observed frequencies	Expected frequencies	χ^2_{cal}	χ^2_{tab}	Phenotypic ratio
Calyx color	*GL 401 × BR 104* (green ×purple)	Green	F_2	200	Green : Purple	184:16	187.5:12.5	1.04	3.84	15:3
			BC_1P_1	100	Green	100	-	-	-	-
			BC_1P_2	100	Green : Purple	69:31	75:25	1.92	3.84	3:1
Peduncle color	*GL 401 × BR 104* (green ×purple)	Green	F_2	200	Green : Purple	184:16	187.5:12.5	1.04	3.84	15:3
				BC_1P_1	100	Green	100	-	-	- -
			BC_1P_2	100	Green : Purple	69:31	75:25	1.92	3.84	3:1
Fruit bearing habit	*W 230 × RMO 1142* (clustered ×solitary)	Clustered	F_2	200	Clustered : Solitary	142:58	150:50	1.71	3.84	3:1
			BC_1P_1	100	Clustered	100	-	-	-	-
			BC_1P_2	100	Clustered : Solitary	54:46	50:50	0.64	3.84	1:
Fruit color	*GL 401 × BR 104* (green ×dark purple)	Light purple	F_2	200	LP:DP:G	108:39:53	112.5:37.5:50	0.42	5.99	9:3:4
			BC_1P_1	100	LP:G	56:44	50:50	0.64	3.84	1:1
			BC_1P_2	100	LP:DP	54:46	50:50	0.64	3.84	1:1
	GL 401 × W 230 (green × white)	Light green	F_2	200	LG:DG:W	105:36:59	112.5:37.5:50	2.18	5.99	9:3:4
			BC_1P_1	100	LG:DG	43:57	50:50	0.36	3.84	1:1
			BC_1P_2	100	LG:W	56:44	50:50	0.64	3.84	1:1
	W 230 × RMO 1142 (white ×red purple)	Light purple	F_2	200	LP:RP:DP:WG:W	69:39:40:36:16	75:37.5:37.5:37.5:12	2.1	9.49	24:12:12:12:4
			BC_1P_1	100	LP:RP:DP	30:47:23	25:50:25	1.34	5.99	1:2:1
			BC_1P_2	100	WG:LP:W	22:62:26	25:50:25	0.48	5.99	1:2:1
Fruit shape	*GL 401 × BR 104* (long ×big round)	Oblong	F_2	200	Oblong:Long:Round	108:57:35	112.5:50:37.5	1.32	5.99	9:3:4
			BC_1P_1	100	Long:Oblong	54:46	50:50	0.64	3.84	1:1
			BC_1P_2	100	Round:Oblong	44:56	50:50	1.44	3.84	1:1
	GL 401 × W 230 (long ×small oval)	Long	F_2	200	Long: Small oval	142:58	150:50	0.67	5.99	3:1
			BC_1P_1	100	Long	100	-	-	-	-
			BC_1P_2	100	Long: Small oval	46:54	50:50	0.64	3.84	1:1

Fruit color: LP-Light Purple, RP-Reddish Purple, DP- Dark Purple, G- Green, LG- Light Green, DG- Dark Green, WG- Whitish Green, and W-White CROSS I-GL 401 × BR 104, CROSS II -GL 401 × W 230 and CROSS III-W 230 × RMO 1142

with interactions results in different shades of the fruits in brinjal. For the improvement of fruit colour in brinjal colour, specific contrasting parents can be selected along with other horticultural traits. The combination of dark or shining purple and white genotypes can give rise to the development of a variety of colours in F_2, but all these genotypes are not homozygous for colour at this stage. Therefore, the selection for this trait can be delayed to the F_3 or F_4 generation of selfing. The intensity of the purple colour can be increased by backcross with the dark coloured parent. For the development of white or green genotypes, parents involving these colours can be used.

The inheritance of fruit shape is also shown in three crosses (Table 3, Fig 1). In *GL 401 × BR 104* (Long× Big Round), oblong fruits of F_1 were segregated into three phenotypic classes of oblong (108), long (57), and round (35) fruits in the F2 that highlighted some kind of the interaction of two genes. The segregation pattern of this cross for fruit shape was following 9:4:3 ratio. However, BC_1P_1& BC_1P_2 progenies were segregated into 1:1 ratios for long: oblong and round: oblong fruits, respectively. In *GL 401 × W 230* (long × small oval), long fruited F_1 produced a 3:1 ratio for long: oval fruits. BC_1P_1 plants had long fruits only, while BC_1P_2 resulted in long: oval fruits in a 1:1 ratio. In *W 230 × RMO 1142* (small oval× big oval), oval

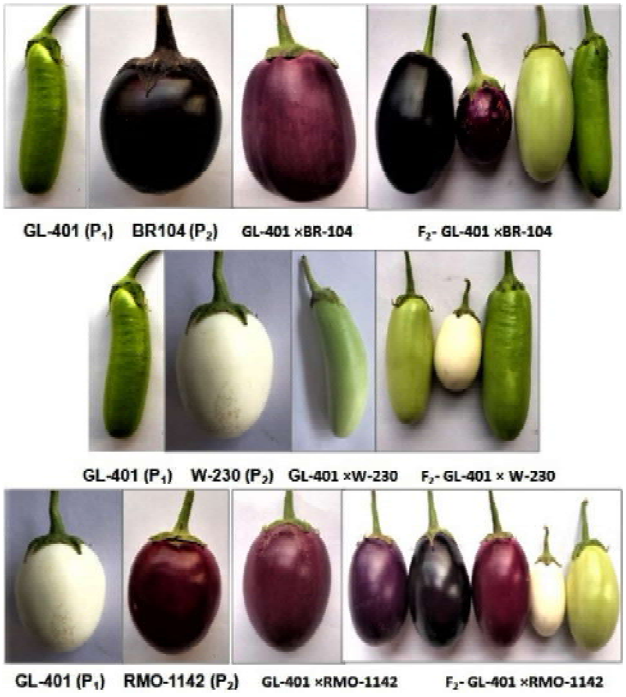

Fig. 1 : Inheritance of fruit colour and shape in brinjal

fruited F_1 did not segregate for fruit shape as both the parents were similar for fruit shape. Phenotypic ratio of the first cross indicated that both round and long fruit shape was controlled by two dominant genes, where the homozygous recessiveness of one gene allows full expression of the other gene and vice versa. However, the dominance of both genes produces oblong fruits. The phenotypic ratios of the second cross confirmed the monogenic control of fruit shape. Overall observation from all the crosses suggested that fruit shape was controlled by two genes with supplementary effects that resulted in oblong fruits. The size of fruits in segregating populations was affected by the type of parents involved in the hybridization. These findings for fruit shape were in agreement with Aravindakshan (2003) and Kamini *et al*. (2007). For the generation of F1 hybrids based on fruit shape, we should either select both the parents with oblong fruits or we can cross a parent with big round fruits to a parent with long fruits for the oblong type. Here, the size of the fruit also affects. The cross between long and oval should result in oblong fruits, but our result for this cross showed long fruit. This may have occurred due to variation in size. In quantitative inheritance also, fruit length and fruit breadth were strongly affec0ted by dominant genes. It means more length and breadth of the fruit in parents, as in cross I, is showing the dominance of both the traits. In contrast, a cross of parents with more length and less breadth is showing the dominance of fruit length only. Therefore, the involvement of both major and minor genes in the expression of the trait cannot be neglected. Our results were in accord with the findings of Kamini *et al*. (2007) and Qiao *et al*. (2011) regarding fruit shape in brinjal. For the improvement of fruit shape, length, and breadth specific parental genotypes such as long ×long, round × round, round × long, small round ×long can be crossed and selections can be practiced for the desired genotypes.

In brinjal, generally, three classes of calyx and peduncle pigmentation are common i.e. completely pigmented, partially pigmented, and green. In the present investigation, the inheritance of calyx and peduncle colour was elucidated from the *GL 401 × BR 104* (green × partially pigmented) (Table 3 and Fig 1). F_1 had green calyx as well as peduncle that segregated into 184 plants with green calyx as

well as peduncle and 16 plants with purple pigmentation. It pointed toward the dominance of green colour for both the traits. The ratio obtained for calyx as well as peduncle colour was following the 15:1 ratio that suggested the digenic inheritance through duplicate factors. Backcross to the parent with pigmented calyx as well as peduncle resulted in a ratio of 3:1 for green and purple traits, while with a parent having green calyx and peduncle resulted in progenies bearing fruits with green calyx and peduncle only. In contrast, Patidar (2015) reported the dominance of purple calyx over the green. They might have selected completely pigmented calyx of one parent and green calyx of the other. Similarly, for fruit-bearing (single or in a cluster), *W 230 × RMO 1142* (cluster× single) produced clustered fruits and segregated into clustered type (142) and solitary bearing (58) in 3:1 ratio in F_2, all cluster bearing plants in BC_1P_1 and 1:1 proportion in BC_1P_2 that unveiled monogenic dominance of clustered fruits in brinjal. These findings were in agreement with the results of Rangaswamy and Kadambavanasundaram (1973).

CONCLUSION

It was concluded from the present investigation that epistatic interactions significantly influenced most of the traits in all crosses. All types of interactions were observed affecting different traits. The type of interactions affecting the expression of a particular trait varied in different crosses. The non-significance of interactions for peduncle length and fruit girth in CROSS I and calyx length in *GL 401 × W 230* pointed towards the occurrence of higher-order interactions or the presence of linkages for these traits. Among the qualitative traits in brinjal, clustered fruit bearing was the monogenic dominant, and the green colour of calyx and peduncle was dominant over purple with a duplicate type of epistasis. Fruit shape was controlled with two genes with incomplete dominance of both round and long fruits. However, in other crosses, long shapes remained dominant over small oval fruits. For the inheritance of fruit colour, dark purple × green and green × white crosses followed the 9:3:4 ratio with incomplete dominance of genes controlling purple and green pigmentation, respectively. However, in red-purple × white cross, trigenic control (ratio of 24:12:12:12:4) was noticed along with incomplete dominance of the above two genes, where each class represented the variable intensity of colour due to homozygous or heterozygous condition of three genes in various combinations.

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Original Research Paper

Morphological and biochemical characterization of *Passiflora quadrangularis* L. - A source of vegetable from East Siang district, Arunachal Pradesh, India

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ABSTRACT

Present research investigation was aimed at morphological and biochemical assessment of *Passiflora quadrangularis* L. commonly known as giant granadilla and locally called as vegetable squash grown as vegetable crop by the *Adi* tribe of Arunachal Pradesh. Seven genotypes collected during survey were characterized for different morphological and biochemical traits. Results showed that average fruit weight was 432.57g/fruit, with juice content 100.11 mL/fruit, vitamin C content 25.79 mg, vitamin A content 1.65 mg, Mean total flavonoids content was 16.75 mg/100 g of fruit juice, total soluble solids 12.04^0 Brix, antioxidant activity (DPPH) 6.07 %, titratable acidity 1.69 %, total carbohydrates 9.95 %, phenol content 338.38 mg/100 g of leaf was noted among the genotypes tested. The mean anthocyanin content in leaf was 1.20 mg/100 g, tendril 0.90 mg/100 g and petiole 1.69 mg/ 100 g among the genotypes. Seed protein profiling of *Passiflora quadrangularis* L. with SDS-PAGE showed diverse molecular weights ranging from 11 KD to 163.53 KD. However, monomorphic banding pattern among the protein profiling of giant granadilla was recorded among the selected genotypes. The results of the study show that the collected genotypes are belonged to *Passiflora quadrangularis* L. and are good source of nutritive value which can be used as source of vegetable.

Keywords : Giant granadilla, *Passiflora quadrangularis*, SDS-PAGE and vegetable source

INTRODUCTION

Passiflora quadrangularis L. commonly known as Giant granadilla, belongs to Passifloraceae family consists of about 700 species and 16 genera and among them only two genera, *Passiflora* and *Tetrapathaea* are cultivated (Feuillet, 2004) and about 520 species of the genus *Passiflora* are distributed to Neotropics and Africa (Ulmer and MacDougal, 2004). The most important genus is *Passiflora*, with the most common commercial species being the purple passion fruit (*Passiflora edulis*) for the fresh market and the more acid yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) for the juice industry (Tripathi *et al.,* 2014). Leaves of purple and yellow passion fruit are used as leafy vegetable in Manipur and also used as folk traditional medicine as anti-diabetic (Singh *et al*., 2014). The total area under passion fruit cultivation is about 0.014 million hectares with a production of 0.082 million tonnes in India during 2020 (Anon, 2020). P. *quadrangularis* L. is a lesser-known member of the genus also grown for its fruit as well as vegetable. It is locally known as vegetable squash by the *Adi tribe* of Arunachal Pradesh.

This novel P. *quadrangular* is helps the rural sectors in mitigating the malnutrition and hence enabling them a quality life. During investigation it was observed that P. *quadrangularis* L. is grown by the *Adi* tribe of Arunachal Pradesh and sold as squash in the market. However, very few systematic, inventory and documentation about the *Passiflora* species found in Northeast India and sporadic attempts have been made on characterization of passion fruit found in Arunachal Pradesh. Therefore, the present research was initiated to explore and document for the morphological and biochemical characteristics features of P. *quadrangularis* L. found in East Siang district of Arunachal Pradesh.

MATERIALS AND METHODS

Survey was carried out in East Siang district of Arunachal Pradesh during the year 2019-2020 which is located at 28p 03'N, 95p 20'N covering an area of 1865 sq. km. and having the altitude of 176.57m above MSL and represent a mild subtropical zone with cool, dry winter, a warm summer and a moderate season. The identification and description of the plant were adopted from De Jesus *et al*. (2017). During the survey, *Passiflora* species *viz*. purple passion fruit, yellow passion fruit and giant granadilla were found growing in East Siang district. Data on passion fruit uses were obtained through interview of knowledgeable elderly people of *Adi* tribe (both genders of 30-75 ages) inhabiting in the East Siang district to which the P. *quadrangularis* L. identified on the basis of vernacular name, regional floras and published literatures (Singh *et al*., 2003). Totally seven genotypes were collected during the survey and the passport information of the collected genotypes are presented in Table 1. The collection of fruits and leaves have been done from different direction of single plant. Fifty fruits were collected from each genotype and every replication had 10 fruits with five replications.Recorded morphological traits *viz*., leaf length (cm), leaf breadth (cm), flower length (cm), number of flowers/node, peduncle length (cm), fruit length (cm), fruit breadth (cm), fruit weight (g), number of fruits/vine, fruit yield (kg/vine), peel weight (g), seed length (cm), seed breadth (cm), number of seeds/fruit and seed weight/fruit. Biochemical traits *viz*., juice content (mL/fruit), total soluble solid content (°Brix), titratable acidity (%) (AOAC, 2006), Vitamin C content (mg/100 g) (Ranganna, 1986), total carbohydrate (%) (Hedge, 1962), reducing sugar (%) (Somogyi, 1952), Vitamin A (mg/100 g) (Bayfield and Cole, 1980), total flavonoid (mg/100 g) (Vijay and Rajendra, 2014), and antioxidant activity (%) (AOAC, 2006). Anthocyanin content (mg/100 g) of leaf, tendril and petiole (Malick and Singh, 1980), vitamin C content of leaf (mg/100 g) (Ranganna, 1986) and phenol content of leaf (mg/100 g) using Folin-Ciocalteu reagent (Malick and Singh, 1980) and chlorophyll content of leaf (mg/g) (Arnon, 1949) and shelf life (days) at room temperature were estimated for the collected genotypes.

Seed protein extraction

Seed protein extraction was as described Lowry *et al*. (1951) in seven genotypes and was carried out protein banding pattern was determined using SDS-PAGE as described by Laemmli (1970). 0.2 g of seeds were soaked overnight in phosphate buffer (pH 7.0) solution. Seeds were crushed with a solution of Tris-HCl 0.06 M (PH 7.4), 10 mM urea, 1 mM EDTA, 0.1% TCA, 2.5% glycerol, 0.5% SDS and 1.25% β-mercaptoethanol. Electrophoresis was performed in vertical electrophoresis unit and gel run at 25 mA. Silver staining was performed as described by Mortz *et al*. (2001) and sensitizing with 0.02% sodium thiosulphate solution. The reaction was stopped with 12% acetic solution. Gel was washed thoroughly but gently with double distilled water until protein bands became clearly visible for bands scoring**.** The electrophorograms developed on protein mobility and density expressed in Rm values. The gels were scored as presence (+) or absence (-) of protein bands. Depending upon the presence (+) or absence (-) of bands, similarity index between the genotypes were calculated (Nei and Li, 1979).

Data analysis

The statistical analysis *viz*., standard error of mean, coefficient of variance and test of significance were performed by following Singh and Chowdhury (1985).

RESULTS AND DISCUSSION

Morphological characters are important for identification and documentation of horticultural traits for crop improvement. A large variability having unique characters was recorded for morphological traits of fruits and other plant parts of the collected giant granadilla (Fig 1a and Fig 1b). There are no significant variations in different qualitative traits recorded. All the accessions had quadrangular stem, large green cordate leaves having entire margin and leaf lamella. Leaf had deep sinus, stipule and heterophylly was absent. All the lines showed axillary tendrils bearing 2.33 to 3.00 flowers/node. Flowers had light red color petals and sepals are green from outside and whitish pink from inside with yellowish-green ovary. The flowers possess yellowish green stamens with violet dots, blue, brown speckled corona. All the lines produced light yellowish brown oblong fruits possessing dark brown seeds. The same qualitative characters were also reported by Lim, 2012. Among the genotypes leaf length varied from- 9.95 to 12.08 cm, leaf width from- 8.89 to 11.14 cm.

Table 1 : List of collected *Passiflora quadrangularis* L. genotypes from East Siang district, Arunachal Pradesh, India and their sources

Genotype code	Source	Latitude	Longitude	Altitude
P. quadrangularis L. P1	Pasighat, Arunachal Pradesh	28^0 03' N	95^0 20' N	156 m
P. quadrangularis L. P2	CHF, Pasighat, Arunachal Pradesh	28^0 04' N	95^0 19' N	183 m
P. quadrangularis L. P3	Baptist Church, Pasighat, Arunachal Pradesh	28^0 05' N	95^0 18' N	192 m
P. quadrangularis L. P4	Agami House, Pasighat, Arunachal Pradesh	28^0 06' N	95^0 31' N	168 m
P. quadrangularis L. P5	Police line, Pasighat, Arunachal Pradesh	28^0 05' N	95^0 32' N	166 m
P. quadrangularis L. P6	Teachers Residence, Pasighat, Arunachal Pradesh	28^0 03' N	95^0 19' N	159 m
P. quadrangularis L. P7	Tekang, Pasighat, Arunachal Pradesh	28^0 04' N	95^0 22' N	212 m

Flowers are 8.40 to 9.41 cm in length which was maximum among other cultivated passion fruit. Significant variation for quantitative traits like peduncle length, fruit length, fruit breadth, fruit weight and peel weight, seed length, seed breadth, number of seeds/fruit and weight of seeds/fruit were recorded (Table 2a and 2b).

This *Passiflora* species is commonly used as vegetable in unripe stage having an average yield of 15.88 to 23.89kg/vine. Fruit juice content of giant granadilla was about 53.39 to 131.04 mL/ fruit which was maximum in comparison to other *Passiflora* species because of bigger size of fruits. This finding was similar with the result of Arjona and Matta, 1991. Based on the yield and yield attributing traits genotypes for leaf length (P7; 12.08 cm), number of flowers per node (P6; 3.67), fruit weight (P1; 488.33), number of fruits/vine (P2; 51.33), peel weight (P1; 352.33), number of seeds/fruit (P1; 172) and flower length (P7; 9.41 cm) were identified. Selecting the genotypes with

Table 2a : Morphological characters of *Passiflora quadrangularis* L. genotypes from East Siang district, Arunachal Pradesh

Genotype	Leaf Length (cm)	Leaf Breadth (cm)	Flower length (cm)	Number of flowers/ nodes	Peduncle length (cm)	Fruit length (cm)	Fruit breadth (cm)	Fruit weight (g)
P1	9.95	8.89	9.15	2.33	2.37	14.60	9.59	488.33
P2	11.64	11.14	8.40	2.67	1.96	13.42	9.11	463.67
P3	10.99	9.48	9.11	2.67	2.50	13.89	11.04	476.67
P4	10.51	9.70	8.77	3.00	2.62	12.19	10.08	408.00
P5	11.25	9.65	9.40	2.33	2.42	11.82	9.84	407.00
P6	11.46	10.19	8.57	3.67	2.52	12.83	10.80	402.67
P7	12.08	10.59	9.41	2.67	2.65	12.43	10.44	381.67
Mean	11.12	9.95	8.97	2.76	2.43	13.03	10.13	432.57
CV (%)	3.18	6.54	5.92	1.14	3.81	4.19	5.96	9.93
SE (m)+	0.78	0.55	0.46	0.38	0.08	0.69	0.52	12.32
C.D (5%)	1.31	1.62	1.36	0.54	0.24	1.59	1.55	3.34

Table 2b : Morphological characters of *Passiflora quadrangularis* L. genotypes from East Siang district, Arunachal Pradesh

Genotype	No. of Fruits/ vine	Peel weight (g)	Fruit yield (Kg/vine)	Seed length (cm)	Seed breadth (cm)	No. of seeds/ Fruit	Seed weight/Fruit (g)
P1	48.00	352.33	23.44	0.77	0.61	172.00	9.33
P2	51.33	320.67	23.86	0.69	0.59	168.00	9.13
P3	42.00	343.33	20.05	0.65	0.61	156.67	8.78
P4	45.33	306.33	18.45	0.68	0.59	157.67	9.03
P5	42.67	304.33	17.36	0.71	0.64	149.67	8.34
P6	45.00	349.67	18.19	0.72	0.64	153.00	9.07
P7	40.67	296.67	15.55	0.71	0.65	168.00	8.83
Mean	45.00	324.76	19.56	0.70	0.62	160.71	8.93
CV (%)	8.04	2.38	4.76	1.10	1.24	2.96	1.60
SE (m)+	4.69	4.90	2.23	0.01	0.02	2.75	0.19
C.D (5%)	2.83	27.11	1.58	0.04	0.04	8.10	0.55

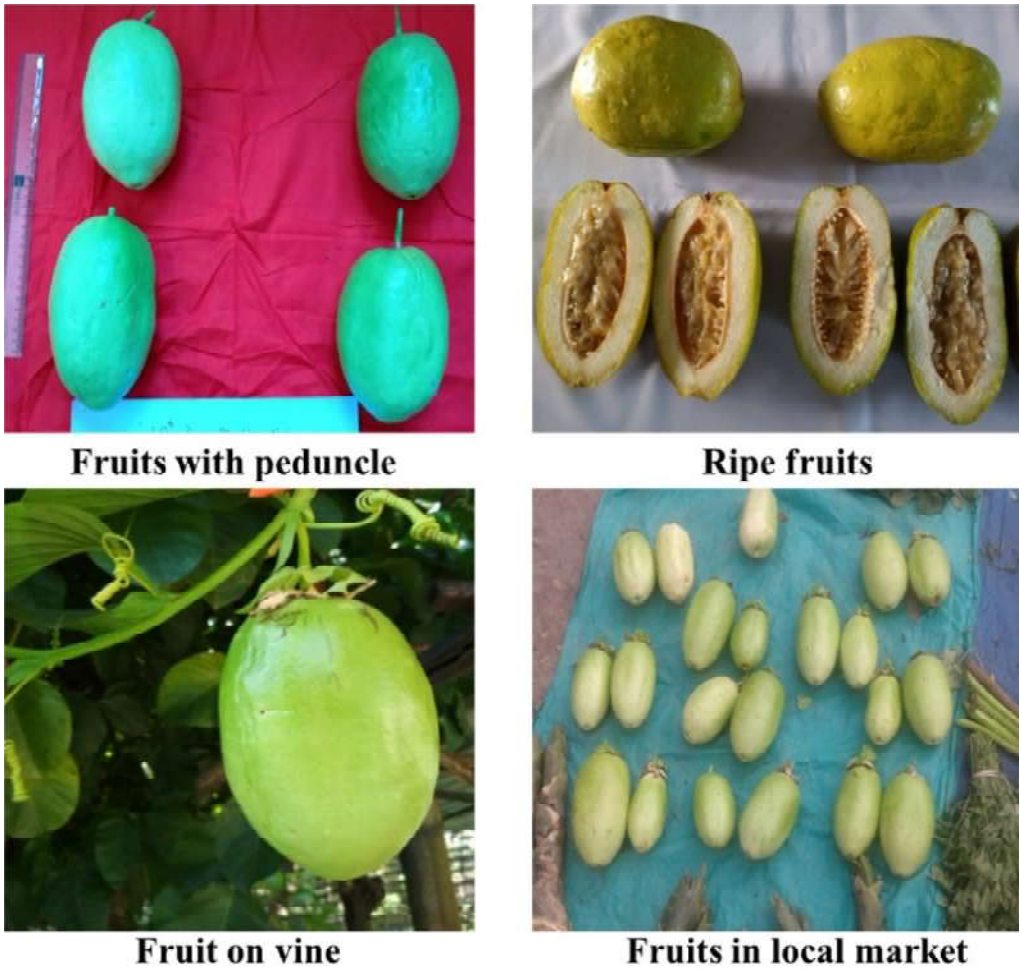

Fig. 1a : Fruits of *Passiflora quadrangularis* L.

Fig. 1b : Leaf, flower and ovary colour of *Passiflora quadrangularis* L.

large leaf and flower aid in imparting maximum photosynthate accumulation to the sink leading to high crop yield.

Biochemical characteristics

Biochemical characterization also revealed that the TSS content of fruit juice was ranging from 10.26 to 13.44 °Brix which is in agreement with data of Ramaiya *et al*. (2021) in P. *quadrangularis*. The higher TSS may be due to the fact the fruit tree is grown under natural water scarce condition without care and management and eventually increasing the TSS content Meghwal *et al*. (2004). In giant granadilla, citric acid is the predominated organic acid followed by malic acid that is about 1.49 to 1.80 % which is in conformity to the data of Velente *et al*. (2011). The high acidity may be due to the prevalence of primary organic acids, such as malic and citric acid, in mature fruits, which accumulate in the mesocarp cells during the fruit development process and are controlled by both genetic and environmental factors. Ascorbic acid varied from 22.45 to 29.53 mg/100 g in fruit juice and 44.78 to 50.15 mg/100 g in fresh leaf and finding similar with Ramaiya *et al*. (2021).

Total carbohydrate content data showed variation from 9.49 to 10.75 % and is in agreement with Shanmugam *et al.* (2018). Reducing sugar content was about 5.00 to 5.68 % and similar results were reported by Patel *et al*. (2014). The increasing sugar is due to the hydrolysis of starch to sucrose as fruit approach to ripening (Pandy and Deen, 2018). Vitamin A (mg/100 g) content was recorded between 1.62 to 1.69 mg/100 g of fruit juice and data was in agreement with Oliveira *et al*. (2014); Homnava *et al*. (1990) and it may be due to biosynthesis genes controls its accumulation and composition in fruit during maturity. The DPPH free radical scavenging antioxidant (%) activity was recorded as 5.91 to 6.28 % which is in conformity with Loizzo *et al.* (2019) and Marroquin *et al.* (2011). As these fruits are known to contain a variety of antioxidant compounds, and ascorbic acid (vitamin C) which implying that fruits high in vitamin C are powerful antioxidants as reported by Esti *et al*. (2002).

Chlorophyll content of leaves were ranging from 1.56 to 1.69 mg/g which was an agreement with Do Valle *et al.* (2018). Phenol content was as 319.67 to 351.32 mg/100 g of fresh leaf and similar data was reported by Rudnicki *et al.* (2005) and Marroquin *et al.* (2011). Anthocyanin (mg/100 g) content in leaf, petiole and tendril varied from 1.17 to 1.24 mg/100 g, 1.59 to 1.76 mg/100 g and 0.85 to 0.94 mg/100 g respectively which was in agreement with Aizza *et al*., 2019 and Reis *et al.,* 2018. The anthocyanin, phenol and chlorophyll concentration of petioles, tendrils and leaves might differ according to a variety of external and internal factors such as genetic, agronomic and climatic factors (Kayesh *et al*., 2013). Shelf life (days) of *Passiflora quadrangularis* genotypes at room

Table 3 : Biochemical parameters in fruits of *Passiflora quadrangularis* L. genotypes from East Siang district, Arunachal Pradesh

Genotype	Juice content (mL/fruit)	Vit. C (mg/100 g)	TSS (° Brix)	Vit. A (mg/100 g)	Total flavonoids (mg/100 g)	Antioxidant Activity (%)	Titratable Acidity (%)	Total carbohydrates (%)	Reducing sugar (%)	Shelf-life (days)
P1	123.78	29.53	13.21	1.67	17.42	6.28	1.80	10.75	5.54	27.22
P2	131.04	29.04	12.74	1.61	16.62	6.22	1.49	9.49	5.54	25.57
P3	124.38	22.45	11.90	1.69	16.37	5.98	1.72	9.83	5.29	22.25
P4	92.67	25.66	13.44	1.62	17.20	6.01	1.69	10.29	5.00	18.67
P5	95.03	25.68	11.73	1.64	15.78	6.09	1.69	9.29	5.14	18.67
P6	53.39	23.26	10.97	1.64	16.40	5.91	1.68	9.51	5.21	19.00
P7	80.49	24.93	10.26	1.67	17.45	6.00	1.77	10.48	5.68	25.67
Mean	100.11	25.79	12.04	1.65	16.75	6.07	1.69	9.95	5.34	22.43
CV (%)	4.65	5.35	3.13	1.75	0.69	2.50	2.42	1.59	0.68	3.11
SE (m)+	2.69	0.80	0.22	0.02	0.07	0.09	0.02	0.09	0.02	1.70
C.D (5%)	7.93	2.35	0.64	0.05	0.20	0.26	0.07	0.27	0.06	1.01

Table 4 : Biochemical parameters in leaves, petioles and tendrils of *Passiflora quadrangularis* L. genotypes from East Siang district, Arunachal Pradesh

Genotype	Leaf				Petiole	Tendril
	Anthocyanin (mg/100 g)	Vit. C (mg/100 g)	Phenol (mg/100 g)	Chlorophyll (mg/g)	Anthocyanin (mg/100 g)	Anthocyanin (mg/100 g)
P1	1.24	47.69	351.32	1.67	1.76	0.94
P2	1.19	50.15	336.33	1.56	1.73	0.92
P3	1.24	47.67	343.00	1.64	1.69	0.91
P4	1.19	44.78	319.67	1.61	1.74	0.89
P5	1.18	46.06	324.33	1.66	1.69	0.92
P6	1.17	48.72	345.00	1.69	1.59	0.88
P7	1.22	46.72	349.00	1.57	1.64	0.85
Mean	1.20	47.40	338.38	1.63	1.69	0.90
CV (%)	1.81	3.75	3.34	1.11	0.80	1.81
SE (m)+	0.01	1.03	12.39	0.01	0.01	0.01
C.D (5%)	0.04	3.03	9.55	0.03	0.02	0.03

temperature was recorded maximum for genotype P1 which is 27.22 days at room temperature. It is due to the thick exocarp which prevents easy decay under biotic and abiotic stress.

Present investigation recorded a total 70 numbers of bands having molecular weights ranging from 11 KD to 163.53 KD. All the seven selected genotypes found in East Siang District, Arunachal Pradesh exhibited monomorphic banding pattern in the protein profiling of giant granadilla. Beena and Beevy, (2015) also reported the highest molecular weight i.e., 69.94 KD was generated by *Passiflora foetida* var. *hispida*, while the lowest (12.95KD) was produced in *Passiflora foetida* var. *gossippifolia* in *Passiflora* species.

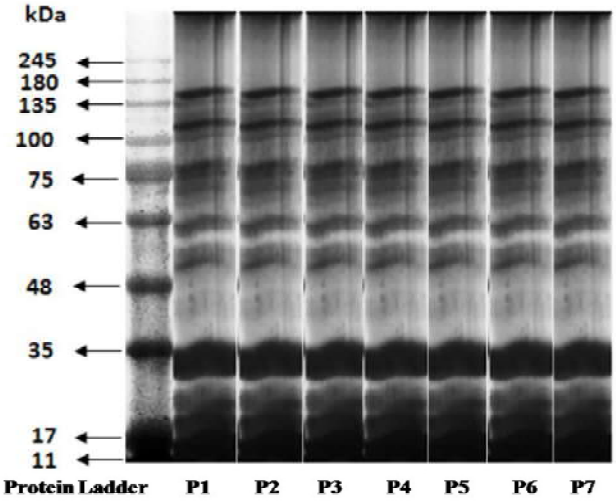

Fig. 2 : SDS-PAGE protein profiling of seven genotypes of *Passiflora quadrangularis* L. from East Siang district, Arunachal Pradesh

CONCLUSION

From the study of morphological, biochemical and seed protein profiling it could be concluded that all seven genotypes belong to same species *i.e.*, *Passiflora quadrangularis* L. locally known as vegetable squash (by *Adi* tribe of Arunachal Pradesh). As its green fruits and leaves are nutritious this novel underexploited *Passiflora* species can be explored for commercial cultivation as a source of vegetable in the future. Because of its higher fruit yielding capability, the fruits also have a lot of potential in the food processing industry.

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Original Research Paper

Advancing fruiting season in *Annona* cv. Arka Sahan through pruning

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ABSTRACT

***Annona* cultivar 'Arka Sahan', an inter-specific hybrid of *Annona atemoya* × *A. squamosa* comes to harvest during August-September under mild tropical climate, which coincides with monsoon rains resulting in poor fruit quality and high susceptibility to anthracnose and fruit fly. An attempt was made to advance the fruiting in this hybrid through pruning during 2016-17 and 2017-18. The effect of three pruning levels (25, 50 and 75% of previous season's growth) at five different times (60, 75, 90, 105 and 120 days after final harvest of previous crop) on flowering and fruiting were compared. Early sprouting, flowering and fruit harvest were recorded in trees pruned to 75% of the past season's growth in both the years. Earliest fruits were harvested 271 (3rd week of June) and 268 (2nd week of June) days after pruning in trees pruned during first week of October in 2016-17 and 2017-18 respectively (P<0.05). Bigger fruits with lesser seeds per 100 g of pulp (P<0.05) were harvested from trees pruned to 75% and 25% levels in the first and second year, respectively, irrespective of pruning time. Tree canopy following pruning at 75%level recorded higher light interception and photosynthetic rate (P<0.05). Pruning time and levels significantly influenced the biochemical constituents of leaf and shoot. The fruiting in cultivar 'Arka Sahan' could be thus advanced by 8-9 weeks to June from the normal season of August-September with comparable or better fruit quality by pruning 75% of the last season's growth during October.**

Keywords: Annona, biochemical constituents, fruit quality, off-season and pruning

INTRODUCTION

Sugar apple (*Annona squamosa* L.), also known as sweet sop, sugar apple, sitaphal or sharifa and as custard apple in India is a native of tropical America and West Indies, introduced to India. The fruits are generally used fresh, while some products like custard powders and ice-creams are prepared from the fruits. 'Arka Sahan' is an inter-specific hybrid between *A. atemoya* (var. Island Gem) × *A. squamosa* (var. Mammoth). It is a vigorous plant. Its mature fruits take about 6-7 days to ripe. The creamy white colour flesh is juicy with mild pleasant aroma and tender with fewer seeds (9/100 g pulp) and large segments. The edible pulp is remarkable for its sweetness with 22.8 per cent total sugars and measures more than 30^0 B as against 24^0 B in Mammoth (Jalikop and Kumar 2007). Flowering in annona occurs on current season growth arising after natural leaf fall during late winter. In annona, flower bud formation is restricted to early shoot development, and is extra-axillary, borne opposite to leaves (George and Nissen 1991). The leaf imposed para-dormancy of axillary bud is present in annona (George and Nissen 1987). Soler and Cuevas (2008) reported off-season (winter season) fruit production through shoot pruning followed by tipping the newly emerged shoots in cherimoya. Normal fruiting time of 'Arka Sahan' grown under the mild tropical climate is August-September, which coincides with monsoon rains resulting in deterioration of fruit quality due to anthracnose incidence and fruit fly infestation during the rainy period. Flowering can be manipulated by modifying the timing of bud break in annona species to get fruit out of season. For this leaf fall is prerequisite to open up the sub-petiolar axillary bud residing under the leaf petiole. We attempted to make the annona hybrid 'Arka Sahan' to flower and fruit early through pruning, which could induce early defoliation, bud sprouting and formation of new shoots and flowers and thus advance fruiting season to

summer months. The pruning techniques were standardized in terms of time and severity, keeping in view the flowering and fruiting behavior of the cultivar 'Arka Sahan'.

MATERIALS AND METHODS

The experiment was conducted at ICAR - Indian Institute of Horticultural Research, Bengaluru (Karnataka state, India) during two consecutive years, 2016-17 and 2017-18. The experimental material consisted of eight-year-old one hundred and twenty uniform plants of *Annona* cv. Arka Sahan planted at a distance of 5m × 5m. The treatments comprised of five pruning times (T_1, T_2, T_3, T_4 & T_5) and three shoot pruning levels (L_1, L_2, & L_3). Pruning was performed after 60, 75, 90, 105 or 120 days after final harvest of the previous crop. Pruning levels consisted of removal of 25 per cent (one-fourth of shoot length), 50 per cent (half of shoot length) or 75 per cent (two-third of shoot length) of the previous season's growth. Each treatment was replicated four times in a factorial randomized block design. Two trees were observed in each replication under each treatment for collection of data. Eight trees that were not pruned and giving new shoot growth naturally by the end of March following leaf abscission during late winter served as external check for comparison of treatment effects against natural fruiting as these could not be fitted effectively into the factorial design involving pruning time and intensity. Standard package of practices were adopted for maintenance of all the trees during the experimentation.

The number of days required for sprouting and flowering was assessed by recording the days taken for the emergence of first sprout and flower respectively after the treatment imposition. The durations of the first and last harvest were calculated from the date of imposing the treatments to the first fruit harvest and the last fruit harvest respectively. The total fruit yield per tree was recorded at harvest by measuring the weight of fruits harvested and values were expressed in kilogram. Fruit weight (g) was recorded using electronic balance. The total soluble solids (TSS) were measured using digital refractometer and expressed as degree Brix. Titrable acidity was estimated by adopting the *titrametric* method of A.O.A.C (1975) using phenolphthalein indicator and the values were expressed in terms of percentage citric acid equivalent. Pulp content (%) of fruit was determined using the following formula:

$$\text{Pulp (\%)} = \frac{\text{Pulp weight}}{\text{Fruit weight}} \times 100$$

The number of seeds per 100 g of pulp was calculated by using the following formula:

$$\text{Number of seeds per 100 g of pulp} = \frac{\text{Number of seeds in fruit}}{\text{Pulp weight of fruit}} \times 100$$

Gas exchange parameters such as net photosynthesis (P_N, µmol m^{-2} s^{-1}), transpiration rate (E, mmolm^{-2} s^{-1}) and stomatal conductance (gs, mmol $m^{-2}s^{-1}$) were recorded in three fully expanded leaves of each plant using portable photosynthesis system (LCpro+, ADC BioScientific limited, UK) during morning hours of clear and sunny conditions between 09:30 h and 11:30 h at two stages *viz.,* fruit set (March, 2018) and rapid fruit growth (May, 2018) stage in the second year (2017-18) of study. Photosynthetically active radiation (PAR) below the tree canopy was measured using the LI-191SA Line Quantum Sensor (Li-Cor, Lincoln, NE) on uniformly overcast days between 12:00 h and 13:00 h at the fruit set and rapid fruit growth stages (FSS and RFGS) during 2017-18. The total leaf chlorophyll content was measured at FSS using spectrophotometer (UV 1650PC, Shimadzu, Japan) at wave lengths of 645 and 663 nm as per Hiscox and Isrealstam (1979). Total sugar in shoot was estimated after the harvest of fruits following the method of Somogyi, (1952).

Statistical analysis was done separately for the parameters studied for each year using OPSTAT (Sheoran *et al.,* 1998) and discussed at $P < 0.05$ for significance of difference between their mean values.

RESULTS AND DISCUSSION

Physiological and biochemical characteristics: Pruning, especially its levels, significantly influenced the amount of light interception at both fruit set stage (FSS) in March and rapid fruit growth stage (RFGS) in May ($P<0.05$) (Table 2). Higher light interception in different treatments could be related to longer shoot length and higher number of leaves and leaf area in 75 per cent pruned trees ($P<0.05$). Differential light interception within tree canopies can also influence vegetative growth, flower initiation, fruit set, fruit size and fruit quality (Marini and Marini, 1983). Higher light interception was also associated with higher photosynthetic rate of leaves at both fruit set and rapid fruit growth stage. Pruning provided open canopy area and resulted in maximum interception of sunlight for

Table 1 : Details of timing and level of pruning

Treatment	Pruning level	Pruning time
T_1L_1 T_1L_2 T_1L_3	25% pruning 50% pruning 75% pruning	60 DAFH* (1st week of October)
T_2L_1 T_2L_2 T_2L_3	25% pruning 50% pruning 75% pruning	75 DAFH (3rd week of October)
T_3L_1 T_3L_2 T_3L_3	25% pruning 50% pruning 75% pruning	90 DAFH (1st week of November)
T_4L_1 T_4L_2 T_4L_3	25% pruning 50% pruning 75% pruning	105 DAFH (3rd week of November)
T_5L_1 T_5L_2 T_5L_3	25% pruning 50% pruning 75% pruning	120 DAFH (1st week of December)

*Days after final fruit harvest

higher rate of photosynthesis (Singh and Singh 2007). Similar results were recorded by Sharma *et al*. (2006) that the light interception was significantly influenced by pruning intensity in mango, being higher for pruned trees than for not pruned ones. The highest value of diffuse light availability below the canopy was recorded for severely pruned trees than for trees not pruned. Higher photosynthetic rate was recorded in trees pruned to 75 per cent level compared to 50 and 25 per cent levels ($P<0.05$) (Table 2). Higher photosynthetic rate reflects more metabolic activity in these leaves which could be attributed to interception of more light by the leaves. The trees pruned to 75 per cent produced longer shoots carrying more leaves, which harvested more light. Similar results were observed by Sharma *et al*. (2006) in mango where higher photosynthetic rate was recorded in leaves of pruned trees than trees not pruned. However, stomatal conductance was not affected much due to pruning in the present study (Data not presented). It ranged from 0.07 to 0.18 mmol m^{-2} s^{-1} among the treatments. The leaf chlorophyll content is considered as an important index of the metabolic activity of plants. At both FSS and RFGS, chlorophyll content exhibited differential pattern in response to different levels of pruning (Table 2). Accumulation of higher chlorophyll content in leaf could be related to the higher light interception which favoured the synthesis of more chlorophyll. Light interception by 75 per cent level pruned trees was higher at both fruit set and rapid growth stages. The lower chlorophyll content in the other treatments may be attributed to limited chlorophyll synthesis for want of conducible environmental conditions (Sritharan *et al*., 2010). Although, there was significant influence of pruning time and pruning levels on chlorophyll content at fruit set stage, no consistent results were evident over the years. At fruit set, the amount of chlorophyll content varied from 1.5 to 3.1 mg/g in the first year and from 1.2 mg to 3.2 mg/g in the second year. At rapid fruit growth stage, in the first year, pruning treatments did not influence the chlorophyll content while in the second year, significant influence was recorded with chlorophyll content varying from 2.0 to 2.8 mg/g ($P<0.05$). Sharma and Chauhan (2003) reported higher chlorophyll content in leaves of pruned peach tree leaves as compared to trees not pruned. However, total leaf chlorophyll content was recorded similar for both pruned and unpruned mango trees during April and July while during November it was recorded highest in pruned trees (Schaffer and Gaye 1989). Presence of higher amount of sugar in shoots of trees pruned at 25 per cent level in the present study, could be attributed to poor translocation of sugar for the growth of shoot or more towards the developing fruits which was also reflected in terms of relatively, smaller shoot and less number of leaves in 25 per cent pruned trees. However, sugar accumulation in shoot was recorded more in the second year (506.2 mg/100 g) over the first year (457.4 mg/100 g) which could be related to the favourable environmental condition prevailed including higher rainfall (average 2.41 mm per month), relative humidity (average 76.32%) and maximum temperature (average 29.74° C) in the second year (October to July) than the first year (rainfall 1.76 mm & relative humidity 68.47%). Shoots that emerged from 75 per cent pruning treatments were longer, which also reflected better translocation and utilization of sugar in the growth of shoot. Overall, total sugar content was affected by pruning levels, the results are in conformity with those of Bagchi *et al*. (2008) who observed that pruning up to 10 cm with complete removal of old leaves showed significant effect on increasing reducing sugars (36.7 mg/g) than other treatments and control.

Growth and yield characteristics: Pruning led to leaf fall followed by sprouting of sub petiolar axillary buds on the shoot. Irrespective of pruning time, the number of days required for sprouting has become shorter with the increase of pruning levelduring both the years with minimum number of days to sprout for those pruned in December first week and October first week in first and second years, respectively ($P<0.05$) (Table3). Early sprouting in trees pruned to 75 per cent level

Table 2 : Effect of pruning time and pruning levels on light interception, photosynthetic rate, total leaf chlorophyll and total sugar in *Annona* cv. Arka Sahan

Treatment	Light interception (%) 2017-18		Photosynthetic rate (µmol m^{-2} s^{-1})	Total leaf chlorophyll (mg g^{-1})		Total sugar (mg/100 g)	
	FSS	RFGS	2017-18	2016-17	2017-18	2016-17	2017-18
T_1L_1	21.8	65.1	7.9	3.1	3.2	399.3	480.0
T_1L_2	41.5	82.6	7.7	2.5	2.5	352.0	415.0
T_1L_3	58.8	92.0	10.8	2.9	3.1	181.5	249.3
T_2L_1	19.6	66.3	5.7	2.3	2.2	372.0	410.8
T_2L_2	40.6	75.9	5.8	2.9	3.0	427.8	504.0
T_2L_3	55.7	90.6	7.2	2.3	2.3	182.5	299.0
T_3L_1	24.4	71.9	4.5	2.3	2.4	467.5	531.0
T_3L_2	32.5	80.2	4.8	2.0	1.8	408.8	472.0
T_3L_3	59.4	85.2	8.7	2.2	2.1	278.8	327.8
T_4L_1	21.0	70.4	7.6	1.7	1.2	589.5	698.0
T_4L_2	47.4	76.9	8.0	1.5	2.9	570.5	573.0
T_4L_3	58.4	84.1	8.7	2.0	3.1	326.5	539.0
T_5L_1	24.6	73.4	6.8	1.8	2.6	458.8	411.0
T_5L_2	40.6	80.5	6.4	1.6	2.5	427.0	498.0
T_5L_3	58.7	92.7	7.5	1.9	1.8	364.0	431.0
External Check	20.3	60.2	7.6	3.0	3.1	350	465
$T_{C.D.\ (P=0.05)}$	-	3.00	1.11	0.03	0.04	6.71	8.39
$L_{C.D.\ (P=0.05)}$	2.81	2.33	0.86	0.03	0.03	5.20	6.50
$T \times L_{C.D.\ (P=0.05)}$	6.28	5.20	1.92	0.06	0.07	11.62	14.53

T: Time of pruning; L: Level of pruning; FSS : Fruit Sel Style; RFGS : Rapid Fruit Growth Style

Table 3 : Effect of pruning time and levels on sprouting, flower initiation, fruit yield and its pattern of *Annona* cv. Arka Sahan

Treatment	Sprouting (days)		Flower initiation (days)		First harvest (days)		Final harvest (days)		Fruit yield per tree (kg)		Fruit yield per TCSA (kg/cm²)	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
T_1L_1	85.3	104.7	100.6	132.8	297.5	307.5	307.5	320.0	12.0	20.2	0.10	0.14
T_1L_2	57.5	60.7	75.7	74.5	285.0	279.5	302.0	306.3	10.1	14.9	0.07	0.09
T_1L_3	23.3	13.7	42.9	26.3	271.0	268.9	299.5	302.5	11.1	16.1	0.09	0.11
T_2L_1	60.5	104.6	77.7	126.1	290.0	291.0	305.0	301.8	10.2	17.7	0.08	0.13
T_2L_2	28.0	90.1	45.4	105.3	277.5	266.0	296.3	295.0	11.2	15.7	0.09	0.10
T_2L_3	17.5	20.0	35.3	33.9	271.0	245.0	287.0	295.0	11.3	17.0	0.08	0.10
T_3L_1	41.6	44.9	54.2	54.8	269.0	276.8	279.0	285.5	9.0	18.9	0.08	0.14
T_3L_2	25.5	32.6	42.9	55.6	269.8	265.8	278.0	284.5	10.0	19.4	0.09	0.14
T_3L_3	16.3	17.6	48.2	42.2	271.3	235.0	287.3	281.0	10.1	16.8	0.09	0.11
T_4L_1	31.5	62.9	44.4	76.1	269.0	261.5	284.0	266.8	8.7	20.5	0.07	0.13
T_4L_2	19.2	39.6	33.8	55.7	270.8	252.5	284.0	276.0	9.3	16.4	0.07	0.10
T_4L_3	16.2	18.4	56.8	44.0	276.3	236.8	285.0	271.5	10.8	15.2	0.08	0.10
T_5L_1	25.2	45.1	38.5	59.8	270.0	246.8	283.0	258.3	9.8	20.6	0.07	0.12
T_5L_2	20.9	33.5	37.8	45.6	271.0	236.5	283.0	255.0	10.5	19.1	0.07	0.12
T_5L_3	14.7	17.3	50.8	40.6	274.5	222.5	283.0	244.8	11.3	16.4	0.08	0.09
External Check	118	135	133	145	321	329	335	338	14.2	20.1	0.09	0.14
$T_{C.D.\ (P=0.05)}$	3.06	5.10	4.39	5.07	3.98	2.55	3.25	4.78	0.21	1.27	0.01	0.01
$L_{C.D.\ (P=0.05)}$	2.37	3.95	3.40	3.93	3.08	1.98	2.52	3.70	0.16	0.98	-	0.01
$T \times L_{C.D.\ (P=0.05)}$	5.29	8.84	7.61	8.78	6.89	4.42	5.64	8.28	0.36	2.20	-	-

T: Time of pruning; L: Level of pruning;

could be attributed to very few leaves or no leaf left on such shoots and with less number of buds available on the shoot, the reserve metabolites from trunk could have contributed to early release of these buds. Similar results were observed in cherimoya (Soler and Cuevas, 2008) and guava (Shaban and Haseeb, 2009), where severely pruned trees gave early sprouting. Also, early flowering occurred in trees pruned to 75 per cent level ($P<0.05$). In a less vigorous cultivar of sugar apple, Balanagar, early shoot growth during winter could be induced under similar climatic condition through chemical defoliation (Chander *et al.,* 2019). Since flowering is on current season growth in *Annona* and concomitant with the shoot growth, early sprouting resulted in early flowering in both the years. However, in the first year, flowering was earlier on trees pruned to 50 per cent level during November and December despite early sprouting in those pruned to 75 per cent level. It was observed that there was continuous vegetative growth in 75 per cent pruned trees. Similar results were reported in custard apple (George and Nissen, 1987), cherimoya (Soler and Cuevas, 2008) and atemoya (Olesen and Muldoon, 2012). Pruning treatments significantly influenced the flowering and fruiting period of *Annona* cv. 'Arka Sahan' (Table 3). Earliest fruits were harvested from the treatments imposed in 1st week of October, at 75 per cent pruning level (T_1L_3) with minimum days (271) to harvest by 2nd week of June in first year ($P<0.05$). A consistent result was recorded for early harvest (2nd week of June) with 75 per cent pruned trees in second year for all pruning time. Early harvest from 75 per cent pruned trees could be attributed to advanced flowering and fruit set in these trees. Observations recorded on final harvest exhibited significant differences with pruning time and pruning level (Table 3). In both the years, the final harvest extended longer for the 25 per cent pruned trees ($P<0.05$). Final harvest in case of 75 per cent pruning was completed earlier than 25 per cent or 50 per cent pruning. Early harvest in these trees could be attributed to earlier induction of flowering and pollination than the other treatments. The results are in conformity with those reported by Vinay *et al.* (2014) in custard apple and Adhikari and Kandel (2015) in guava. Higher yield was obtained from trees pruned during 1st week of October at 25 per cent level (T_1L_1) while for rest of the pruning treatments greater yield was recorded from 75 per cent pruned trees in the first year. However, in the second year, maximum yield per tree was obtained from 25 per cent pruned trees (Table 3). Higher yield in respective years could be attributed to bearing of larger size of fruits and occurrence of prevailing congenial environmental conditions during the fruit growth. Also, accumulation of more sugars in the shoot of 25 per cent pruned trees at harvest reflect more availability of assimilates to fruits on these trees. Fruits were harvested near to normal season from trees pruned to 25 per cent level which could have advantage of prevailing congenial environmental condition than other treatments. The results are in conformity with Kumar *et al.* (2010) in peaches and Choudhary and Dhakare (2018) in sugar apple, where heavy pruning (90 cm) gave lesser yield than light or trees not pruned but medium pruning (30-45 cm) recorded higher yield per tree. The yield per TCSA was not influenced much with pruning treatments, which could be attributed to lesser effect of pruning treatments on trunk growth (Table 3). In the second year, comparatively higher yield per tree was recorded although there was not much improvement in trunk growth. Higher yield in second year could be more related to the increase of yield per tree rather than trunk circumference.

Fruit quality characteristics: There was consistent significant effect of pruning levels on fruit weight and pulp content in both the years ($P<0.05$) (Table 4). Higher fruit weight and pulp content in 75 per cent pruned trees could be attributed to the better growth of shoot with higher number of leaves which resulted in higher synthesis of photosynthates in these shoots. The higher amount of accumulated photosynthates could have contributed for bigger size of fruits. Similar results were reported in custard apple (Olesen and Muldoon, 2009; Choudhary and Dhakare, 2018) and ber (Gupta and Gill, 2015). However, in the second year, trees pruned at 25 per cent level recorded the maximum fruit weight and pulp content which could be due to availability of sufficient stored carbohydrates, confirmed with the estimation of higher sugar in the developed shoot. Similar trend was observed on other fruit quality parameters including fruit volume, fruit length, fruit width and fruit circumference with the pruning treatments imposed over two years (data not presented). Results indicated that irrespective of pruning time, comparatively fewer seeds per 100 g of pulp were recorded in 75 per cent and 25 per cent pruning levels in the first and second years, respectively ($P<0.05$). As the fruit size including fruit weight, volume, and pulp content was recorded more in these treatments this could have lowered the proportion of seed per unit of pulp. Similar findings were also observed by Chander and Kurian (2019) in sugar apple and Teaotia and Singh (1971) in guava where lesser percentage of seed was recorded in heavier fruit obtained from pruned trees. Pruning

Table 4 : Effect of pruning time and pruning levels on fruit quality attributes of *Annona* cv. Arka Sahan

Treatment	Fruit weight (g)		Pulp content (%)		Seeds per 100 g of Pulp		TSS (°B)	
	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18	2016-17	2017-18
T_1L_1	248.1	432.9	59.9	80.1	29.1	12.4	36.3	33.9
T_1L_2	301.5	350.8	69.5	70.5	22.7	15.4	35.2	32.0
T_1L_3	316.5	363.6	64.2	76.2	21.9	16.1	34.5	31.8
T_2L_1	257.9	394.0	63.6	80.3	24.1	12.1	37.0	33.6
T_2L_2	265.6	361.5	66.3	75.2	25.6	15.2	35.4	33.0
T_2L_3	310.7	421.4	67.7	73.3	20.3	15.0	32.8	31.8
T_3L_1	278.9	468.7	65.8	79.0	27.1	12.4	35.5	33.8
T_3L_2	308.9	452.8	66.8	78.6	24.2	15.4	36.7	32.8
T_3L_3	289.7	394.5	68.2	66.2	22.2	18.2	35.0	31.3
T_4L_1	262.5	456.0	66.5	80.0	26.1	13.5	36.3	32.6
T_4L_2	250.8	402.7	68.5	77.4	24.2	13.4	36.1	32.4
T_4L_3	275.0	385.8	70.8	74.3	19.2	15.5	35.3	31.7
T_5L_1	262.9	451.2	62.3	79.1	25.7	13.5	36.0	33.5
T_5L_2	273.1	408.7	65.8	69.7	28.6	13.1	35.7	32.9
T_5L_3	278.7	441.4	66.6	73.9	23.9	16.1	35.9	32.2
External Check	300	375	61.4	66.1	23.2	15.8	31.8	30.2
$\mathbf{T}_{\text{C.D. } (P=0.05)}$	-	36.73	2.72	-	-	-	0.60	-
$\mathbf{L}_{\text{C.D. } (P=0.05)}$	18.23	28.45	2.11	2.34	2.62	1.39	0.47	0.54
$\mathbf{T \times L}_{\text{C.D. } (P=0.05)}$	-	-	-	5.22	-	-	1.04	-

T: Time of pruning; L: Level of pruning

influences quality of the fruits by regulating carbohydrate allocation to the developing fruits (Palanichamy *et al*., 2011). Early pruning during October-November resulted in increased level of total soluble solids (TSS) of the fruit than the later or trees not pruned ($P<0.05$) (Table 4). The prevailing congenial temperature during fruit growth and maturation could have contributed for accumulation of more sugar in the developing fruits as the fruits come to harvest earlier in these pruned trees. In both the years, comparatively higher value of prevailing average maximum temperature (31.11, 31.50°C) was recorded from flowering to fruit maturity (February to June) for October-November pruned trees than the late pruned trees wherein lesser average maximum temperature (30.73, 30.33°C) was recorded from flowering to fruit maturity (April to August). The results are in conformity with those of Kadam *et al*. (2018) in custard apple cv. Dharur-6 where fruits from light pruned (20 cm) trees recorded maximum TSS content. In contrast, heavy pruning resulted in accumulation of more TSS in grapes (Zabadal *et al*., 2002) and peach (Chitkara *et al*., 1991). There were no consistent trends of acidity content of fruit pulp although higher level of acidity was observed in trees pruned to 75 per cent level (data not presented). Chitkara *et al*. (1991) and Kumar *et al*. (2010) recorded increased acidity level with the increase of pruning severity in peaches. Similar results were obtained by Mehta *et al*. (2012) in guava and Kadam *et al*. (2018) in custard apple cv. Dharur-6.

CONCLUSION

Induction of off-season crop with better quality is a new technique in sugar apple production that could enable the growers to get better market and profitability. Fruiting could be advanced by 8-9 weeks to June with pruning at 75 per cent level during October in *Annona*cv. Arka Sahan from the normal fruiting season of August - September.

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Original Research Paper

Standardisation of soil volume wetting for drip irrigation in mango (*Mangifera indica* L.,)

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ABSTRACT

Field experiments were conducted in mango for four years during 2017-2020 at ICAR-Indian Institute of Horticultural Research to standardise optimum soil volume wetting for drip irrigation. Wetting soil volume upto 70% recorded higher mean fruit yield of 34.8 kg/plant (9.68 t/ha)and with further increase in the level of soil volume wetting irrigation (upto 80%), there was a decline in the mango yield (7.40 t/ha). Similarly, significantly increased response was observed in fruit weight upto 70% soil volume irrigation (226 g) although there were no significant differences in the TSS of the fruit. Significantly higher water use efficiency was observed for 30% soil volume wetting irrigation (274.1 kg/m^3) and further no significant differences were observed in water use efficiency between 50% and 70% soil volume wetting irrigations indicating that in areas of water scarcity, it is enough to scheduling the irrigation only upto 50% soil volume wetting in mango for economising the water (232.1 kg/m^3).

Keywords: Mango yield, scheduling irrigation, soil volume wetting, water use efficiency

INTRODUCTION

A properly designed and operated drip irrigation system has to supply the water amount required by the crop and should also wet enough soil volume. The wetting patterns which develop from dripping water onto the soil depend on discharge and soil type. Two of the key factors in the design of micro-irrigation systems to obtain the maximum benefits are the amount of water used and the volume of soil to be wetted.

The partial soil wetting pattern by micro irrigation requires assessment of the percentage of soil volume that is wetted (Sne, 2006). Distance between emitter on lateral pipe and distance of lateral pipes from each other should be determined based on the degree of wetted soil diameter by emitters. Duration of irrigation also depends on the fact that at what time after commencement of irrigation, the wetting front reaches depth of plant's root or a multiple of it. Distance of outlets, discharge rate and time of irrigation in drip irrigation have to be determined so that volume of wetted soil is close to volume of plant's root as much as possible. This is because volume of wetted soil surface and moisture depends on soil texture and layering, soil homogeneity, dripper flow rate, primary moisture of soil, consumption water and land slope. A truncated ellipsoid is assumed to best represent the geometry of the wetted soil volume under an emitter. The restricted volume of the wetted soil under drip irrigation and depth-width dimensions of this volume are of considerable practical importance. The volume of the wetted soil represents the amount of soil water stored in the root zone, its depth dimension should coincide with the depth of the root system while its width dimension should be related to the spacing between the emitters and lines. The parameters which influence the wetted soil volume are the available water holding capacity of the soil and the peak daily crop water use representing specific field conditions. The irrigation interval and the management-allowed deficit are additional parameters which affect the wetted volume and could be changed depending on crop sensitivity as well as water and irrigation equipment accessibility (Li *et al*., 2004).

Irrigation water applied should be adequate for crop water use in irrigation interval. The applied water should not be beyond crop root zone to avoid deep

percolation. Although the wetted soil is based on soil type, flow rate, and crop water use, the horizontal and vertical water movements are related to both emitter flow rate and soil intake rates. As such there is a need to optimise the wetted volume taking into account soils, crop, crop stage and seasons.

Mango is the main fruit crop of India and is extensively cultivated under rain fed conditions (68%) with wider spacing without much inputs. At present mango is cultivated in an area of 22, 93,000 ha with a production of 2, 07, 98,000 MT, the productivity being 9.66 t/ha (Anon. 2019). Most of the fruit development of on-season mango fruits takes place during the dry season and farmers have to irrigate mango trees to ensure high yields and good quality. Mango responds well to irrigation especially during fruit set to fruit development. Mango fruit production and quality at fruit growth stage were significantly affected under different irrigation water amounts. Variation in soil water content not only had effects on fruit size, but also on fruit yield (Wei *et al*., 2017). Deficit irrigation strategies are needed to increase water use efficiency and solve the problem of fruit weight reduction during development (Srikasetsarakul *et al*., 2011). Further, the amount of water to be irrigated and the per cent soil volume to be wetted need to be standardized to a given crop situation for enhanced water use efficiency especially under scarce situations. Keeping these points in view, efforts were made to standardise the optimum soil volume wetting irrigation for mango.

MATERIALS AND METHODS

Field experiments were conducted for four years during 2017 to 2020 at ICAR- Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru located at a latitude of 13°8’12"N and a longitude of 77°29’45"E, to standardize the optimum wetted soil volume for irrigation in 18 years old mango (variety Raspuri) spaced at 6m x 6m. The maximum temperature during the experimental period ranged from 24^0C to 36^0C and the minimum temperature ranged between 10^0C to 22^0C. The period between March to May are the warm months with higher temperatures and evaporation while the period between November to January were the cooler months with low temperature and evaporation. The average relative humidity was higher during September and October months. The average rainfall of the location is around 850 mm with two peak periods of rainfall during June-July and September-October months.

Pre-experimental soil had a pH of 4.73 with moderate salts (1.00 dSm^{-1}). The organic carbon content of the soil was good (2.91 %). The available nitrogen (471.4 kg/ha), available phosphorus (23.8 kg/ha) and the available potassium content of the soil was on higher side (350 kg/ha).

The experiment involved comparison of three levels of soil volume wetting irrigation (30%, 50% and 70%) with normal drip irrigation (80% soil volume wetting) as control in RBD design with six replications. The mango crop was maintained with recommended package of practices except for irrigation.

The evaporation data was collected from USWB Class A open pan evaporimeter of meteorological observatory situated in the experimental farm of ICAR-IIHR. Irrigation scheduling was done based on pan evaporation data as per the treatments coinciding with the period from fruit set to fruit development stages.

The volume of the active root zone (soil volume) was arrived by excavating the moist soil carefully (without damaging the roots) around the base before experimentation in the representative plants. Plastic barriers were introduced to the required length and depth of the root zone to demarcate the required per cent wet and dry zones. Three different percentages of the surface soil areas were wetted by the use of single (for 30% and 50% soil volume wetting) and double drip laterals (for 70% soil volume wetting). The amount of rain was taken into account and irrigation paused or reduced accordingly. Total water applied was measured and water applied per tree was calculated based on application time and nominal flow rate. The calculated amount of water for each irrigation was either partially wetted or fully wetted in the root zone depending on the treatment. An irrigation level of 80% ER was fixed based on the results of earlier experiments in mango and water use was calculated to wet the required per cent soil volume in the active root zone based on the wetted area basis as per the treatments.Soil moisture variations were monitored both in dry and wet zones periodically through gravimetric method.

Mango was applied with recommended FYM with a fertilizer dose of 730g N, 180g P_2O_5 and 680g K_2O per plant per year and the crop was managed with recommended package of practices except for irrigation. Plant hoppers were controlled using

Imidacloprid 0.3% and powdery mildew with wettable Sulphur. All the growth and yield parameters were recorded in mango each year. The canopy volume in mango was computed as per standard procedure (Mark *et al*., 2002).

At harvest, yield was determined separately for each tree in alltreatments by the use of a mechanical balance. Water use efficiency (kg fruits $m^{-3}$of water applied) was worked out based on the total water applied through drip irrigation according to FAO recommendations (Doorenbos and Kassam, 1979).

Dropped fruits under all trees in the experiment were collected, counted and weighed. Fruit drop was recorded periodically in number and weight. After harvest the number of all dropped fruits per tree and all harvested fruits were added up to estimate the total fruit retention. The retention rate was calculated as the percentage of fruits attached to the tree at harvest as compared to the calculated initial fruit set.

The mean data was analysed as per standard statistical procedures (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Soil moisture

The moisture studies in the root zone during different periods indicated that there exist significant variations in the soil moisture across the treatments. In the wet zone, the soil moisture increased with increase in the per cent volume of soil irrigated. The highest soil moisture in the wet zone (14.5 %) was recorded with 80% soil volume wetting with a record of 179.9% increased moisture over the dry zone. It was noticed that even with 50% soil volume wetting, the per cent soil moisture difference in the wet zone was over 158.3 % as compared to dry zone.The higher moisture with increased level of irrigation meeting higher volumes of soil may be attributed to the fact that increasing the water application rate allowed more water to distribute in horizontal direction, while decreasing the rate allows more water to distribute in vertical direction for a given volume applied (Li *et al*., 2004). Moreshet (1983) attributed this to the differences in the water depletion as well to the root density distribution pattern between the partially irrigated and that of the fully irrigated one.

Plant growth in mango

The growth parameters in general increased upto 50% soil volume wetting and declined thereafter. Further at 80% soil wetted volume irrigation,significantly lower canopy spread of the plant was observed compared to lower levels of soil volume wetting suggesting that the growth in mango is not favoured much with irrigation above 70% soil volume wetting. Vellame (2015) attributes this to the plant acclimation which is caused by an increase in root concentration in the irrigated area. After a period of acclimation, if the entire root system is wetted, soil water extraction becomes proportional to the percentage of wetted area after a short period of time.

Fruit retention in mango

The fruit retention in mango was significantly influenced by different wetted volumes of irrigating the

Table 1 : Mean soil moisture variation in dry and wet root zones in mango basin

Irrigation treatment	Soil moisture in wet zone (%)	Soil moisture in dry zone (%)	% increase in soil moisture in wet zone over dry zone
30% soil wetted volume irrigation	7.71	3.06	152.0
50% soil wetted volume irrigation	11.96	4.63	158.3
70% soil wetted volume irrigation	12.77	5.01	154.9
80% soil wetted volume irrigation	14.50	5.18	179.9
S.Em±	1.05	0.52	
C.D (P=0.05)	3.35	NS	

Table 2 : Percent wetted soil volume irrigation in influencing the plant growth characters in mango

Treatment	Plant height (m)		Canopy volume (m^3)		Girth (cm)		Primary branches/plant		Secondary branches/plant	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
30% soil wetted volume irrigation	3.20	3.34	35.64	31.98	64.38	70.00	3.00	3.00	3.38	3.38
50% soil wetted volume irrigation	3.70	3.98	53.06	45.94	64.13	67.75	4.00	4.00	2.95	2.94
70% soil wetted volume irrigation	3.52	3.70	46.10	37.74	67.75	70.25	2.50	2.50	3.30	3.30
80% soil wetted volume irrigation	3.44	3.58	38.24	34.22	63.50	67.25	2.75	2.75	3.05	3.04
S.Em±	0.19	0.19	4.10	4.50	3.38	3.90	0.42	0.42	0.27	0.28
C.D (P=0.05)	NS	NS	12.79	NS	NS	NS	NS	NS	NS	NS

soil with increase in the retention as the percent soil wetting increased with irrigation although the trend was not continuous. Significantly higher fruit retention (49.3 %) was observed at 70% soil volume wetting irrigation which although was found at par with 50% soil volume wetting irrigation (47.2%), differed significantly from the other two levels. The increase in fruit retention with increased moisture levels may be attributed to the reduction in fruitlet drop as a consequence of favourable moisture conditions. These differences may also be attributed to the accumulation of abscisic acid in buds at floral initiation in optimizing leaf water potential and sap flow besides optimizing carbohydrate availability and cytokinin in sustaining differentiation activity in growing buds (Makhmale Sandip *et al*., 2015). Similar observations of maximum fruit retention at harvest stage and delayed maturity in the mango trees with irrigation was also observed by Malshe *et al*., (2020).

Yield attributing characters and the fruit yield

The number of mango fruits per plant increased significantly with increase in soil volume wetting irrigation upto 70% (191.9 fruits/plant) decreasing thereafter suggesting that mango responds only upto this level of soil moisture. Higher fruit number with 70% soil volume wetting irrigation may be attributed to higher fruit retention with reduced fruit drop owing to favourable soil moisture conditions at the critical phase. Morshet *et al*., (1983) also observed that there was a considerable difference in flower abscission between irrigation levels especially at the beginning

Table 3 : Mean fruit retention and fruit number per plant in mango as influenced by different wetted soil volume irrigation during different years

Treatment	Fruit retention in plant (%)				Fruit no. /plant				
	2017	2018	2019	Mean	2017	2018	2019	2020	Mean
30% soil wetted volume irrigation	38.9	39.7	47.9	42.2	120.6	205.7	136.6	65.9	132.2
50% soil wetted volume irrigation	41.2	36.1	38.8	38.7	124.2	198.7	155.3	131.5	152.4
70% soil wetted volume irrigation	44.2	38.2	59.1	47.2	154.5	214.0	230.4	168.8	191.9
80% soil wetted volume irrigation	45.3	37.8	64.7	49.3	66.6	177.0	169.8	146.7	140.0
S.Em±	2.8	3.7	2.8	1.6	18.4	22.7	18.5	25.5	11.91
C.D (P=0.05)	NS	NS	8.7	4.73	56.1	NS	56.2	NS	36.23

Table 4 : Mean fruit yield as influenced by percent soil volume wetting irrigation in mango

Treatment	Fruit yield (kg /plant)					Fruit yield (t/ha)				
	2017	2018	2019	2020	Pooled Mean	2017	2018	2019	2020	Pooled Mean
30% soil wetted volume irrigation	22.1	36.6	21.8	12.0	21.4	6.13	10.17	6.05	3.32	5.93
50% soil wetted volume irrigation	22.4	34.9	28.3	25.0	25.8	6.21	9.68	7.87	6.95	7.15
70% soil wetted volume irrigation	33.7	40.2	45.9	30.4	34.8	9.35	11.18	12.76	8.43	9.68
80% soil wetted volume irrigation	12.2	31.7	31.2	31.5	26.6	3.38	8.79	8.66	8.73	7.40
S.Em±	3.5	3.8	4.0	4.7	2.0	0.97	1.06	1.11	1.30	0.56
C.D (P=0.05)	10.7	NS	12.2	14.2	6.1	2.96	NS	3.38	3.96	1.70

of the flowering season. The flower abscission rate in the partially irrigated trees was higher than in the fully irrigated trees while the abscission of fruitlets was lesser in the partially irrigated treatment.

The mean fruit yield per plant increased significantly with increase in soil volume wetting irrigation upto 70% decreasing there after indicating the graded response to moisture levels in mango. Significantly higher mean fruit yield of 34.80 kg/plant (9.68 t/ha) was recorded with 70% soil volume wetting irrigation. This suggests that it is worth giving irrigation to meet 70% level of evaporation demand in areas where water is not scarce. The results also suggests that with further increase in the level of soil volume wetting irrigation (upto 80%), there was a decline in the mango yield (7.40 t/ha) indicating that beyond 70% of soil volume wetting, it is a luxury consumption for the plant. The increase in mean fruit yield with 70% soil volume wetting irrigation over the control (80% soil volume wetting) was 26.1 per cent indicating the deleterious effects of excess irrigation in mango that too with loss of precious irrigation water (24.5%). Earlier studies in mango also revealed that meeting 70% evaporative demand is the best for higher fruit yield and quality (Srinivas *et al*., 2016).

Fruit weight and TSS

The number of fruits rather than the fruit size influences the total yield. Higher fruit yield and favorable fruit size distribution are counteracting and the exact control of both parameters by means of irrigation seems to be difficult. While there is a negative correlation between the number of fruits onthe tree and the average fruit size, the influence of irrigation on fruit size remains important.

Significantly increased response for irrigation in mango fruit size was observed upto 70% soil volume irrigation (226 g) and decreased there after suggesting that beyond this level, the rate of increase in fruit size is only marginal. Lesser fruit weight at lower levels of irrigation may be attributed to the water stress for the full growth of the fruit. Noitsakis *et al.,* (2016) also inferred that higher level of water stress was observed when 50% irrigation water of fully watered pomegranate plants was applied resulting in a significant decrease in mean fruit weight and diameter.

The TSS in mango fruit was although not significantly influenced by different irrigation wetted volumes of soil, relatively higher T.S.S. of 18.84^0 B was observed at 50% as compared with 80% (17.76^0 B) although found at par with rest of the treatments. Further, this may be attributed to the ability of mango to survive short periods of water deficits as a result of drought tolerance that reduces vegetative growth allowing better penetration of light into the canopy.

Water use efficiency

A perusal of the amount of water used / ha during different years for each of the treatment showed that there was a considerable difference across the treatments. The amount of water used / ha under 80% of soil wetted volume was substantially higher (78.7 m^3/ha) as compared to 30%, the latter depicting a saving of 67.5 % water. Similarly, 50% wetted soil volume irrigation showed a saving of 46.2% water

Table 5 : Mean fruit weight and total soluble solids as influenced by percent soil volume wetting irrigation in mango

Treatment	Mean fruit weight (g)					T.S.S. (^{0}B)				
	2017	2018	2019	2020	Mean	2017	2018	2019	2020	Mean
30% soil wetted volume irrigation	181.4	177.9	156.7	172.9	172.2	19.64	16.66	18.16	18.3	18.2
50% soil wetted volume irrigation	183.5	178	181.7	185.4	182.2	19.40	17.96	20.52	17.44	18.84
70% soil wetted volume irrigation	218	192.8	197.4	183.5	197.9	17.98	17.76	19.32	19.28	18.58
80% soil wetted volume irrigation	214.3	179.7	183.2	230.2	201.8	18.54	15.84	17.48	19.12	17.76
S.Em±	13.71	6.873	4.69	18.01	7.5	0.79	0.48	0.72	0.52	0.28
C.D (P=0.05)	NS	NS	14.27	NS	22.7	NS	1.49	NS	NS	NS

Table 6 : Water use efficiency in mango (over four years) as influenced by different levels of per cent soil volume wetting irrigation

Treatment	Water used (m^3/ha)					Mean water used (litres/ plant)	Savings in water (%)	WUE (kg/m^3)				
	2017	2018	2019	2020	Mean			2017	2018	2019	2020	Mean
30% soil wetted volume irrigation	25.28	33.18	34.73	8.9	25.52	91.8	67.5	242.3	306.6	174.3	373.3	274.1
50% soil wetted volume irrigation	41.90	54.84	57.67	14.9	42.33	152.3	46.2	148.2	176.5	136.4	467.3	232.1
70% soil wetted volume irrigation	58.98	76.71	81.03	20.8	59.38	213.6	24.5	158.6	145.7	157.5	405.8	216.9
80% soil wetted volume irrigation	77.03	95.15	112.75	29.7	78.70	283.1	-	43.9	92.4	76.8	293.7	126.7
S.Em±	-	-	-		-	-	-	23.3	16.8	20.1	93.3	27.0
C.D (P=0.05)	-	-	-		-	-	-	70.8	51.0	61.1	-	82.2

compared to normal (80% soil wetted volume) irrigation indicating that by following the 50% soil volume wetting, nearly double the area of the crop can be irrigated.

Significant variations were observed in the mean water use efficiency across the treatments. Higher water use efficiency was observed for 30% soil volume wetting irrigation (274.1 kg/m^3) differing significantly with other levels suggesting that more yield could be obtained per unit amount of water used with the treatment. Further, as the per cent soil volume wetting irrigation increased, the water use efficiency decreased

drastically. This may be attributed to the fact that evaporation is minimised by restriction in wetted soil area and such reduction is influenced by the number of days after the beginning of partial irrigation, atmospheric evaporative demand and plant phonological stage (Vellame *et al.*, 2015).

It was noted that there was non-significant differences in the WUE between 50% (232.1 kg/m^3) and 70% soil volume wetting irrigation (216.9 kg/m^3) indicating that in areas of water scarcity it is worth irrigating only upto 50% of soil volume wetting so that we can also save another 21.7% water. Spreer *et al*. (2009) also inferred that water use efficiency was always significantly higher in the deficit irrigation treatments as compared to the control. Further, Wei *et al.* (2017) also concluded that when the soil moisture content was controlled at about 65±70% of the field water moisture capacity, water demand in the growth and development of mango could be ensured and maximum production efficiency of irrigation and the best quality of fruit could be achieved.

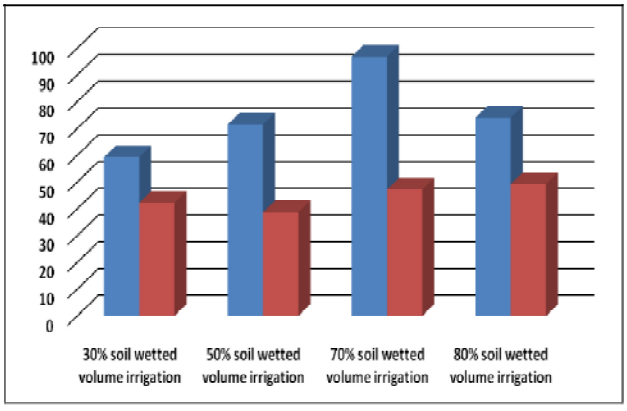

Fig 1. Mean fruit yield and water use efficiency in mango (over four years) as influenced by different levels of per cent soil volume wetting irrigation

CONCLUSION

Wetting soil volume upto 70% recorded higher mean fruit yield of 34.8 kg/plant (9.68 t/ha) and with further increase in the level of soil volume wetting irrigation (upto 80%), there was a decline in the mango yield (7.40 t/ha). Similarly, significantly increased response was observed in fruit weight upto 70% soil volume irrigation (226 g). Significant differences were not observed in water use efficiency between 50% and 70% soil volume wetting irrigations indicating that in areas of water scarcity, it is enough scheduling the irrigation only upto 50% soil volume wetting in mango for economising the water (232.1 kg/m^3).

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Original Research Paper

Assessment of growth and yield parameters in arecanut (*Areca catechu* L.) through correlation and path analysis under hilly zone of Karnataka

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ABSTRACT

Arecanut (*Areca catechu* L.) commonly called as betel nut is a high value commercial crop of coastal and Malnad region of Kerala and Karnataka. The present study was carried out at Agricultural and Horticultural Research Station, Sringeri, UAHS Shivamogga in 2018. The study attempts the correlation studies in the germplasm will help to understand the mutual relationship among various traits and thereby assist in selecting the character contributing to the yield. In addition to this the selection for yield directly is ineffective as yield is affected by many other traits. The highest positive significant for the association of fruit yield per palm was with the fresh kernel weight per palm (0.96g) followed by dry weight of husk per palm (0.89g) and fresh weight of husk per palm (0.89g). Path analysis revealed that nineteen out of thirty-four characters recorded that fruit volume (2.40cc) had highest positive direct effect on fruit yield per palm followed by fresh fruit weight (2.17g) and breadth of leaf sheath (2.11m). It can be concluded that growth and yield characters may be considered in selection criteria for the improvement of yield in arecanut.

Keywords : Arecanut, correlation, path analysis and yield

INTRODUCTION

The coastal and Maland region of Karnataka has tremendous potential for cultivation of arecanut due to favourable soil and climate, it is mostly confined to 28° north and south of the equator. It grows within a temperature range of 14 °C to 36 °C. Susceptibility to low and diverse temperature, it requires ample supply of soil moisture and plentiful of rainfall throughout the year (1,500-5,000 mm). It can be grown in a soil type such as laterites, red loamy and alluvial. The depth of soil may not be less than 1 m. The soil should be well drained.

Arecanut (*Areca catechu* L.) is a high value commercial crop of India, which is also called betel nut. It is widely distributed in Philippines, Indonesia, Sri Lanka, Southern China, Taiwan and Java. India stands first in the world in arecanut production followed by Myanmar, Bangladesh, China and Indonesia. (INDIASTAT, 2020). A total of 11.08 lakh tons of arecanut was produced from 7.43 lakh ha In India with a productivity of 1491 kg per ha (INDIASTAT, 2020). Area and production in different states indicate that Karnataka, Kerala and Assam occupy 80 per cent of area and production followed by Meghalaya, West Bengal, Tamil Nadu, Mizoram and Odisha. The little effort has been identifying the genetic potential of arecanut genotypes in the region. The natural genetic variation for most of the yield contributing characters is considerable in this crop in the region and there is a need for the breeders to restructure the materials for increasing the production and productivity. Correlation study in yield and yield attributing characters/traits will be of value in selection of traits during improvement. Path analysis provides an effective means of finding out direct and indirect causes of association and permits a critical examination of given correlation and measures the relative importance of each factor. It gives more accurate pattern of trait association through direct and indirect effects.

MATERIALS AND METHODS

Ten arecanut genotypes such as Sumangala, Sringeri local, Mohit Nagar, SAS-1, Hirehalli Dwarf, Keladi Local, Sagar Local, Thirthahalli Local, Sreemangala

and Mangala as test entries with three replications, each having three palms of eight years old were evaluated at Agricultural and Horticultural Research Station, Sringeri, which is located in the Western Ghats and represents the typical hill zone (9) of Karnataka and lies at $13^0$25' North latitude and 75^0 25' East longitude with an altitude of 980 m above mean sea level during 2018. The observation on growth and yield characters were recorded at the time of maturity.

Phenotypic correlations of 34 characters both growth and yield quantitative characters namely, kernel breadth (mm), fresh weight of husk (g), number of bunches per palm, husk thickness (mm), dry weight of husk (g), fresh nut yield per palm (g), recovery percentage (%), bunch weight per palm (g), fresh kernel weight per palm (g), fresh weight of husk per palm (g), dry weight of husk per palm, (g) number of inflorescence, plant height (m), crown length (m), girth (m), inter nodal length (m), number of fronds , number of leaflets , length of oldest leaf (m), breadth of oldest leaf (m), length of leaf sheath (m), breadth of leaf sheath (m), number of female flowers per inflorescence, number of nuts per palm, fruit length(mm), fruit breadth (mm), fresh fruit weight (mm), kernel length (mm) , fruit volume (cc), dry weight of kernel (mm), Total chlorophyll content (µg /g) and number of nuts per inflorescence fruit yield per palm (g) presented in Table 1 and 2. Mean data was subjected for study of correlation and path coefficient as suggested by Miller *et al*. (1958) and Dewey and Lu (1959) respectively.

RESULTS AND DISCUSSION

The analysis of variance showed significant differences among the genotypes for all the characters studied. The extent of variability present in the germplasm provides scope for the crop improvement programme and also depends on the extent of heritability for a trait. Range of variation observed for all the traits indicated the presence of sufficient amount of variation among the genotypes for all the characters studied. The genotype Mangala recorded higher mean value for traits like fruit length, fruit breadth, husk thickness, fresh weight of husk, fresh nut yield, bunch weight, fresh weight of kernel, dry weight of kernel, fresh weight of husk per palm, dry weight of husk per palm and number of inflorescences. SAS-1 recorded the lowest value for fruit length and kernel length. Sringeri Local recorded the lowest value for fruit breadth and fruit volume. Sumangala recorded higher value for fruit volume and lowest value for number of nuts per inflorescence. Mohit Nagar recorded higher value for fresh fruit weight, kernel breadth, fresh weight of kernel, dry weight of kernel and dry weight of husk while lower value for recovery percentage. The minimum kernel weight was observed in Hirehalli Dwarf. The higher kernel weight was observed in Sumangala, Mohit Nagar cultivars which has been reported earlier (Ananda and Rajesh, 2004) [2]. Phenotypic expression of any traits largely depends on the genotype of the plant and influences environmental variation but generally, higher environmental influence suppresses the complete expression of genes. Phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters studied but, this needs a good understanding of the association of different traits with yield and their association among themselves. The correlation analysis helps in examining the possibility of improving yield and its attributing traits through an indirect selection of their highly correlated component traits. In this investigation, correlation coefficients were worked out on ten genotypes of arecanut.

The study of the association of component characters with a complex trait like yield is very helpful for ease of gainful selection in any breeding programme. It has been established that the structure of yield must be probed through its components rather than yield. The concept of correlations was elaborated by Fisher (1918) and Wright (1921).

The association of fruit yield per palm was positive significant with the kernel breadth (0.39), fresh weight of husk (0.65), number of bunches per palm (0.68), husk thickness (0.69), dry weight of husk (0.40), fresh nut yield per palm (0.68), recovery percentage (0.36), bunch weight per palm (0.68), fresh kernel weight per palm (0.97), fresh weight of husk per palm (0.89), dry weight of husk per palm (0.90) and number of inflorescence (0.66) (Archana, 2017) and Rajesh (2007) for per cent nut set, the number of female flowers per inflorescence. Since these associated characters were in the

desirable direction, it indicated that simultaneous selection for these characters would be rewarding in improving the dry kernel yield. Talukder *et al.* (2011) observed that nut weight showed a positive and significant correlation with husk weight, the volume of water, shell weight, kernel weight and kernel thickness in coconut. Highly significant positive correlations were observed among whole nut weight, dehusked nut weight and copra weight by Natarajan *et al.* (2010).

The remaining characters are positive but non-significant *viz.,* plant height (0.09), crown length (0.26), girth (0.12), inter nodal length (0.24), number of fronds (0.07), number of leaflets (0.10), length of oldest leaf (0.22), breadth of oldest leaf (0.30), length of leaf sheath (0.13), breadth of leaf sheath (0.31), which is mainly due to an increase in crown length would accommodate a greater number of leaves which in response produce high quantities of photosynthates. The number of female flowers per inflorescence (0.01), number of nuts per palm (0.46), fruit length (0.34), fruit breadth (0.62), fresh fruit weight (0.34), kernel length (0.16), fruit volume (0.19), dry weight of kernel (0.27) The results were confirmed with the findings of Rajesh (2007). In arecanut, plant height, husk thickness, kernel breadth and dry weight of kernel are important characters to be accounted for gaining improvement in yield per palm. Since these characters had a high direct association on dry kernel yield at the phenotypic level. This indicated that in arecanut production of nuts is not affected due to the individual nut weight and *vice versa*.

Total chlorophyll content (-0.10) and number of nuts per inflorescence (-0.30) had a negative association with fruit yield per palm but it was very low and non-significant and none of the characters showed negative correlation with yield/plant. Therefore, there may not be any problem in increasing the yield of arecanut through any of the characters under study. Anand *et al*. (2005) noticed the fresh fruit weight, dry kernel weight, dry kernel recovery, dry fruit weight was correlated positively with kernel yield while husk thickness had negative association with kernel yield in exotic accessions (Ananda *et al*., 2005). Similarly, characters like fresh fruit weight, dry kernel weight and dry kernel recovery had high magnitude of correlation with kernel yield and production of nuts in arecanut varieties during initial bearing in coastal region of Karnataka (Ananda *et al*., 2001) while negative correlations were reported between the dry nut weight and dry husk weight with kernel yield. Therefore, all the characters were found helpful in increasing the yield of arecanut. (Table 1 and 2).

The coefficient of correlation does not give the true picture under complex situations. Under such situations, path coefficient analysis provides a mean to determine the direct influence of one variable (cause) upon another variable (effect). For the establishment of cause and effect relationship path coefficient analysis offers an opportunity for partition of correlation coefficient into component of direct and indirect effects (Wright, 1921) and path coefficient analysis is the effective measure of direct and indirect causes of association and also depicts the relative importance of each factor involved in contributing to the final product that is yield (Dewey and Lu, 1959).Path coefficient analysis was carried out by taking fruit yield per plant as dependent variable. Positive and negative, direct and indirect effect of yield components on fruit yield per plant is presented in table 3 and 4.

The present investigation path analysis revealed that nineteen out of thirty-four characters recorded that fruit volume (2.40) had highest positive direct effect on fruit yield per palm followed by fresh fruit weight (2.18) and breadth of leaf sheath (2.12). Remaining characters had negative direct effect, among them number of inflorescences per palm (-0.25) had highest negative direct effect on fruit yield per palm followed by number of fronds (-2.22) and fresh husk weight per palm (-1.51). Rajesh (2007) observed the direct effects on dry kernel yield via nut set, breadth of leaflet, internodal length, the number of leaves, the number of inflorescences per palm, length of leaf, fresh fruit weight. The traits *viz*., crown length, internodal length and leaf breadth were negatively contributed towards dry kernel yield. Similar results were observed by Bavappa and Nair (1982). The local arecanut cultivar of South Kanara in coconut cultivars, such trends have been reported by Renuga (1999) and Jerard (2002), Ganesamurthy *et al.* (2002) in coconut and Natarajan *et al*. (2010) in Arecanut. Therefore, it can be concluded that these characters can be considered in selection criteria for the improvement of yield in arecanut. The residual effect (0.067) obtained was less than 0.5, suggesting that some of the characters have not been included, which may be responsible to enhance the fruit yield of arecanut.

Table 1 : Estimates of phenotypic correlation coefficient for growth and yield attributing traits of arecanut

Trait	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
X_1	1.000	0.80**	0.56**	0.93**	0.51**	0.32	0.89**	0.88**	0.82**	0.48**	0.38*	-0.07	0.29	0.09
X_2		1.000	0.40*	0.80**	0.56**	0.45*	0.85**	0.83**	0.92**	0.52**	0.39*	-0.09	0.25	0.26
X_3			1.000	0.59**	0.71**	0.73**	0.59**	0.55**	0.52**	0.82**	0.44*	-0.09	-0.09	0.12
X_4				1.000	0.40*	0.47**	0.94**	0.89**	0.82**	0.51**	0.31	-0.09	0.35	0.20
X_5					1.00	0.70**	0.51**	0.59**	0.64**	0.86**	0.36*	0.07	-0.17	0.07
X_6						1.000	0.55**	0.46*	0.59**	0.72**	0.18	0.04	-0.11	0.09
X_7							1.000	0.92**	0.92**	0.52**	0.38*	-0.07	0.32	0.22
X_8								1.000	0.86**	0.58**	0.43*	0.07	0.19	0.30
X_9									1.000	0.54**	0.32	-0.13	0.21	0.13
X_{10}										1.000	0.37*	-0.01	-0.03	0.31
X_{11}											1.00	0.04	-0.28	0.68**
X_{12}												1.00	-0.25	-0.09
X_{13}													1.00	0.01
X_{14}														1.00

*Level of significance at 5% ** Level of significance at 1%

Where,

X_1=Plant height (m) X_2= Crown length (m) X_3=Girth (m) X_4=Internodal length (m) X_5= No. of fronds. X_6= No. of leaflets. X_7=Length of oldest leaf (m) X_8=Breadth of oldest leaf(m) X_9=Length of leaf sheath (m) X_{10}=Breadth of leaf sheath (m). X_{11}=No. of bunches/ palm (m). X_{12}=Total Chlorophyll (µg/ml) X_{13}= No. of female flowers per inflorescence. X_{14}=Yield (g/ palm)

Table 2 : Estimates of phenotypic correlation coefficient for yield and yield attributing traits of arecanut

Trait	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	X_{20}	X_{21}
X_1	1.000	0.40*	0.22	0.42*	0.45*	0.75**	0.63**	0.69**	0.61**	0.86**	0.82**	0.32	0.69**	0.44*	0.57**	0.62**	0.62**	0.36	-0.33	-14	0.33
X_2		1.000	0.49**	0.74**	0.76**	0.18	0.56**	0.41*	0.37*	0.55*	0.39*	0.60**	0.53**	0.67**	0.70**	0.69**	0.50**	-0.54	0.23	-0.18	0.62**
X_3			1.000	0.86**	0.63**	-0.32	0.24	0.25	0.22	0.37*	0.40*	0.05	0.06	0.18	0.14	0.12	-0.07	-0.29	-0.07	0.12	0.19
X_4				1.000	0.71**	-0.01	0.58**	0.58**	0.57**	0.60**	0.62**	0.44*	0.39*	0.38*	0.41*	0.35	0.25	-0.07	0.12	-0.05	0.33
X_5					1.00	0.01	0.34	0.36*	0.36	0.60**	0.57**	053**	0.62**	0.63**	0.60**	0.73**	0.39*	-0.25	0.18	0.21	0.69**
X_6						1.000	0.47	0.46**	0.44*	0.55**	0.52**	0.26	0.48**	0.25	0.38*	0.40*	0.55**	0.05	-0.12	-0.03	0.16
X_7							1.000	0.84**	0.89***	0.74**	0.73**	0.68**	0.70**	0.53**	0.66**	0.55**	0.67**	0.03	0.09	-0.31	0.38*
X_8								1.000	0.92**	0.81**	0.84**	0.71**	0.75**	0.42*	0.56**	0.59**	0.69**	-0.13	0.07	-0.27	0.29
X_9									1.000	0.71**	0.84**	0.72**	0.78**	0.37*	0.49**	0.45*	0.53**	0.15	0.10	-0.23	0.27
X_{10}										1.000	0.86**	0.61**	0.79**	0.37**	0.81**	0.78**	0.76**	-0.39*	0.03	0.02	0.64**
X_{11}											1.00	0.58**	0.76**	0.43*	0.52**	0.59**	0.52**	-0.17	-0.06	0.06	0.39*
X_{12}												1.00	0.81**	0.71**	0.75**	0.72**	0.71**	0.11	0.56**	-0.02	0.68**
X_{13}													1.00	0.70**	0.78**	0.82**	0.72**	-0.05	0.15	-0.11	0.66**
X_{14}														1.00	0.97**	0.89**	0.77**	-0.32	0.41*	0.16	0.96**
X_{15}															1.00	0.91**	0.87**	-0.33	0.32	-0.01	0.89**
X_{16}																1.00	0.81**	-0.33	0.25	0.14	0.89**
X_{17}																	1.00	-0.38	0.29	-0.08	0.66**
X_{18}																		1.00	0.22	-0.11	-0.30
X_{19}																			1.00	0.33	0.45*
X_{20}																				**1.00**	0.36*

*Level of significance at 5% ** Level of significance at 1%

Where,

X_1=Fruit length (mm) X_2= Fruit breadth (mm) X_3=Fruit volume (cc) X_4=Fresh fruit weight (g/fruit) X_5=Husk thickness (mm). X_6=Kernel length (mm) X_7=Kernel breadth (mm) X_8= Fresh weight of kernel(g/fruit)X_9=Dry weight of kernel (g/palm) X_{10}=Fresh weight of husk (g/palm) X_{11}=Dry weight of husk (g/palm) X_{12}= Fresh nut yield (g/palm) X_{13}=Bunch wt (g/palm)X_{14}=Fresh kernel weight (g/ palm) X_{15}=Fresh husk weight(g/palm) X_{16}=Dry husk weight (g/palm) X_{17}=No. of Inflorescence X_{18}= No. Nuts per Inflorescence X_{19}=Total nuts per palm X_{20}= Recovery percentage(%) X_{21}= Yield (g/ palm).

Table 3 : Direct and Indirect effects of growth parameters on kernel yield

Trait	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}
X_1	0.75	0.59	0.42	0.69	0.38	0.24	0.67	0.65	0.61	0.36	0.28	-0.05	0.22	0.09
X_2	-0.69	-0.87	-0.35	-0.69	-0.49	-0.39	-0.74	-0.72	-0.80	-0.45	-0.34	0.08	-0.22	0.26
X_3	-0.53	-0.38	-0.94	-0.55	-0.67	-0.68	-0.56	-0.52	-0.49	-0.77	-0.41	0.09	0.08	0.12
X_4	-1.25	-1.08	-0.79	-1.34	-0.55	0.63	-1.27	-1.21	-1.11	-0.69	-0.42	0.12	-0.48	0.20
X_5	-1.12	-1.24	-1.59	-0.91	-2.22	-1.57	-1.14	-1.30	-1.44	-1.91	-0.81	-0.16	0.39	0.07
X_6	0.20	0.27	0.44	0.28	0.43	0.61	0.34	0.28	0.36	0.44	0.11	0.03	-0.06	0.09
X_7	-0.58	-0.56	-0.39	-0.62	-0.34	-0.36	-0.66	-0.61	-0.61	-0.34	-0.25	0.05	-0.21	0.22
X_8	0.70	0.67	0.44	0.72	0.47	0.37	0.74	0.79	0.69	0.46	0.34	0.05	0.16	0.30
X_9	1.16	1.30	0.73	1.15	0.91	0.84	1.29	1.21	1.40	0.76	0.45	-0.19	0.29	0.13
X_{10}	1.03	1.11	1.74	1.09	1.82	1.52	1.10	1.22	1.15	2.11	0.79	-0.24	-0.08	0.31
X_{11}	0.38	0.39	0.44	0.31	0.36	0.18	0.38	0.43	0.32	0.37	0.99	0.04	-0.28	0.68
X_{12}	-0.01	-0.01	-0.013	-0.01	0.01	0.01	-0.01	0.01	-0.02	-0.02	0.01	0.13	-0.03	-0.09
X_{13}	0.07	0.06	-0.02	0.09	-0.04	-0.02	0.07	0.05	0.05	-0.01	-0.07	-0.06	0.24	0.01
X_{14}														1.000

*Level of significance at 5% ** Level of significance at 1%

Where,

X_1=Plant height (m) X_2= Crown length (m) X_3=Girth (m) X_4=Internodal length (m) X_5= No. of fronds. X_6= No. of leaflets. X_7=Length of oldest leaf (m) X_8=Breadth of oldest leaf(m) X_9=Length of leaf sheath (m) X_{10}=Breadth of leaf sheath (m). X_{11}=No. of bunches/ palm (m). X_{12}=Total Chlorophyll (µg/ml) X_{13}= No. of female flowers per inflorescence X_{14}=Yield (g/ palm)

Table 4 : Direct and Indirect effects of yield components on kernel yield

Trait	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}	X_{13}	X_{14}	X_{15}	X_{16}	X_{17}	X_{18}	X_{19}	X_{20}	X_{21}
X_1	0.12	0.05	0.03	0.52	0.06	0.09	0.08	0.09	0.07	0.11	0.10	0.04	0.08	0.05	0.07	0.07	0.07	-0.04	-0.04	-0.01	0.33
X_2	-0.14	-0.34	-0.17	-0.25	-0.26	-0.03	-0.19	-0.14	-0.13	-0.19	-0.14	-0.20	-0.18	-0.23	-0.24	-0.23	-0.17	0.02	-0.08	0.06	0.62
X_3	0.53	1.18	2.40	2.07	1.51	-0.78	0.59	0.61	0.54	0.89	0.97	0.12	0.14	0.44	0.35	0.30	-0.18	-0.69	-0.17	0.28	0.19
X_4	-0.64	-1.12	-1.39	-1.51	-1.08	0.01	-0.88	-0.89	-0.86	-0.91	-0.94	-0.68	-0.59	-0.58	-0.63	-0.54	-0.39	0.11	-0.17	0.08	0.33
X_5	-0.13	-0.22	-0.18	-0.20	-0.29	-0.002	-0.09	-0.10	-0.10	-0.17	-0.16	-0.15	-0.18	-0.18	-0.17	-0.21	-0.11	0.07	-0.05	-0.06	0.69
X_6	0.64	0.08	-0.28	-0.01	0.01	0.85	0.40	0.39	0.38	0.47	0.44	0.22	0.41	0.22	0.33	0.35	0.47	0.04	-0.10	-0.02	0.16
X_7	0.41	0.37	0.16	0.38	0.22	0.31	0.65	0.55	0.58	0.48	0.48	0.44	0.46	0.35	0.43	0.36	0.44	0.02	0.06	-0.20	0.38
X_8	-0.44	-0.26	-0.16	-0.37	-0.23	-0.29	-0.53	0.63	-0.58	-0.51	-0.53	-0.44	-0.47	-0.26	-0.35	-0.32	-0.43	0.08	-0.04	0.17	0.29
X_9	-0.64	-0.39	-0.24	-0.59	-0.38	-0.47	-0.95	-0.97	-1.05	-0.75	-0.89	-0.76	-0.82	-0.39	-0.51	-0.48	-0.56	-0.16	-0.11	0.24	0.27
X_{10}	0.46	0.29	0.19	0.32	0.32	0.29	0.39	0.54	0.38	0.53	0.46	0.33	0.42	0.39	0.43	0.42	0.41	-0.21	0.02	0.01	0.64
X_{11}	-0.84	-0.40	-0.42	-0.64	-0.58	-0.53	-0.75	-0.86	-0.86	-0.88	-1.02	-0.59	-0.78	-0.44	-0.53	-0.61	-0.53	0.17	0.07	-0.07	0.39
X_{12}	0.69	1.31	0.11	0.76	1.15	0.56	1.48	1.54	1.58	1.34	1.27	2.17	1.77	1.56	1.64	1.58	1.56	0.25	1.22	-0.04	0.68
X_{13}	0.54	0.42	0.47	0.30	0.49	0.38	0.56	0.59	0.62	0.63	0.60	0.65	0.79	0.56	0.62	0.65	0.57	-0.04	0.12	-0.09	0.69
X_{14}	-0.13	-0.21	-0.06	-0.12	-0.20	-0.08	-0.17	-0.13	-0.12	-0.23	-0.13	-0.23	-0.23	-0.32	-0.31	-0.28	-0.25	0.10	-0.13	-0.05	0.96
X_{15}	-0.12	-0.15	-0.03	-0.10	-0.13	-0.08	-0.13	-0.12	-0.10	-0.17	-0.11	-0.16	-0.17	-0.21	-0.21	-0.19	-0.18	0.07	-0.06	0.00	0.89
X_{16}	0.11	0.12	0.02	0.06	0.13	0.07	0.10	0.09	0.08	0.15	0.11	-0.18	0.15	0.16	0.17	0.18	0.15	-0.06	-0.04	0.02	0.89
X_{17}	-0.15	-0.12	0.02	-0.06	-0.10	-0.14	-0.16	-0.17	-0.13	0.18	-0.13	-0.02	-0.18	-0.19	-0.22	-0.20	-0.25	0.09	-0.07	0.02	0.66
X_{18}	0.05	0.01	0.04	0.01	0.03	-0.01	-0.01	0.072	-0.02	0.05	0.02	0.00	0.01	0.04	0.04	0.65	0.05	-0.14	-0.03	0.02	-0.30
X_{19}	-0.000	0.00	-0.00	0.00	0.00	-0.00	0.00	0.00	0.00	0.00	-0.00	-0.00	0.00	0.00	0.00	0.00	0.00	-0.00	0.00	0.00	0.45
X_{20}	-0.00	-0.00	0.00	-0.00	0.00	-0.00	-0.00	-0.00	-0.00	0.00	0.00	-0.23	-0.00	0.00	-0.00	0.00	-0.00	-0.00	0.00	0.00	0.36

*Level of significance at 5% ** Level of significance at 1%

Where,

X_1=Fruit length (mm) X_2= Fruit breadth (mm) X_3=Fruit volume (cc) X_4=Fresh fruit weight (g/fruit) X_5=Husk thickness (mm). X_6=Kernel length (mm) X_7=Kernel breadth (mm) X_8= Fresh weight of kernel(g/fruit)X_9=Dry weight of kernel (g/palm) X_{10}=Fresh weight of husk (g/palm) X_{11}=Dry weight of husk (g/palm) X_{12}= Fresh nut yield (g/palm) X_{13}=Bunch wt (g/palm)X_{14}=Fresh kernel weight(g/ palm) X_{15}=Fresh husk weight(g/palm) X_{16}=Dry husk weight (g/palm) X_{17}=No. of Inflorescence X_{18}= No. Nuts per Inflorescence X_{19}=Total nuts per palm X_{20}= Recovery percentage(%) X_{21}= Yield (g/ palm)

CONCLUSION

The study of the association of component characters with a complex trait like yield is very helpful for ease of gainful selection in any breeding programme. The association of fruit yield per palm was positively significant with most of the morphological characters under study. Path analysis revealed that nineteen of thirty-eight characters recorded fruit volume had highest positive direct effect on fruit yield per palm followed by fresh fruit weight and breadth of leaf sheath. It can be concluded that these characters may be considered in selection criteria for the improvement of yield in arecanut.

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Original Research Paper

Growth and yield enhancement of carrot through integration of NPK and organic manures

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ABSTRACT

A pot experiment was conducted at Horticulture Experimental Area, Gomal University, Dera Ismail Khan, Pakistan to investigate the combined effects of NPK and organic manures on growth and yield of carrot, for two consecutive years. The experiment was laid out in CRD with six treatments and four replications. Five different organic manures such as poultry manure (PM), sewage sludge (SS), farmyard manure (FYM), press mud (PrM) and goat manure (GM) were applied in combination with NPK, each at recommended levels for two successive years. A fertilizer check (control) was also included as treatment where no fertilizer and manure were used. The study revealed significant improvements in almost all growth and yield attributes by combined application of NPK and organic manures. Among different combinations, NPK + PM surpassed all other treatments by giving maximum leaves per plant (8.73 and 8.13), leaf length (38.17 and 36.77cm), root length (29.30 and 24.83cm), root diameter (3.10 and 3.27cm), root weight per plant (142.40 and 142.00g), total biomass per plant (169.33 and 166.67g) and root yield (56.67 and 56.83 t/ha), during both the experimental years. Similarly, NPK combination with green manure and sewage sludge also produced better results pertaining to carrot growth and production for two consecutive years. It was also observed during the study that control treatment showed poorest findings and placed at lowest levels.

Keywords: Carrot, NPK, organic manures, root length, root weight and total biomass

INTRODUCTION

Carrot is one of the major vegetable crops grown throughout the world (Cho *et al*., 2021) and considered to be an important economical vegetable as it has large yield per unit area (Sikora *et al*., 2020). In Pakistan, carrot is one of the cheaply available vegetables and is equally used by poor and rich people (Amjad *et al.*, 2013). Besides, vitamin A and fiber carrot is also enriched with carbohydrates, protein, minerals, fibers, iron and so on (Khomich *et al*., 2020). From therapeutic point of view carrot is more useful in curing human diseases especially eye sight (Nagraj *et al*., 2020). This root vegetable is used for different purposes in daily human diet and its roots are eaten uncooked in steamed or boiled vegetable salad and can also be used in soup and other food stuff (Rahman *et al*., 2020). According to survey in Pakistan (2017-18) the carrot was grown on area of 13.95 thousand ha and its total production was 241.91 thousand tones (Noor *et al.*, 2020). The proper application of nutrients increase the soil fertility and crop production (Silveria and Kohmann, 2020). Plants and crops fulfill their nutritional requirements by the uptake of minerals largely through soil (Vijayprabhakar *et al.*, 2020). Balanced nutrition application is considered as an important factor to boost production. Both soil fertility and crop production are adversely affected by misuse of fertilizers without any significant knowledge (Pandey *et al*., 2020). Generally, most carrot growers use inorganic fertilizers to realize higher yields. The rising level of inorganic fertilizers adversely affect the human health (Toor *et al.*, 2020) soil texture and structure. So, the farmers tried the integrated plant nutrients which significantly increased the fertility of

soil and crop production (Singh *et al*., 2020). There are several organic soil amendments which include materials such as chicken manure, cattle manure, cocoa pod husk, compost and solid waste (Ameen, 2020). So, the mineral fertilizers can be substituted by organic manures. Manure application provides nutrients, enhances water holding ability, soil structure and porosity, moisture retention, bulk density, enhance the microbial growth and crop quality (Goel *et al.,* 2020). Organic fertilizers are cheaper than inorganic sources, thus farmers can easily afford the cost of organic fertilizers (Hafez *et al*., 2020) In order, to achieve high yield and quality product, the proper use of mineral fertilizers and organic manure are of considerable importance. They also display a vital role in avoiding harmful effects on soil and environment as well (Fallah *et al*., 2020) The effectiveness of the combined application of mineral and organic fertilizers assigned to the increased efficiency of mineral fertilizer and the balanced supply of all the essential nutrients. Integrated use of organic and inorganic fertilizers can improve crop productivity and sustain soil fertility (Hammad *et al*., 2020) However, the main important issue is that the organic fertilizers are slowly available to the crops as compared to the inorganic fertilizers. Recently, the researchers focused to practice the combination of mineral fertilizers and organic manures. The combination of both the organic and inorganic fertilizer increase the soil fertility, crop production and decrease the level of soil pollution (Karmakar *et al*., 2020). Taking into consideration the beneficial aspect of integrated fertilizers, an experiment was conducted to study the response of growth and yield of carrot towards the combined effect of NPK dose and organic manures.

MATERIALS AND METHODS

The two years study to investigate the integrated use efficiency of different organic manures in addition to NPK on growth and production of carrot was carried out at Horticulture Experimental Area, Gomal University, Dera Ismail Khan, Pakistan. Experimental site is located between 32º 4' N (latitude), 71º 2' (longitude) and 173 m (altitude) above sea level. Climatic conditions of the study area are arid, subtropical, and continental with an average rainfall ranging 180-300 mm. The trial was conducted in pots using CRD layout with six treatments (i.e.) T_1:Control (no fertilizers), T_2: NPK (100:100:125 kg ha^{-1}) + FYM (30.0 t ha^{-1}), T_3:NPK + PM (10.0 t ha^{-1}), T_4:NPK + GM (15.0 t ha^{-1}), T_5: NPK + PrM (20.0 t ha^{-1}) and T_6:NPK + SS (20.0 t ha^{-1}) each treatment replicated four times. All pots were filled with equal and uniform amount (20.0 kg) of river soil along with respective quantities of NPK and organic manures. A set of pots without any additives (manures and fertilizers) treated as control. The required quantity of mineral fertilizers (phosphorus and potash) were applied in the form of Single super phosphate and Sulphate of potash at sowing, while different manures were incorporated well before sowing of seeds (10 days). Nitrogen was applied in the form of urea in two splits i.e., before sowing and after one month of sowing. Five seeds of carrot (local variety) were sown on 20th October, each year in pots and all cultural practices were performed uniformly.

Data on various attributes pertaining to plant growth and yield including number of leaves per plant, leaf weight and length, root weight, length, diameter, plant biomass and yield were recorded, and statistical analysis was done as per ANOVA techniques, while means' comparison was done by Duncan's multiple range (DMR) test.

RESULTS AND DISCUSSION

Application of NPK and organic manures significantly influenced number of leaves per plant during both the experimental years (Table1). Application of NPK + PM recorded the significantly higher number of leaves per plant (8.73 and 8.13) during both the years. It was followed by the application of NPK + GM (8.17 and 7.60). The study also showed statistically on par number of leaves per plant by applying SS (7.83 and 7.33), FYM (7.73 and 7.27) and PrM (7.60 and 7.07) in addition to NPK. The control treatment recorded the least number of leaves per plant was (4.53 and 3.27). The obtained results showed that the integrated mineral and organic manure increased the number of leaves by providing macro and micro nutrient to plants. The increase in the number of is attributed to the use of variant nature of the organic manures. The obtained results are in accordance to previously reported literature (Singh *et al.*, 2007). Kirad *et al*. (2010), also recorded 8.26 and 16.06 leaves per plant. The addition of various organic fertilizers along with NPK greatly increased the leaf length of the carrot. The results related to the combined effect of organic fertilizers along with NPK on the leaf length are shown

in Table 1). Among the treatments, the longest leaves (38.17 and 36.77 cm) were produced by the combination of NPK + PM, followed by NPK + GM (35.17 and 36.50 cm) and NPK + SS (34.13 cm). Significantly shortest leaves (17.33 and 15.70 cm) were found in control treatment, during two years of experimentation. The results of this experiment are also supported by numerous references already cited in literature (Singh *et al.*, 2007, Singh *et al.*, 2020 and Sunandarani and Mallareddy, 2007). Data pertaining to weight of carrot leaves (Table 1) expressed significant variations by comparing organic manures, as well as comparison over control for two succeeding years. During 1st year, highest and statistically leaf weight per plant (25.00g) was recorded with T_3, which remained on par with only T_4 (24.33 g) only. During second year significantly higher leaf weight per plant (23.67 g) was recorded in T_4, which remained on par with T_3 (23.00 g) and T6 (22.67g). The significantly lowest values of 9.0 and 7.67 gm were recorded with T_1 during first and second year respectively. It can be concluded from the results that the application of organic manures in combination with NPK substantially increased the weight of carrot leaves. The combined introduction of manures along with NPK raised the leaf weight 163.7% to 226.1% over control in the first year, while the same was 144.4% to 162.9% in the next year, higher in NPK + PM (first year) and NPK + GM (second year), while during both years the minimum increase was noted NPK + PrM. This might be attributed to the combination of inorganic and organic fertilizers that decreased the loss of nutrients. The proper use of the integrated manures and fertilizers increased the leaf weight by providing higher rate of nutrients availability (Toor *et al*., 2020).

The different treatments significantly influenced root length, root diameter, root weight, biomass weight and root yield (Table 2). Application of poultry manure (PM) in addition to NPK produced significantly higher values for root length (29.30 and 24.83 cm), which

Table 1 : Effect of NPK and organics manures on leaf characters in carrot in response of NPK and organic manures

Treatment	No. of leaves per plant		Leaf length (cm)		Leaf weight (g per plant)	
	I year	II Year	I year	II Year	I year	II Year
T_1	4.53	3.27	17.33	15.70	9.00	7.67
T_2	7.73	7.27	33.87	32.73	22.33	21.33
T_3	8.73	8.13	38.17	36.77	25.00	23.00
T_4	8.17	7.60	36.50	35.17	24.33	23.67
T_5	7.60	7.07	32.70	32.17	22.00	20.22
T_6	7.83	7.33	34.77	34.13	23.00	22.67
LSD (0.05)	0.239	0.289	0.670	1.013	0.878	1.434

Table 2 : Effect of NPK and organics manures on root characters and yield

Treatment	Root length (cm)		Root diameter (cm)		Root weight (g/plant)		Biomass weight (g/plant)		Root yield (t/ha)	
	I year	II Year	I year	II Year	Iyear	II Year	Iyear	II Year	I year	II Year
T_1	12.03	10.80	1.43	1.22	47.33	38.33	56.33	46.00	18.93	15.33
T_2	22.00	19.57	2.79	2.50	128.00	114.33	150.33	135.63	51.04	45.73
T_3	29.30	24.83	3.27	3.10	142.40	142.00	169.33	166.67	56.83	56.67
T_4	26.03	23.87	2.93	2.90	141.33	136.67	166.0	160.00	55.15	51.75
T_5	23.77	20.73	2.80	2.67	128.67	120.33	150.73	140.57	53.83	45.90
T_6	25.17	21.83	2.83	2.73	130.33	129.37	153.0	152.33	54.37	48.83
LSD (0.05)	1.829	1.157	0.133	0.176	5.116	4.598	2.876	4.608	1.301	1.936

remained on par with only T_4 during the second year of experimentation. Among different organic fertilizers, poorest results (22.00 and 19.57 cm root length) were recorded in NPK + FYM. However, the shortest roots (10.80 cm and 12.03 cm) were found in control treatment. The current study revealed that the use of organic manure in conjunction with NPK significantly enlarged carrot roots, thereby advocating positive impact on root growth from the combined use of manures and fertilizers. These results are supported by previously work done in literature (Sunandarani and Mallareddy, 2007)

Root length and diameter greatly contributes to carrot weight and yield. Amongst different organic manures applied in addition to NPK, poultry manure (PM) superseded other treatments by producing maximum root diameter (3.27 and 3.10 cm), respectively for two successive years. It was followed by NPK + GM (2.93 and 2.90 cm) and NPK + SS (2.83 and 2.73 cm) respectively for two years. The lowest root diameter (1.43 and 1.22 cm) was recorded in control treatment. The study showed that the combined use of organic manures together with NPK substantially increased the carrot root diameter. Addition of PM proved superior amongst treatments,while FYM was least effective that might be due to lower nutrient concentrations in FYM as well as its slow release and delayed decomposition. From the obtained results it was concluded that the integrated nutrients increased the root diameter (Toor *et al*., 2020).

Maximum root weight per plant (142.4 and 142.0 g) was recorded in combined application of NPK and PM, which was followed by NPK + GM (141.33 and 136.68 g) for two years. Addition of FYM along with NPK resulted in poor root weight (128.00 and 114.33 g) during both the cropping seasons. However, control treatment, where no fertilizers (chemical + organic) were mixed into the soil showed lowest root weight per plant (47.33 and 38.33 g), respectively for two consecutive years. The results of this study showed that the combined use of organic and mineral fertilizers substantially increased the root weight of carrot, which might be attributed to the well solubilization of plant food, contributing to the increased nutrient uptake. These results suggested that combination of organic manures and mineral fertilizers with appropriate ratios can significantly increase the root weight (Vijayaprabhakar *et al*., 2020).

Perusal of data presented in Table 2 indicated that biomass of carrot plants was significantly affected by integrated use of NPK and organic manures, during both the years. Amongst different treatments, significantly higher biomass per plant (169.33 and 166.67g) was recorded in plants amended with NPK + PM than other treatments during two years of cropping. It was followed by the combined use of NPK with GM (166.00 and 160.00 g) and SS (153.0 and 152.33 g). The lowest biomass weight (56.33 and 46.00g) was recorded in control treatment. The results revealed that the effectiveness of NPK supplied with PM and GM was remarkable, suggesting that these organic sources provided more nutrients to plants. These results are in the agreement with previously report literature (Singh *et al.*, 2020).

Considerable variations existed in carrot root yield due to combined application of inorganic and organic fertilizers, for two years study (Table 2). Application of NPK + PM recorded significantly higher root yield (56.83 and 56.67 t/ha) than all the treatments in both the years. It was followed by NPK + GM (55.15 and 51.75 t/ha) and NPK + SS (54.3 and 48.83 t/ha). Among integrated treatments, NPK + FYM produced statistically lowest yield (51.04 and 47.73 t/ ha), respectively during both the experimental years. Combination of organic and inorganic treatments recorded the higher yield to the tune of 170-200 and 198-269 per cent than the control treatment during both the years. The study exposed that amongst various combinations, NPK + PM surpassed rest of the treatments in enhancing root yield. The NPK incorporation with manures significantly increased the root yield, which might be attributed to the plant nutrient solubilization leading to increased macro and micronutrients uptake. The advantage of the use of mixture of organic and mineral fertilizers is it increase the efficiency of the fertilizers, minimized the nutrient loss and enhanced the yield of carrot (Vijayaprabhakar *et al*., 2020).

CONCLUSION

It is concluded that collective application of NPK and organic manures has significantly improved vegetative growth and yield of carrot, as compared to control. Integration of NPK and poultry manure (both at recommended levels) has out yielded all other combinations and control in almost all parameters. Hence, for getting more root yield of

carrot, poultry manure must be incorporated into the soil in addition to NPK. Moreover, use of goat manure along with NPK is also a viable combination for getting higher root yield of carrot.

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Original Research Paper

Effect of different growth media on biometric parameter of brinjal and chilli seedlings under shade net house

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ABSTRACT

The study was undertaken for two consecutive years (2017 and 2018) to evaluate the effect of different growth media on various growing parameters and incidence of insect pest on brinjal and chilli seedlings. Seedlings were grown in protray using six types of growing media. The highest germination percentage (71.11), plant height (11.05 cm), number of leaves (5.81) and percentage healthy seedlings (89.82) were observed with vermiculite + perlite + vermi-compost (1:1:2) during both the years in brinjal. Similarly in chilli, highest germination percentage (66.33), plant height (9.81 cm), number of leaves (5.62) and percentage healthy seedlings (87.61) were observed with vermiculite + perlite + vermi-compost (1:1:2). There was significantly low incidence of whitefly in brinjal (1.49 whitefly/leaf) and chilli (1.65 whitefly/ leaf) seedling grown in media with vermiculite + perlite + vermi-compost (1:1:2). Hence, vermiculite + perlite + vermi-compost (1:1:2) was found as optimum growth media for growing of chilli and brinjal seedlings. The findings of this study recommend the use of vermiculite + perlite + vermi-compost (1:1:2) as growth media for raising nursery by farmers as it had significant positively effect on plant growth parameters of seedlings that lead to increase production of chilli and brinjal.

Keywords: Brinjal, chilli, germination percentage, growth media and growth parameters

INTRODUCTION

Brinjal (*Solanum melongena* L.) and chilli (*Capsicum annum* L.) are the principal crops of Solanaceae family grown in sub-tropics and tropics. Both are popular vegetable crops of Punjab, India. In India area under brinjal and chilli cultivation is estimated at 758.0and 399 .0 thousand hectares with total production of 13154 & 4393 metric tonnes, (NHB, 2021) In Punjab, brinjal occupies 5.47-thousand-hectare area with production of 139.79 thousand tonnes and average yield of 225.72 q ha^{-1} during 2018-19. Similarly, chilli is grown over an area of 8.78 thousand hectare with production of 17.63 thousand tonnes and average yield of 20.09 q ha^{-1} during 2020-21 (Anonymous 2021). Both vegetables are first grown in nursery and thereafter transplanted in the fields. Therefore, growing of healthy nursery is prerequisite for a productive crop stand that depends on the growth medium in which these nursery plants are grown and which cause a serious challenge to brinjal and chilli growers.

Germination of the seed is pre-requisite for raising good nursery. All soils used as a growing media are not always perfect for the germination of seeds and subsequent growth of seedling. Growth medium used for potted plants plays an important role on various growth parameters like plant height, number of leaves, spike length, number of florets per spike, spike diameter and yield, *etc.* by providing proper nutrient supply, moisture and adequate aeration besides physical support to the growing plant (Bhardwaj *et al*., 2019). Growing media used for nursery production of brinjal and chilli contain a variety of organic and inorganic ingredients. Organic ingredients include peat moss, bark, coconut coir, rice hulls, *etc*. Inorganic components include perlite, pumice, vermiculite, sand, hydrogel, *etc*. Soils are generally unsatisfactory for producing plants in containers because of improper aeration, drainage and poor water holding capacity (Najar *et al*., 2015). The selection of growing media by farmers mainly depends on cost, their availability and plant requirements. Substrates used commonly in

nursery raising are peat, cocopeat, perlite, vermiculite, hydrogel, rockwool (Gruda *et al.,* 2016; Savvas and Gruda, 2018).Growing media is not only a place where seeds are sown and seedlings raised, but also a source and reservoir of plant nutrients (Dahanayake *et al.,* 2012).

A good growing media should be composed of mixtures that are tender enough for seeds to easily germinate, retain moisture, drain excess water and provide sufficient plant nutrients for seedling growth (Choudhary and Deena, 2020). Brinjal and chilli are attacked by various insect-pests, causing significant losses to brinjal and chilli farmers resulting in low productivity .These crops are attacked by number of insect pests right from nursery stage till harvesting (Salve *et al.,* 2020). According to Kumar *et al.,* (2019) jassid (*Amrasca biguttula biguttula)* and whitefly (*Bemisia tabaci*) were recorded as major pests in nursery crop of brinjal. The damage of 15-20% caused by jassid and whitefly on brinjal had also reported by (Gangwar *et al*., 2014; Singh, 2015; Borah *et al*., 2017). Subhashree *et al.,* (2018) reported aphid (*Aphis gossypii*) and whitefly (*Bemesia tabaci*) as major pests of nursery crop of chilli. There is also need to study the incidence of these insect pest on nursery plants of brinjal and chilli grown in different growing media. The present study was carried out to explore the most suitable growing media of sowing for raising chilli and brinjal seedlings in nursery .

MATERIALS AND METHODS

The present study was conducted to investigate the effect of different growing media on brinjal and chilli seedlings under shade net house during the years 2017 and 2018 at Dr. D R Bhumbla Regional Research Station, Ballowal Saunkhri, District SBS Nagar, Punjab. The experiment was conducted in CRBD with six treatments with four replications. Six growing media M_1 :[Vermiculite + Perlite + Vermi-compost (1:1:2); M_2 :Vermiculite + Perlite + Cocopeat (1:1:2) ; M_3:Sand + Soil + Farm yard manure (1:1:2); M_4 : Farm yard manure + Vermi-compost (1:1); M_5 :Farm yard manure and M_6 :Vermi-compost were used.

Brinjal seeds of PBH-3, were sown one in each plug/ cell into 98 cells of trays filled with the different growth media. Similarly, chilli seeds of variety Punjab Sindhuri were also sown as described for brinjal. Thereafter, growth and development parameters were measured using twenty-five (25) randomly tagged seedlings from each replication throughout the study. After 5 days of sowing, the number of normal seedlings germinated were counted and expressed in percentage. At the 30 DAS (days after sowing), the length of seedling was measured and the average length was calculated and expressed in centimeter. The seedling girth was measured using vernier caliper and mean girth was expressed in centimeter. The total number of leaves in the plants were counted and recorded. After 30 DAS the number of healthy seedlings were counted and expressed in percentage.

Three leaves from upper, middle and lower canopies of five seedlings selected randomly were collected and observed with the help of magnifying glass (10×) for the presence of insect pest. Mean population of the insects was expressed as number of insect/leaf/plant in each replication. All analyses of data sets were performed using the statistical analysis (Gomez and Gomez, 1984). Data on various growth parameters and insect pest was recorded.

RESULTS AND DISCUSSION

Germination: The germination percentage of both brinjal and chilli seeds were significantly affected by the growth medium (Table 1 and 2). The significantly higher germination percentage for brinjal was found to be 71.11% with Vermiculite + Perlite + Vermi-compost (1:1:2), while lowest germination (60 %) was recorded in Vermiculite + Perlite + Cocopeat (1:1:2).Other growth media germination percentage varied from 61-66%. Similarly for chilli, significantly higher germination was found to be 66.33% with Vermiculite + Perlite + Vermi-compost (1:1:2), while lowest germination (40.20 %) was recorded in Vermiculite + Perlite + Cocopeat (1:1:2) (Table 2). Low germination in Vermiculite + Perlite + Cocopeat (1:1:2) may be due to low water retention capacity and low nutrient availability (Meena *et al.,* 2017). Growing media greatly influences seed germination, seedling emergence and growth of seedlings in a nursery because these media serve reservoir of moisture and plant nutrients. These results are in line with Mahala and Sharma (2020) who reported that media containing Vermiculite + Perlite + Vermi-compost (1:1:2) resulted in highest germination (62.40%) while lowest germination (43.24 %) was observed in media containing Vermiculite + Perlite + Cocopeat (1:1:2) in tomato seedlings.

Seedling height: The seedling height was also influenced by different treatments in both brinjal and chilli. Growing media (Vermiculite + Perlite + Vermi-compost (1:1:2) had recorded the significantly higher values for seedling length (11.05 cm) than other treatments. The lowest seedling height (7.41 cm) was recorded with Vermiculite + Perlite + Cocopeat (1:1:2) (Table 1) with 30 days old seedlings. Data on effect of media on chilli seedling height depicted that significantly higher values (9.80 cm) was observed with Vermiculite + Perlite + Vermi-compost (1:1:2), while chilli seedling raised with Vermiculite + Perlite + Cocopeat (1:1:2) media showed lowest plant height (7.01cm). These results were in conformation with Mahala and Sharma (2020) who also reported media containing Vermiculite + Perlite + Vermi-compost (1:1:2) resulted in highest plant height (11.82 cm) in tomato seedling.

Seedling girth : The significantly higher seedling girth (0.17 cm and 0.15) was recorded with M_1 i.e. Vermiculite + Perlite + Vermi-compost (1:1:2) in brinjal and chilli, respectively, while the lowest values were recorded in M_5 in both the crops.

Number of leaves/seedlings: The number of leaves per seedling was also influenced by different media treatments in both brinjal and chilli. The number of leaves per seedling (5.81) was significantly higher in 30 days old seedlings grown in the Vermiculite + Perlite + Vermi-compost (1:1:2) and the lowest number of leaves (3.62) was found in Vermiculite + Perlite + Cocopeat (1:1:2) during pooled analysis in brinjal seedlings. For chilli seedlings, significantly higher number of leaves (5.62) was observed when chilli seedlings were raised with Vermiculite + Perlite + Vermi-compost (1:1:2) and lowest number of leaves (3.63) was observed when Vermiculite + Perlite + Cocopeat (1:1:2) was used as media for raising chilli nursery (Table 2). The possible reason was nutritional contribution of the media that produced maximum number of leaves.

Healthy seedling: The per cent healthy seedling was one of the prime growth parameters that was significantly variable among different growth media (Table 1). Per cent healthy seedling was highest in brinjal and chilli i.e. 89.82 and 87.61, respectively in media containing Vermiculite + Perlite + Vermi-compost (1:1:2), whereas the lowest percentage of healthy seedlings was 60.43 and 51.58 in brinjal and chilli, respectively grown in Vermiculite + Perlite + Cocopeat (1:1:2). This might be due to the variation of available nutrients in the selected growth media. Mahala and Sharma (2020) also reported that media containing Vermiculite + Perlite + Vermi-compost (1:1:2) resulted in maximum per cent healthy (83.39 %) seedling in tomato which is in line with our studies.

Insect pest incidence: Brinjal and chilli seedlings grown on different media were critically analyzed for the incidence of any insect pest (Table 3). There was significant difference in incidence of jassid (0.68-1.23 jassid/leaf) and whitefly (.49-3.20 whitefly/leaf) when brinjal seeds are raised in different growth media. Lowest incidence of whitefly (1.49 whitefly/leaf) and jassid (06.8 jassid /leaf) was recorded in Vermiculite + Perlite + Vermi-compost (1:1:2). Similarly in chilli seedlings there was significant difference in whitefly and aphid incidence among the treatments (Table 3). There was significant low incidence of whitefly (1.65 whitefly/leaf) and aphid (0.28 jassid/leaf) in Vermiculite + Perlite + Vermi-compost (1:1:2). Thus,

Table 1 : Effect of different growing media on biometric parameters of brinjal seedlings under shade net house condition (Pooled data)

Treatment	Germination (%)	Plant height (cm)	Seedling girth (cm)	Number of leaves/seedlings	Percentage of healthy seedling
M_1	71.11	11.05	0.17	5.81	89.82
M_2	60.06	7.41	0.11	3.62	60.43
M_3	66.92	9.77	0.14	5.11	83.42
M_4	64.70	9.33	0.14	4.09	78.01
M_5	61.75	8.02	0.10	5.05	65.22
M_6	63.43	9.67	0.11	4.01	70.87
CD (5%)	1.576	1.024	0.016	0.093	1.568

Table 2 : Effect of different growing media biometric parameters of chilli seedlings under shade net house (Pooled data)

Treatment	Germination (%)	Plant height (cm)	Seedling girth (cm)	Number of leaves/seedlings	Percentage of healthy seedling
M_1	66.33	9.80	0.15	5.62	87.61
M_2	40.20	7.01	0.12	3.63	51.58
M_3	63.58	8.41	0.12	5.01	81.87
M_4	60.01	8.18	0.13	4.84	76.41
M_5	50.84	7.65	0.10	4.11	63.99
M_6	58.35	7.87	0.10	4.81	69.57
CD (5%)	0.816	0.219	0.017	0.276	0.975

Table 3 : Effect of different growing media on insect pest incidence of brinjal and chilli seedlings under shade net house (Pooled data)

Treatment	Insect pest incidence			
	Brinjal		Chilli	
	Mean No. of whitefly/leaf	Mean No. of jassid/leaf	Mean No. of whitefly/leaf	Mean No. of aphids/leaf
M_1	1.49 (1.58)	0.68 (1.30)	1.65 (1.63)	0.28 (1.13)
M_2	3.20 (2.05)	1.18 (1.48)	3.22 (2.05)	0.95 (1.40)
M_3	1.73 (1.65)	0.72 (1.31)	1.57 (1.60)	0.43 (1.20)
M_4	1.87 (1.69)	0.77 (1.33)	1.68 (1.64)	0.48 (1.22)
M_5	3.25(2.06)	1.23 (1.49)	3.07 (2.02)	1.03 (1.43)
M_6	1.65(1.63)	0.67 (1.29)	1.68 (1.64)	0.38 (1.18)
CD (5%)	0.08	0.09	0.09	0.10

*Value in parenthesis is square root transformation

it can be concluded that significant difference in incidence of insect pest occurs on these seedlings that may be attributed to different growth factors. These findings are in agreement with Islam *et al.* (2017), who reported that infestation behaviour of the whiteflies could be affected by the quantity of plant released volatile organic compounds that depends on availability of nitrogen content in the plant. Above results are also in agreement with studies conducted by Mahala and Sharma (2020) who reported that there was effect of growing media on the insect pest incidence in tomato seedling.

The results of present studies showed growing media had a pronounced effect on plant growth parameters like germination per cent, plant height, number of leaves, percentage of healthy seedlings, insect pest incidence on brinjal and chilli seedlings. The overall evaluation indicated that the growing media containing Vermiculite + Perlite + Vermi-compost (1:1:2) proved the best growing media in both brinjal and chilli nursery plants. This result was parallel to the finding of Nissi (2018) who stated that vermiculite and perlite improve water holding capacity, permeability and airflow in the media thus improving germination, development and rooting system of plants. Combination of vermicompost, vermiculite and perlite showed a significant positive effect on germination, seedling growth owing to improving physical condition of the media and providing increasing nutritional availability. These results are akin to the findings of Meena *et al.*, 2017, where soil + vermicompost + vermiculite (1:1:1) provided highest performance of seedling growth of papaya. The vermicompost increased leaf area and biomass in various plants have

been reported by Yadav *et al*., (2012), which agree with findings of current study. Results of these studies are in line with Kumar *et al*. (2019) which depicted jassid and whitefly as the major sucking pests of brinjal both in nursery and field crop. Similarly these results are also in accordance with Saini *et al.* (2017), where chilli crop was attacked by whitefly and aphid in nursery leading to 10-15% incidence by these sucking pests.

CONCLUSION

Considering the above-mentioned results, it may be concluded that planting seeds of brinjal and chilli in growing media containing Vermiculite + Perlite + Vermi-compost in the ratio 1:1:2 resulted in maximum germination of healthy seedling, enhanced growth of subsequent seedling and lowest incidence of insect pest. The findings of this study recommend use of Vermiculite + Perlite + Vermi-compost (1:1:2) as growth media for raising chilli and brinjal nursery by farmers as it has significant positively effect on plant growth parameters.

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Original Research Paper

Effect of nano and macro iron sprays on growth, flowering, seed and oil yielding attributes in calendula (*Calendula officinalis* L.)

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ABSTRACT

The investigation was executed with nine treatments *viz*. nano forms of ferrous sulfide (7, 14, 21, 28 ppm) and macro ferrous sulphate (0.2, 0.4, 0.6, 0.8 per cent) along with control, and were applied as foliar sprays after 30 days of transplanting on two varieties of calendula namely Fiesta Gitana Mix' and 'Fiesta Yellow' during 2018 and 2019. The experiment was laid out in factorial randomized block design with three replications. Application of 0.8 % $FeSO_4$ recorded maximum number of branches (26.75), plant height (29.73 cm), plant spread (45.17 cm), number of leaves (22.63) and seed test weight (15.63 g) and number of flowers per plant (134.04). However, application of 0.2% macro $FeSO_4$ resulted in early bud appearance (50.50 days) and higher flower diameter (8.09 cm). 'Fiesta Gitana Mix' outperformed over 'Fiesta Yellow' for most of the vegetative and floral characters. The 'Fiesta Yellow' variety with oil content (13.97%) had an edge over 'Fiesta Gitana Mix'.

Keywords: Calendula, Ferrous sulphate, Flowering, Nano iron, Oil content and Seed yield.

INTRODUCTION

Calendula officinalis is a member of the family Asteraceae. It is cultivated in Eastern Europe, West Asia, Germany and USA. It is also known as pot marigold, calendula, ringer blume, *souci des jardins* in different countries (Sahingil, 2019). It is an economic plant for its beautiful flowers, herbal and cosmetic products. It is also used traditionally as culinary and medicinal herb. The petals are edible and can be used afresh in salads or dried and used in coloring cheese or as a replacement for saffron. The petals color varies from yellow to orange and has an aromatic scent (Saffari and Saffari, 2020). A yellow dye has been extracted from the flowers. Skin products from calendula are used to treat minor cuts, burns and skin irritations and other ailments. These various uses are attributed by constituents such as flavanol glycosides, triterpene oligoglycosides, saponins and sesquiterpene glucoside. Flower heads are sources of carotenoids which help for improved vision, normal growth and development and flavanoids which possess anti-viral and anti-cancer properties (Khalid and Teixeira da Silva, 2010). An increasing interest in calendula cultivation has been witnessed in recent years as an oil-bearing plant whose seeds were reported to contain unique poly unsaturated fatty acids which have the potential to be used in paint, coatings and pharmaceutical industries (Krol and Paszko, 2017).

Nano-fertilizers are currently a novel technology that allows for much more absorption by miniaturization of the particle size in nano scales. High absorbability and consumption both through the soil and the leaves are the characteristics of these types of fertilizers. The slow-releasing property of nano-fertilizers has a major contribution to their optimal use (Alamdari *et al*., 2021). This enables nano-particles (NPs) to boost the plant's metabolism. Application of nano-fertilizers promoted growth, development, antioxidant activity, stress tolerance and total phenol content (TPC) in many crops with lesser concentration.

Iron NPs due to their nano size as well as magnetic characteristics are considered as special nano-fertilizers. The bio-compatibility as well as interaction between plants and the Fe nano-particles had led to a great deal of attentions. The Fe nano-particles effect plants in two ways, lower concentrations of FeNPs had positive effects on the growth and physiology of crop

plants, whereas, high concentrations had toxic effects on plants. Fe nano-particles were reported with nutrient absorption promotion as well as photosynthetic efficiency enhancement. The use of nanotechnological inventions in calendula production having potential as landscaping, ornamental as well as medicinal plant can prove as beneficial research environmentally, economically and aesthetically. Therefore, the present investigation was planned to assess the impact of different concentrations of nano and macro forms of iron on growth, flowering, seed yield and oil content in *C. officinalis*.

MATERIALS AND METHODS

The experimental site was situated in the *Tarai* region of Uttarakhand, India at 29^0 N latitude and 79.3^0 E longitudes in the foot hills of the Himalaya at an altitude of 243.84 m above mean sea level. The soils of the experimental field were sandy loam having pH 6.68, organic carbon (0.60%), available N, P and K as 231.91, 18.34 and 135.97 $Kgha^{-1}$, respectively. Well rotten farmyard manure @ 5 kg/ m^2 was incorporated into soil at the time of bed preparation. Calendula seeds of two varieties namely "Fiesta Gitana Mix" and "Fiesta Yellow" were sown in well prepared nursery beds. Upon germination, 25-day-old seedlings were transplanted in the experimental field at a spacing of 60 cm × 30 cm. The experiment was conducted in factorial randomized block design with nine treatments replicated thrice. Five plants per treatment per replication were randomly selected for observations.

Nano and macro iron treatments

For nano-iron treatments, a stock solution of 28 ppm nano FeS was diluted with distilled water to make four different concentrations (7, 14, 21 and 28 ppm). For ferrous sulphate solution, different quantities (2, 4, 6 and 8g) of $FeSO_4$ salt were dissolved separately in 1000 ml of slaked lime water to prepare solutions of required concentrations. Nano-iron (iron sulfide) solutions of 7, 14, 21 and 28 ppm and ferrous sulphate solutions of 0.2, 0.4, 0.6 and 0.8 per cent concentrations were sprayed 30 days after transplanting. All other cultural conditions such as hoeing, weeding, irrigation, etc were kept uniform for all the treatments.

Oil extraction from seeds

The oil from seeds of calendula was extracted using solvent extraction method. Soxhlet apparatus was used for extraction using hexane as a solvent. The pooled data for both the years 2018 and 2019 were statistically analyzed using the software 'OPSTAT'(8).

RESULTS AND DISCUSSION

Data presented in Table 1 indicated that vegetative traits such as number of branches, plant height, plant spread and number of leaves significantly affected by treatments, varieties and their interaction.

Irrespective of the varieties, spray of $FeSO_4$ recorded significantly more number of branches (26.75) in T_9 than rest of the treatments, however, it was recorded minimum (14.50) in control (T_1). The variety Fiesta Yellow had maximum number of branches (20.07) over variety Fiesta Gitana Mix (18.45). Among the interaction, a greater number of branches (29.58) were recorded in V_1T_9 followed by V_2T_6 (25.17) which were statistically at par with other treatments. However, number of branches was recorded minimum (12.00) in variety Fiesta Gitana Mix sprayed with 21 ppm nano FeS (V_1T_2). Torabian *et al*. (2018) reported that increased growth in sunflower grown under saline condition by application of $FeSO_4$ both in normal and nano form which is due to increased leaf area, net CO_2 assimilation, sub-stomatal CO_2 concentration, chlorophyll content, etc. Likewise, Yuan *et al*. (2018) also reported that iron NPs promoted plant growth of *Capsicum annum* by increasing chloroplast numbers and grana stacking. In the present investigation, the lesser number of branches in nanoparticle treated plants against macro iron treatment might be due to their insufficient quantity as compared to macro forms.

Irrespective of varieties, the maximum plant height was recorded in T_9 (29.73 cm) which was at par with T_8 (29.42 cm) and T_7 (27.86 cm) but significantly higher than the rest of the treatments. However, minimum was recorded in T_2 (21.19 cm). The plant height was significantly higher in variety Fiesta Gitana Mix (26.18 cm) than variety Fiesta Yellow (23.63 cm). Among the interaction, maximum plant height (31.96 cm) was recorded in V_1T_9 combination followed by V_1T_8 (30.84), and V_1T_7 (30.34) and it was minimum (20.54 cm) in V_2T_2.Treatmnts T_9 (31.96 cm) and T_8 (28.00 cm) showed significant effect on plant height over nano iron and control in varieties Fiesta Gitana Mix and Fiesta Yellow, respectively. However, among

Table 1 : Effect of different concentrations of nano and macro forms of iron on vegetative characters in calendula varieties 'Fiesta Gitana Mix (V_1)' and 'Fiesta Yellow (V_2)'

Treatment	No. of branches			Plant height (cm)			Plant spread (cm)			Number of leaves		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Control)	12.75	16.25	14.50	23.09	20.88	21.98	40.71	40.04	40.38	20.08	17.83	18.96
T_2 (7 ppm nano FeS)	12.00	17.50	14.75	21.83	20.54	21.19	40.08	41.79	40.94	18.50	17.92	18.21
T_3 (14 ppm nano FeS)	13.25	17.42	15.33	23.21	20.96	22.09	39.46	38.92	39.19	17.75	18.33	18.04
T_4 (21 ppm nano FeS)	14.25	15.42	14.83	22.17	22.17	22.17	40.33	41.38	40.86	18.50	19.25	18.88
T_5 (28 ppm nano FeS)	13.75	17.00	15.38	24.75	21.50	23.13	41.38	37.00	39.19	22.16	17.25	19.71
T_6 (0.2% $FeSO_4$)	21.92	25.17	23.54	27.46	25.71	26.59	41.75	44.67	43.21	26.92	17.33	22.13
T_7 (0.4% $FeSO_4$)	23.83	24.83	24.33	30.34	25.37	27.86	43.13	42.83	42.98	23.75	19.58	21.67
T_8 (0.6% $FeSO_4$)	24.75	23.17	23.96	30.84	28.00	29.42	44.75	43.79	44.27	26.83	19.00	22.92
T_9 (0.8% $FeSO_4$)	29.58	23.92	26.75	31.96	27.50	29.73	45.38	44.96	45.17	25.42	19.83	22.63
Mean	18.45	20.07		26.18	23.63		41.88	41.71		22.21	18.48	

Factor	C.D. (5%)	SEm	C.D. (5%)	SEm	C.D. (5%)	SEm	C.D. (5%)	SEm
Variety (V)	0.876	0.611	0.931	0.323	0.67	0.48	1.359	0.471
Treatments (T)	3.738	1.295	1.976	0.684	2.939	1.018	2.882	0.999
V×T	2.015	1.832	1.0436	0.968	1.612	1.440	4.076	1.412

nano iron treatments, T_5 (24.75cm) and T_4 (22.17 cm) had maximum plant height in variety Fiesta Gitana Mix (V_1) and Fiesta Yellow (V_2), respectively. Both NPs treatments were more effective than control but less effective as compared to iron in normal form. The effect of nano iron might be due to increased chlorophyll content which increased photosynthesis, in turn, growth of plants (Ghafari and Razmjoo, 2015). Askary *et al.* (2017) reported increased growth, photosynthetic pigments and total protein contents in peppermint with application of FeO_3 (30µM NPs). Yuan *et al*. (2018) observed low concentration of iron NPs promoted plant growth due to increased chloroplast, number of grana stacking and regulation of vascular bundles. Increase in plant height in Cress was observed by Salarpour *et al*. (2013) upon applying 5g nano iron chelate + foliar spray of iron. Enhanced plant height due foliar spray of iron NPs has been reported (Elfeky *et al.,* 2013).

Irrespective of the varieties, maximum plant spread (45.17cm) was recorded in T_9 which was statistically at par with T_8 (44.27cm), T_6 (43.21cm) and T_7 (42.98cm) but significantly higher than rest of the treatments. However, minimum plant spread (39.19 cm) was recorded in both, T_3 and T_5. Maximum spread of plants (41.89 cm) was recorded in variety Fiesta Gitana Mix and minimum in variety Fiesta Yellow (41.71cm). Among the interaction plants' spread was maximum (45.38 cm) in V_1T_9 followed by V_2T_9 (44.96 cm), V_2T_8 (44.75cm) and V_2T_6 (44.67 cm). However, least plant spread (37.00 cm) was recorded in V_2T_5. Among nanoparticles treatments, T_5 in variety Fiesta Gitana Mix and T_2-in variety Fiesta Yellow showed higher plant spread over control. Yuan *et al*. (2018) reported improved overall plant growth in capsicum as a result of iron nanoparticles application was due to enhanced chloroplast, grana stacking as well as development of vascular bundles. Pirzad and Shokrani (2012) reported improved plants growth in calendula due to application of iron NPs (1.5 l/ha). In the present investigation, positive influence of NPs for plant spread over control might be due to reduced nutrient loss as reported by Hu *et al*. (2017) in *Citrus maxima* plants.

Irrespective of the varieties, maximum number of leaves (22.92) was recorded in T_8 which was statistically at par with T_6 (22.13) T_7 (21.67) and T_9 (22.63) but significantly higher than rest of the treatments. However, minimum number of leaves (18.04) was recorded in T_3. The variety Fiesta Gitana Mix recorded maximum number of leaves (22.21) than variety Fiesta Yellow (18.48). Among the interaction, more number of leaves (26.92) was recorded in V_1T_6 combination followed by V_1T_8 (26.83) and V_1T_9 (25.42) whereas least number of leaves (17.25) was recorded in V_2T_5. However, higher dose of nanoparticle resulted in more number of leaves. Praveen *et al*. (2018) reported improved growth of mustard plants treated with NPs (Fe_3O_4) mainly due to enhanced availability of iron.

Calendula plants when sprayed with different treatments of iron showed significant response for days to earlier bud appearance, days to bloom, flower diameter and number of flowers per plant (Table 2). The effect of treatments was significant, whereas, varieties and treatments-varieties interactions on days to early bud appearance were non-significant. Among the treatments, irrespective of varieties, maximum days to bud appearance (52.06 days) was recorded in control (T_1) which was statistically at par with by T_2 (50.92 days) but significantly higher than rest of the treatments. However, minimum number of days to bud appearance (48.63 days) was recorded in T_9. Tayade *et al*. (2018) reported early initiation of spike in tuberose with 0.4% $FeSO_4$.

The days to bloom was significantly influenced by varieties, however, treatment and variety-treatment interaction was non-significant. Significantly more days to bloom (65.00 days) was recorded in variety Fiesta Gitana Mix than variety Fiesta Yellow (64.59 days). Tayade *et al*. (2018) reported early opening of first floret in tuberose with 0.4% $FeSO_4$. Goshwami *et al*. (2021) reported that application of 10 ppm of Gold-nanoparticle was found best for number of flowers, flower diameter , flower weight , minimum days to flower bud initiation and flowering duration.

Irrespective of varieties, maximum diameter of flower (8.09 cm) was recorded in T_6 which statistically par with T_3 (7.92 cm) but significantly higher than rest of the treatments, whereas, minimum diameter of flower (7.43 cm) was recorded in T_9. Barring the treatments, significantly higher flower diameter (7.94 cm) was recorded in variety Fiesta Gitana Mix than variety Fiesta Yellow (7.52 cm). Among interaction maximum diameter of flower (8.33 cm) was recorded for treatment V_1T_6 andV_1T_7 and were statistically at par with V_1T_3 (8.28 cm) V_1T_5 (8.11 cm) but

Table 2 : Effect of different concentrations of nano and macro forms of iron on flowering characters in calendula varieties 'Fiesta Gitana Mix (V_1)' and 'Fiesta Yellow (V_2)'

Treatment	Days to bud appearance			Days to Bloom			Flower diameter (cm)			No. of flowers per plant		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Control)	53.25	50.88	52.06	67.67	62.33	65.00	7.80	7.37	7.58	90.00	101.75	95.88
T_2 (7 ppm nano FeS)	50.92	50.92	50.92	65.67	62.33	64.00	7.82	7.59	7.70	95.13	108.17	101.65
T_3 (14 ppm nano FeS)	51.17	49.50	50.33	67.33	64.33	65.83	8.28	7.57	7.92	116.88	95.75	106.31
T_4 (21 ppm nano FeS)	50.13	50.50	50.31	68.00	65.00	66.50	7.53	7.76	7.65	102.88	115.00	108.94
T_5 (28 ppm nano FeS)	49.63	50.50	50.06	66.00	64.67	65.33	8.11	7.56	7.83	106.02	127.00	116.51
T_6 (0.2% $FeSO_4$)	49.33	51.67	50.50	66.33	66.33	66.33	8.33	7.85	8.09	102.88	111.75	107.31
T_7 (0.4% $FeSO_4$)	50.50	49.46	49.98	67.33	67.00	67.17	8.33	7.14	7.74	91.63	90.58	91.11
T_8 (0.6% $FeSO_4$)	51.08	49.25	50.17	65.33	66.33	65.83	8.03	7.23	7.63	115.00	111.42	113.21
T_9 (0.8% $FeSO_4$)	48.50	48.75	48.63	65.00	63.00	64.00	7.27	7.60	7.43	131.92	136.17	134.04
Mean	50.50	50.16	-	66.52	64.59	-	7.94	7.52	-	105.81	110.84	-
Factor	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm	C.D. (5%)		SEm
Variety (V)	NS		0.538	1.891		0.655	0.093		0.032	NS		2.378
Treatments (T)	1.553		0.254	NS		1.390	0.197		0.068	14.559		5.044
V×T	NS		0.761	NS		1.965	0.279		0.097	NS		7.133

Table 3 : Effect of different concentrations of nano and macro forms of iron on flower characters in calendula varieties 'Fiesta Gitana Mix (V_1)' and 'Fiesta Yellow (V_2)'

Treatment	Flower weight (g)			Duration of flowering (days)			Flower yield per plant (g)			Flower yield (tons/ha)		
	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Control)	3.78	2.70	3.24	59.42	65.00	62.21	328.69	275.19	301.94	18.26	15.29	16.77
T_2 (7 ppm nano FeS)	4.10	3.16	3.63	61.42	64.67	63.04	390.32	395.25	392.78	21.68	21.96	21.82
T_3 (14 ppm nano FeS)	3.76	3.60	3.68	59.92	62.83	61.38	485.23	349.55	417.39	26.96	19.42	23.19
T_4 (21 ppm nano FeS)	4.12	2.37	3.25	58.92	62.17	60.54	423.30	272.95	348.13	23.52	15.16	19.34
T_5 (28 ppm nano FeS)	4.70	2.50	3.60	60.83	62.33	61.58	498.45	336.34	417.39	27.69	18.69	23.19
T_6 (0.2% $FeSO_4$)	4.27	3.01	3.64	60.75	60.75	60.75	486.54	361.46	424.00	27.03	20.08	23.56
T_7 (0.4% $FeSO_4$)	4.52	3.26	3.89	60.17	60.00	60.08	455.07	295.08	375.07	25.28	16.39	20.84
T_8 (0.6% $FeSO_4$)	3.56	3.22	3.39	61.92	60.75	61.33	422.25	368.80	395.52	23.46	20.49	21.97
T_9 (0.8% $FeSO_4$)	2.64	2.88	2.76	62.00	64.08	63.04	362.40	388.95	375.68	20.13	21.61	20.87
Mean	3.94	2.97	-	60.59	62.51	-	428.03	338.17	-	23.78	18.79	-

Factor	C.D. (5%)	SEm	C.D. (5%)	SEm	C.D. (5%)	SEm	C.D. (5%)	SEm
Variety (V)	0.236	0.082	1.907	0.661	24.607	8.525	1.367	0.473
Treatments (T)	0.5	0.173	NS	1.402	52.2	18.085	2.899	1.004
V×T	0.707	0.245	NS	1.982	73.822	25.576	4.1	1.42

significantly higher than rest of the interactions. Pirzad and Shokrani (2012) reported that iron NPs @ 1.5 l/ha increased capitulate diameter and in calendula with 0.4% of $FeSO_4$ (Tayade *et al*., 2018).

A perusal of data presented in Table 2 indicated the significant effect of treatments and non-significant effect of varieties as well as treatment-variety interaction on number of flowers. Irrespective of varieties, maximum number of flowers (134.04) was recorded in T_9 which was significantly higher than rest of the treatments, whereas, minimum number of flower (91.11) recorded in T_7. Enhancement in number of flowers might have attributed by increased leaf chlorophyll content, increased enzymatic activity in leaves, etc. as influenced by iron NPs in Durum wheat (Ghafari and Razmjoo, 2015) and in saffron plants (Farahani *et al*., 2015).

The treatments, varieties and their interaction had significantly influenced the average flower weight, duration of flowering, flower yield per plant and flower yield (Table 3). Irrespective of varieties, average flower weight was recorded maximum (3.89 g) in T_7 which was statistically at par with T_3 (3.68 g), T_6 (3.64 g), T_2 (3.63 g) T_5 (3.60 g) and T_8 (3.39 g) but significantly higher than control (T_1). However, it was recorded minimum (2.76 g) in T_9. The variety Fiesta Gitana Mix had significantly higher average flower weight (3.94 g) than variety Fiesta Yellow (2.97 g). Among interaction, maximum individual flower weight (4.70 g) was recorded in V_1T_5 which was at par with V_1T_7 (4.52 g), V_1T_6 (4.27 g) and V_1T_2 (4.10 g), whereas it was recorded minimum (2.37 g) in V_2T_4. Bakhtiari *et al*. (2015) reported enhanced spike weight of wheat due to application of nano iron oxide (0.04%). Higher concentration of NPs (1000ppm) enhanced plant growth in *Hydrangea paniculata* (Karunakaran *et al*., 2017).

Non-significant effect of treatments, treatment-variety interaction but significant effect of varieties on duration of flowering was observed. The variety Fiesta Yellow had significantly higher duration of flowering (62.51 days) than variety Fiesta Gitana Mix (60.59 days).

Irrespective of varieties, maximum flower yield per plant (424g) was recorded in T_6 which was at par with all the treatments but significantly higher than control whereas minimum flower yield per plant (301.94 g) was recorded in control (T_1). Barring treatments, variety Fiesta Gitana Mix (V_1) had significantly higher flower yield per plant (428.03 g) over variety Fiesta Yellow (V_2) (338.17 g). Maximum flower yield per plant among interactions (498.45 g) was recorded in V_1T_5 which was statistically at par with V_1T_6 (486.54 g) V_1T_3 (485.23 g) V_1T_7 (455.07 g) but significantly higher than remaining treatments and control in both the varieties. However, the minimum flower yield per plant (272.95 g) was recorded in V_2T_4. These significant results might be due to reduced nutrient loss and strong adsorption ability as reported by Hu *et al*. (2017) and increased ability of plants to overcome stressed conditions (Elfeky *et al*., 2013).

Irrespective of varieties, estimated flower yield was recorded highest in T_6 (23.56 t/ha) which was at par with all the treatments except control (T_1) and T_4 (19.34 t/ha) with control (T_1) being recorded for least flower yield (16.77 t/ha). The variety Fiesta Gitana Mix had significantly higher estimated flower yield (23.78 t/ha) than variety Fiesta Yellow (18.79 t/ha). Among interaction estimated flower yield was recorded maximum (27.69 t/ha) in V_1T_5 which was statistically at par with V_1T_6 (27.03 t/ha), V_1T_3 (26.96 t/ha) and V_1T_7 (25.28 t/ha) but significantly higher than rest of the treatments. However, it was found minimum (15.16 t/ha) in V_2T_4.This might be due to increased nutrients uptake and enhanced enzymatic activities in peppermint (Askary *et al*., 2017) and increase in biomass production with iron application (Torabian *et al*.,2018), where, foliar application of $FeSO_4$ in nano and normal form increased leaf area, shoot dry weight, net carbon dioxide (CO_2) assimilation rate, sub-stomatal CO_2 concentration, chlorophyll content, iron (Fe) content and decreased sodium (Na) content in leaves of sunflower.

The data on test weight of seeds showed significant effect of treatments, varieties and their interactions (Table 4). Irrespective of varieties, test weight of seeds was recorded highest in T_9 (15.63 g) which was statistically at par with treatments T_6 (15.25 g), T_7 (15.22 g) and T_4 (14.90 g) but significantly higher than rest of the treatments. However, it recorded minimum in control (T_1) (12.84 g). Barring treatments, variety Fiesta Gitana Mix (V_1) had significantly higher test weight of seeds (14.65 g) than variety Fiesta Yellow (V_2) (14.11 g). Among interaction, maximum test weight of seeds (16.10 g) was recorded in V_1T_8, which was at par with V_1T_9 (16.10 g), V_1T_6 (15.72 g), V_2T_7 (15.69 g), V_2T_5 (15.67 g) but significantly higher than

Table 4 : Effect of different concentrations of nano and macro forms of iron on seed yield and oil content in calendula varieties 'Fiesta Gitana Mix (V_1)' and 'Fiesta Yellow (V_2)'

Treatment	Test weight of seeds (g)			Oil content in seeds (%)		
	V_1	V_2	Mean	V_1	V_2	Mean
T_1 (Control)	13.04	12.64	12.84	11.75	12.84	12.30
T_2 (7 ppm nano FeS)	13.20	12.82	13.01	12.67	13.74	13.20
T_3 (14 ppm nano FeS)	13.45	13.93	13.69	12.37	14.34	13.35
T_4 (21 ppm nano FeS)	15.40	13.15	14.27	12.43	15.44	13.93
T_5 (28 ppm nanoFeS)	14.14	15.67	14.90	14.69	15.03	14.86
T_6 (0.2% $FeSO_4$)	15.72	14.78	15.25	12.03	12.85	12.44
T_7 (0.4% $FeSO_4$)	14.75	15.69	15.22	13.49	14.28	13.88
T_8 (0.6% $FeSO_4$)	16.10	13.12	14.61	12.39	13.91	13.15
T_9 (0.8% $FeSO_4$)	16.07	15.20	15.63	13.53	13.27	13.40
Mean	14.65	14.11	-	12.81	13.97	-

Factor	C.D. (5%)	SEm	C.D. (5%)	SEm
Variety (V)	0.322	0.111	0.496	0.174
Treatments (T)	0.682	0.236	1.051	0.369
V×T	0.965	0.334	NS	0.522

rest of the treatments combinations and was minimum (12.64) in control. Increased test weight of seeds was observed with increasing concentration of nano iron particles. It may be opined that enhanced test weight of seeds may be attributed by more accumulation of iron in seeds (Rawat, 2017). Ghafari and Razmjoo (2015) reported increased 1,000 grain-weight due to application of nano iron in wheat. The increased oil content due to nano iron application in calendula (Amuamuha *et al*., 2012) and in chamomile (Elfeky *et al*., 2013) observed earlier.

CONCLUSION

The oil content of seeds was significantly affected by variety and treatment but non-significantly affected by variety-treatment interaction. Irrespective of varieties, oil content of seeds was recorded highest in T_5 (14.86%) and it was at par with T_4(13.93%) and T_7 (13.88%) but significantly higher than rest of the treatments. However, minimum oil content (12.30%) was obtained in control (T_1).The variety Fiesta Yellow (V_2) had significantly higher oil content of seeds (13.97%) than variety Fiesta Gitana Mix (V_1) (12.81%). It is apparent that higher seed oil content of calendula was obtained from nano iron treatments with lesser concentrations. The treatment of 21 ppm nano FeS with variety Fiesta Yellow had performed better for seed oil content and was significantly higher than result obtained with normal or macro iron among both varieties. Also, variety Fiesta Yellow had recorded significant result though all other flowering attributes were lesser than variety Fiesta Gitana Mix (V_1) and can be considered with perspective of seed oil of calendula.

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Original Research Paper

Optimization of nitrogen application and planting geometry for production of cut chrysanthemums (*Chrysanthemum morifolium* Ramat.)

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ABSTRACT

Nutrition and planting geometry are the two key factors affecting the production and quality of cut stems in chrysanthemum. The present investigation was undertaken to standardize the nitrogen nutrition and planting geometry for chrysanthemum var. "Yellow Star" cultivated for cut flowers. The data revealed the proportionate increase in plant height, chlorophyll content, days to bud appearance and days to 50% inflorescence anthesis and length of cut stem with increase in nitrogen dose and row spacing. However, flower diameter, number of flowers per stem, cut stem diameter, vase life, and water absorbed by cut flower decreased proportionately with increase in nitrogen dose and row spacing. Application of N@100 Kg ha^{-1} to chrysanthemum planted at 20x10 cm spacing produced cut stems of acceptable length, more number of flowers of bigger size and optimum postharvest longevity. The amount of nitrogen can be reduced to 1/3rd to grow cut chrysanthemums planted at twice the row spacing for longer cut stems of appreciable vase life.

Keywords: Chrysanthemum, fertilization, nitrogen, planting geometry, Yellow Star

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.), commonly known as 'pot mums' belonging to the family 'Asteraceae' ranks third in world cut flower trade and has retained first position in China and Japan, being its primary centers of origin (Datta and Janakiram 2015). In India, chrysanthemum is cultivated in 28.32 thousand ha with an average annual production of 537.56 thousand MT (loose flowers) and 18.52 lakh (cut stems) (Anonymous 2022). The production of quality cut stems of chrysanthemum depends on several cultural practices, the most influential being the nitrogen (N) nutrition and availability of optimum space for the plants to manifest its vigorous vegetative and reproductive growth stages.

Chrysanthemum, being a heavy feeder requires N application in splits for the first seven weeks of its vegetative growth, that is essential for adequate accumulation of N in branches and leaves for utilization at later stage during reproductive phase (Crater, 1992). The analysis of mature leaf samples revealed 4.6-6.0% N, which is considerably less then accounted during the active growth period (Muniz *et al.,* 2009; Stern *et al.,* 2008). Most of the ornamental plants utilize either or both $N\text{-}NH_4^+$and $N\text{-}NO_3^-$ depending upon their stage of growth and development (Bernstein *et al.,* 2005). The quality of chrysanthemum cut stems can be influenced by the $N\text{-}NH_4^+/N\text{-}NO_3^-$ratio that affect postharvest behaviour of cut stems (Ramos *et al.,* 2013). The availability of optimum space for proper growth and development of chrysanthemum is a key factor determining the quality and productivity of cut stems. An ideal planting geometry influence several factors such as the density of plants per unit area, light interception within the plant canopy, resource utilization, ease in performing various cultural operations, optimum ground to canopy ratio, suppression of weed growth and most important being the productivity of the crop.Optimum utilization of resources is the need of the hour to safeguard and effectively utilize the available resources for successful production of high value and low volume floriculture crops, in particular chrysanthemum.

However, information regarding N-nutrition and planting geometry in cultivation of cut chrysanthemum needs to be determined under the subtropical climatic conditions of India. The study is deemed as significant for the small and marginal flower growers, to yield maximum productivity of quality cut stems from limited land-holdings. Therefore, the present need-based investigation was undertaken to standardize the N nutrition and optimum planting geometry for cultivation of chrysanthemum var. "Yellow Star" for cut stems under subtropical conditions of North India.

MATERIALS AND METHODS

The experimental was conducted at the Research Farm (33°55' N latitude; 75°54' E latitude; 247m above msl receiving 700 mm annual rainfall), Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana, during the year 2018. The relative humidity ranged between 63.0-76.0%. The mean minimum evaporation during the period of crop growth was recorded during November (2.1 mm) and maximum during July (129.9 mm). The soil texture was classified as sandy loam with pH 7.75, 7.79 and 7.82 recorded from 15 cm, 30 cm and 45 cm soil depth respectively.

The chrysanthemum variety "Yellow Star" was selected for the study as it is popular and commercially cultivated by flower growers for yielding cut flowers and for exhibition purpose. The flowers are yellow, with compact decorative type of inflorescence. Healthy disease free rooted cuttings of uniform height (3 inches) and age (25 days old) with well developed root system were planted during first week of August.

The treatments for planting geometry were designed at 3 spacing levels: S_1 (10×10 cm), S_2 (15×10 cm) and S_3 (20×10 cm) accommodating 100, 66 and 50 plants per square meter area respectively The treatments for N-doses comprised 4 differential applications: N_1 control (0 kg N ha^{-1}), N_2 reduced fertilizer (100 Kg ha^{-1}), N_3 conventional fertilizer (200 Kg ha^{-1}), N_4 excessive fertilizer (300 Kg ha^{-1}). The experiment comprised of 12 treatments executed in factorial randomized block design (FRBD), replicated thrice. The straight fertilizers *viz*. Urea, Single Super Phosphate (SSP) and Muriate of Potash (MOP) were taken as the sources of N, P_2O_5 and K_2O, respectively. Entire dose of P and K were applied each @ 200 Kg ha^{-1} as basal dose and graded levels of N were applied in two splits doses. Two splits doses one 30 days after transplanting and other 45 days after transplanting.

Various growth characteristics such as plant height (measured at 30, 60 and 90 days after transplanting), leaf area and total chlorophyll content were recorded. Total chlorophyll content of leaves was determined using method purposed by Witham *et al.* (1971):

$$\text{Total chlorophyll (mg/g)} = \frac{20.2\,(A_{645}) + 8.02\,(A_{663})\ \text{Final volume of DMSO}}{1000 \times \text{weight of tissue}}$$

The floral characters such as days to flower bud appearance, days to 50 % flowering, flower diameter, number of flowers per stem, cut stem diameter and length of the cut stem were recorded and the average values were computed for data analysis. Data was subjected to statistical analysis by SPSS v. 22 (IBM) software.

RESULTS AND DISCUSSION

Plant height, leaf area and total chlorophyll content

The mean plant height recorded at 30, 60 and 90 DAP with respect to differential application of N-levels showed progressive increase with subsequent application of N, compared to control (Table 1). The mean plant height was recorded least in control, however, the percent increment in plant height with increasing N-levels was more pronounced in plants during first 30 DAP. The highest percent increment (40.6%) of plant growth during first 30 DAP can be attributed due to ample availability of space and sunlight for the plants to grow that were supplemented with higher doses of N-levels. Nitrogen is considered as an important factor for building plant biomass through photosynthesis and subsequent translocation of carbohydrates for vegetative growth (Evans and Clarke, 2019).The subsequent percent increase in plant height decreased relatively (29.1% and 22.4%) at 60 and 90 DAP respectively. The availability of space and competition for air and sunlight tend to become a limiting factor, resulting in decrease in plant growth during later period (Woodson and Boodley 1983). The N requirement of Chrysanthemum is highest during first 7 weeks after transplanting (Fernandes *et al.,* 2012), and N uptake thereafter tend to decrease (Yoon *et al.,* 2000). The further N requirement is pooled mostly from the accumulated nitrate in stems and the petioles (MacDonald *et al.,* 2013). Conversely, the mean plant height decreased progressively in the plants

planted at narrower to wider spacing. However, the percent decrease in plant height was more pronounced at 30 DAP as compared to observations recorded at later monthly quarters. The plants planted at narrow spacing tend to compete for sunlight, as a consequence began to outgrowin length and appear taller compared to the plants planted at relatively wider spacing (Lavhaji, 2007).

The total chlorophyll content measured from the mature leaf tissue showed a significant 29.5% increment at the highest N-level (N4) compared to control. Similarly plants grown at wider spacing recorded 13.0% increase in chlorophyll content compared to plants grown at narrow spacing. Nitrogen, is an essential element for synthesis of amino acids and proteins, besides structurally important component of chlorophyll, and is considered essential for transportation of metabolites for synthesis of chlorophyll (Tucker, 2004).

The leaf area per plant was significantly influenced by the application of different N-levels and varying plant spacing (Table 2). The maximum leaf area/plant (1435.37 cm^2) was recorded in plants supplemented with N2 fertilizer treatment, and the minimum (992.67 cm^2) leaf area was measured in control. Further, planting distance also exhibited a significant effect on leaf area per plant. The plants planted at S3 spacing showed mean maximum leaf area (1266.80 cm^2) while the minimum leaf area (1172.69cm^2) was observed in plants planted at S1 spacing, irrespective of the N-levels. However, the interaction between N-level and spacing revealed non-significant differences for leaf area.

Flowering characteristics

Chrysanthemum plants delayed by 2.3 days to bud appearance with subsequent increase in N-levels (Table 3). However, the mean days taken to bud emergence at highest N-level was found insignificant compared to control plants. Plants planted at twice the row spacing showed 3.0 % delay in days to bud appearance compared to plants that were planted at narrower spacing. With the onset of short days, the accumulated leaf N is remobilized to developing buds to show color (Macz *et al.,* 2007). It has been proposed that N is not a decisive factor in initiation and development of floral primordia, but may alter (delay) the timing of its emergence (Withrow, 1945). Nitrogen affect the reproductive development of photosensitive short day plants (SDPs) that initiate buds with the onset of SDs, however, the split applications of N may slightly prolong the vegetative phase with the continuous synthesis and availability of accumulated photosynthates in the plant tissues which is utilized for flower bud growth and ultimately initiation of flowering.

With subsequent increase in application of N-levels, the mean number of days taken to 50% flowering were found delayed by one week at highest N-level, but was found insignificant compared to control (Table 3). However, plants planted at narrower spacing showed earliness in days to 50% flowering compared to plants at wider spacing. The application of higher N-level during early period of onset of SDs and cool nights delayed flower bud initiation, and subsequent stages of inflorescence anthesis indicating the potential affect of exogenous N in timing of transition of vegetative to reproductive phase rather than inhibiting the onset of flowering. The plants planted at wider spacing also exhibited delayed flowering due to less competition for space, sunlight, water and nutrients in soil, that aid in prolonging the vegetative phase and delayed flowering (Nagaraja, 2013).

The flower diameter showed 19.1% decrement at highest N-level, however, differed significantly with the mean diameter of flower that was recorded least in plants under control treatment. Chrysanthemum plants planted at twice the row spacing showed 9.23% increment in diameter of the flower compared to the plants that were planted at narrower spacing. The results present a contradictory observation to the synergistic effect of increasing N-level on flower diameter. The plants devoid of N dose (control) measured least flower diameter which was in accordance with the findings of Nell *et al.* (1989) and Adams *et al.* (1970) who reported reduction in flower diameter in chrysanthemum with lower N-levels.

The average number of flowers per stem recorded a significant decrease (44.7%) in the plants subjected to highest N-level. The control plots showed least number of flowers per stem which were found at par at highest N-level. However, the plants planted at wider spacing showed a significant 30.0% increment in number of flowers per stem compared to the plants planted at narrower spacing. The higher N doses likely induce succulence in plants with weak stems, causing reduction in flower

number. The results are in accordance with observations made by Vijayakumar *et al.* (1988), recording greater number of China aster flowers at lower (300 kg N ha^{-1}) N doses.

The diameter of cut stem recorded 22.3% decrement at higher N level, that differed significantly compared to control measured with least diameter of the cut stem (Table 4). Plants raised at wider spacing showed 11.57% increase in cut stem diameter, however, the increment was found non-significant compared to the plants grown at narrower spacing. The N application increased the diameter of cut stems that tend to become heavily lignified resulting in higher girth of stems (Withrow, 1945).

Post-harvest characteristics

The mean length of cut stem was measured least in plants under control (no N application). The stem length showed 7.58% increment in plants at highest N-level and was found statistically significant compared to stem lengths recorded in plants under control treatment. However, the mean length of stems showed a significant reduction (6.99%) in the plants that were grown at wider spacing. The higher N-levels resulted in more cell elongation and differentiation of the vascular tissues that caused increase in length of cut stems.

The cut stems harvested from plots applied with N2-fertilizer dose reported mean 45.6% increase in vase life compared to the control pot recording mean minimum vase life (14.8 days) (Fig1). However, the cut stems taken from plants grown at different spacing revealed improvement (15.5%) in mean vase life (Fig 2). The higher N increased the conductance that is a determining factor in enhancing the longevity of the cut stems (Roude *et al.,* 1991). Higher conductance resulted in damaging of the root system thereby limiting the water uptake. Adequate availability of N during growth period maintains the level of carbohydrates that determines the post harvest longevity of cut stems (Drüge, 2000). However, excess N is detrimental for the post harvest longevity of cut stems due to accumulation of excess salts and production of endogenous ethylene (Roberts *et al.,* 1984) that reduced the vase life of cut stems.

Total water absorbed by cut stem showed 34.6% decrement with subsequent additions of N. The cut

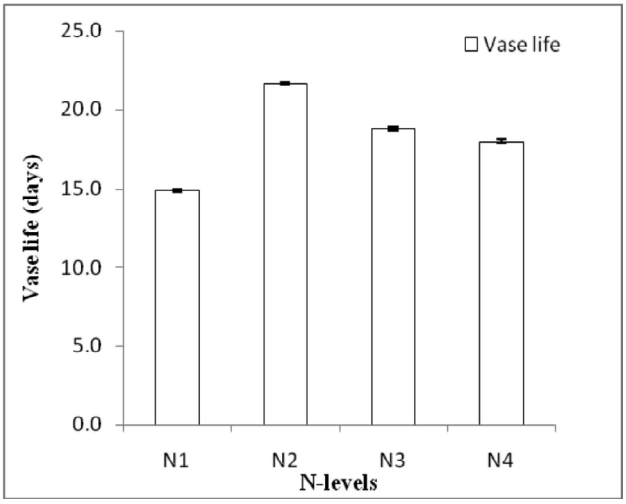

Fig. 1 : Response of N nutrition on vase life of cut stems

*error bars represent standard error (SE±0.071)

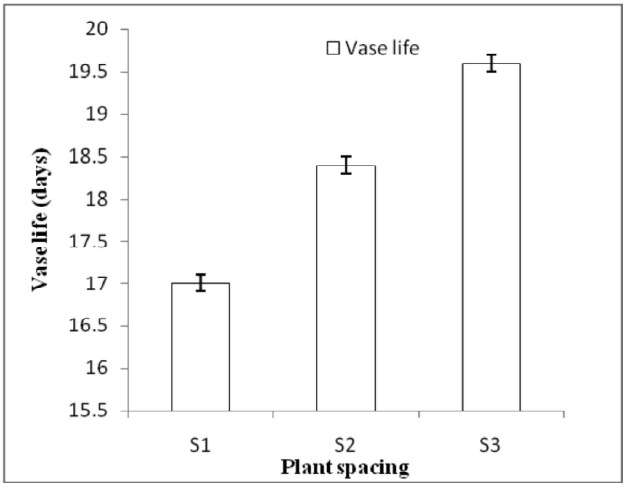

Fig. 2 : Response of plant spacing on vase life of cut stems

*error bars represent standard error (SE±0.1)

stems harvested from plants devoid of N showed least uptake of water, that was less (18.2%) than the water absorbed by the cut stems at highest N nutrition. The interaction effect of N and plant spacing enhanced the water absorption, the highest water absorption was recorded in cut stems harvested from chrysanthemum plants raised at wider spacing. The water absorbed by the cut stems is proportionate to their longevity. However, as stated above, the water uptake by cut stems decreased due to build up of excess salts with subsequent higher applications of N, supported from the findings of Nell *et al.* (1989); proposed to terminate N fertilization in chrysanthemum 3 weeks prior to flowering to reduce the conductance and increase the longevity of cut stems.

Table 1 : Response of varying N-levels and plant spacing on vegetative characters of chrysanthemum

N- level	Plant height (cm) 30 DAT				Plant height (cm) 60 DAT				Plant height (cm) 90 DAT			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
N1	13.1	11.7	10.8	11.8 a	48.8	46.9	44.4	46.7	72.0	66.6	63.3	67.3 a
N2	15.1	13.6	12.1	13.6 b	53.0	78.9	49.9	51.6	78.9	76.8	72.5	76.1 b
N3	16.7	14.0	13.5	14.7 c	56.9	54.3	53.5	54.8	81.3	79.6	76.2	79.0 c
N4	18.2	16.4	15.2	16.6 d	62.1	60.3	58.6	60.3	84.9	82.7	79.5	82.4 d
Mean	15.7 a	13.9 b	12.9 c		55.2	60.1	51.6		79.2 a	76.4 b	72.8 c	
Significance of fixed factors												
N	*				NS				*			
S	*				NS				*			
N x S	NS				NS				NS			

Each value represent a mean of n = 3 replicates.; ns: not significant; Different letters within a column indicate significant differences between values at *: $p < 0.05$

Table 2 : Response of varying N-levels and plant spacing on chlorophyll content and leaf area of chrysanthemum

N- level	Chlorophyll content (mg/g)				Leaf area (cm^2)			
	S1	S2	S3	Mean	S1	S2	S3	Mean
N1	0.80	0.91	1.03	0.91 a	948.13	986.33	1043.56	992.67 a
N2	1.31	1.42	1.55	1.4 b	1117.41	1170.00	1214.18	1167.19 b
N3	1.51	1.62	1.71	1.6 c	1242.76	1293.57	1343.07	1293.13 c
N4	1.66	1.77	1.84	1.7 d	1382.45	1424.33	1466.37	1424.38 d
Mean	1.32 a	1.43 b	1.53 c		1172.69 a	1218.56 b	1266.80 c	
Significance of fixed factors								
Nitrogen (N)	*				*			
Spacing (S)	*				*			
N x S	NS				NS			

Each value represent a mean of n = 3 replicates.; ns: not significant; Different letters within a column indicate significant differences between values at *: $p < 0.05$

Table 3 : Response of varying N-levels and plant spacing on flowering characters of chrysanthemum

N- level	Days to flower bud appearance				Days to 50 % flowering				Flower diameter (cm)				Number of flowers/stem			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
N1	93.0	95.3	97.0	95.1 a	110.3	111.6	112.0	111.3 a	8.1	8.7	9.0	8.6 a	4.6	5.2	6.2	5.3 a
N2	87.6	89.3	90.3	89.1 b	100.0	102.3	103.6	102.0 b	11.6	12.6	13.2	12.5 b	10.2	11.9	13.0	11.0 b
N3	88.6	90.6	91.3	90.2 c	104.6	106.6	108.3	106.5 c	11.3	11.9	12.2	11.8 c	8.3	10.5	11.4	10.1 c
N4	91.0	93.0	94.6	92.8 d	107.3	108.0	108.6	108.0 d	10.5	10.9	11.1	10.8 d	7.6	8.3	9.4	8.4 d
Mean	90.0 a	92.0 b	93.3 c	-	105.5 a	107.1 b	108.1 b		10.3 a	11.0 b	11.3 b		7.6 a	8.9 b	10.0 c	
Significance of fixed factors																
Nitrogen (N)	*				*				*				*			
Spacing (S)	*				*				*				*			
N x S	NS				NS				NS				NS			

Each value represent a mean of n = 3 replicates; ns: not significant; Different letters within a column indicate significant differences between values at *: $p < 0.05$

Table 4 : Response of varying N-levels and plant spacing on post-harvest characters of cut stems

N- level	Cut stem diameter (mm)				Length of cut stem (cm)				Total water absorbed by cut stem (ml)				Weight of cut stem (g)			
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
N1	3.1	3.4	3.7	3.4 a	66.0	60.7	56.9	61.2 a	61.6	69.0	73.9	68.1 a	48.2	54.7	59.6	54.2 a
N2	7.2	7.4	7.8	7.5 b	74.0	71.0	70.0	71.7 b	110.3	124.5	135.4	123.4 b	80.7	87.2	92.6	86.8 b
N3	6.1	6.4	6.7	6.4 c	75.2	73.9	71.5	73.5 b	86.4	91.1	98.3	91.9 c	72.4	76.6	80.1	76.4 c
N4	5.6	6.0	6.2	5.9 d	78.7	76.9	73.4	76.3 c	83.7	87.0	93.3	88.0 d	66.2	70.8	75.2	70.8 d
Mean	5.5 a	5.8 b	6.1 c		73.4 a	70.6 a	67.9 b		85.5 a	92.9 ab	100.2 b		66.9 a	72.3 b	76.9 c	
Significance of fixed factors																
Nitrogen (N)	*				*				*				*			
Spacing (S)	*				*				*				*			
N x S	NS				NS				NS				NS			

Each value represent a mean of n = 3 replicates.; ns: not significant; Different letters within a column indicate significant differences between values at *: $p < 0.05$

CONCLUSION

It can be concluded that application of N@100 Kg ha^{-1} to chrysanthemum plants planted at 20x10 cm spacing accommodating 50 plants per square meter yielded best quality cut stems of acceptable length and optimum post-harvest longevity. Thus, compared to the conventional practice of N application (300 Kg ha^{-1}) adopted by farmers, the amount of N can be reduced to $1/3^{rd}$ to grow cut stems of chrysanthemums planted at twice the row spacing for optimum growth and flowering. The application of lower dose of N will likely reduce the production cost and lessen the environmental impact of leaching of N without compromising on quality and yield of chrysanthemum for commercial cultivation.

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Original Research Paper

Standardization of container type, substrate and nutrition for potted plant production of China aster [*Callistephus chinensis* (L.) Ness.] var. Arka Archana

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ABSTRACT

A study was conducted at the ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru for three consecutive seasons during 2019-20, to standardize the container type, substrate combination and nutrition for potted plant production of China aster var. Arka Archana. The treatments comprised of two type of containers (plastic and coir), three substrates {Red soil + FYM + Sand (1:1:1 v/v), Arka Fermented cocopeat (AFC), AFC + Vermicompost (1:1 v/v)} and four nutrition concentration (160:30:180 ppm N:P: K, 128:24:144 ppm N:P: K, 96:18:108 ppm N:P: K and Jeevamrutha @ 3%) laid out in factorial completely randomized design with three replications. Plant height at flowering (33.12 cm), number of primary branches (12.4), plant spread (536.64 cm^2), number of flowers/plant (26.47), flower size (5.26 cm) and uptake of major, secondary and minor nutrients were maximum in the plants grown in 6" plastic pots using the substrate combination of soil +sand +FYM (1:1:1 v/v/v) along with the weekly application of nutrient solution of 96:18:108 ppm NPK/plant. This production protocol resulted in a dense canopy and highly floriferous potted plants. The benefit cost ratio of potted China aster production was 1.70. This technology can be adopted by the nurserymen for large-scale commercial potted plant production.

Keywords: Containers, substrate, China aster, nutrients, floriferousness, potted plant

INTRODUCTION

Potted plants are an important segment in commercial floriculture occupying the second place after cut flowers with the demand increasing over the years @ 20-25%. Global flower pots and planters' market is expected to reach USD 2,208.3 million by the end of 2024 from USD 1,869.6 million in 2018 (Anon., 2019). Rapid urbanization and shrinking land area for conventional gardening and emergence of many high-rise buildings in the city landscape has led to a huge market demand for flowering and foliage potted plants. Potted plants are displayed in outdoor spaces, in spaces that are the extension of the house and indoors to improve indoor air quality. It has been reported that potted-plants can reduce total volatile organic compound loads, a major class of indoor pollutants, by 75% (<100 ppb) and also CO and CO_2 contents.

Flowering potted plants like rose, chrysanthemum, aster, carnation, marigold etc. are in good demand for their aesthetic appeal beside other associated positive benefits. China aster [*Callistephus chinensis* (L.) Ness.], a member of the family Asteraceae, is a commercial flower crop of India grown mainly for loose and cut flower purpose over an area of 3500 ha in Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, Maharashtra and West Bengal (Kumari *et al*., 2018). This crop can also be grown in containers. China aster var. Arka Archana released from ICAR-IIHR, Bengaluru is an early bloomer, with spreading plant type, bearing semi double white flowers, the plant form and floriferous nature makes it a suitable candidate for potted plant production.

Most of the consumers prefer eco-friendly products considering the health benefits while using these in indoor closed spaces. Flowering potted plant production mainly relies on the selection of appropriate containers, potting media and optimum nutrition, which tends to influence the crop canopy and

floriferousness. Alternate containers like coir pots are gaining popularity with certain section of consumers. An ideal potting substrate must possess unique physical and chemical characteristics favoring maximum water retention between irrigations while being well drained in order to avoid drought and root asphyxia (Caron and Nkongolo, 1999). Application of balanced nutrients to the potted plants, its solubility, availability, frequency of application is some of the factors that require standardization. The present study was undertaken to determine the effects of type of containers, substrate and nutrient levels on quality potted plant production of China aster var. Arka Archana.

MATERIALS AND METHODS

The study on potted plants of China aster var. Arka Archana was done at the ICAR - Indian Institute of Horticultural Research, Bengaluru, Karnataka, India. The pot plant experiment was conducted for a period of three seasons, i.e., February – May, 2019, July – October, 2019 and November 2019 – February, 2020 (Fig. 1). The pots were kept under open condition with full sunlight. Physical and chemical properties of initial and post-harvest media composition were analysed. The treatment details are as follows; Factor A: Type of pots (P_1: 6" plastic pot; P_2: 6" coir pot); Factor B: Substrate (S_1: red soil + FYM + sand (1:1:1 v/v), S_2: Arka Fermented cocopeat (AFC), S_3: AFC + Vermicompost (1:1 v/v)}; Factor C: Nutrition concentration (N_1- 160:30:180 ppm, N_2- 128:24:144 ppm, N_3 - 96:18:108 ppm N:P:K; N_4 - Jeevamrutha @ 3% (organic source). For treatments N_1, N_2 and N_3, secondary and micronutrients applications were kept unchanged. Nutrient solution application was scheduled at weekly intervals @ 50 ml / pot. The experiment was conducted in factorial CRD design with three replications and ten pots per replication. One month old seedlings at 4-6 leaves stage were transplanted in the centre of each pot @ one seedling/ pot and watered. Thereafter regular need-based watering was done, depending on the media and season. The texture and porosity of the growing medium were important considerations in deciding the frequency of irrigation. Pots containing substrate with cocopeat, cocopeat + vermicompost retained moisture for longer period compared to soil + sand + FYM media. Pinching was done one month after transplanting. Prophylactic sprays of plant protection chemicals ensured no severe infestation of pest and diseases during the period of experimentation.

The substrates Arka Fermented Cocopeat (AFC), soil and FYM were characterized as per the standard procedures. Soil physical and chemical properties were estimated by following standard procedures (Jackson, 1973). For nutrient uptake studies, nitrogen (N) contents in the plant samples were analyzed after mineralization with sulfuric acid by Kjeldahl method (Jackson, 1973). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were estimated digesting with a triacid mixture (9:4:1 HNO_3: $HClO_4$: H_2SO_4) as described by Jackson (1973).

Observations were recorded at flowering stage on the vegetative and flowering parameters *viz*., plant height (cm), number of primary branches, number of secondary branches, plant spread (cm^2), leaf area (cm^2), internodal length, number of flowers $plant^{-1}$, flower diameter (cm) and duration of flowering. Economic analysis of potted plant production of China aster was done considering the market price ranging from Rs. 45-70 per pot, depending upon the type of pot (plastic or coir), appearance and display life for computing gross returns from economic produce. Pooled analysis of the data for three seasons on different growth and yield parameters was done using statistical software WASP 2.0 (Web Agri Stat Package, ICAR-Central Coastal Agricultural Research Institute, Goa).

RESULTS AND DISCUSSION

Vegetative parameters: Plant height was recorded at the time of flowering varied significantly for the factors container type, substrate and nutrient doses (Table 1) and for their interaction effect (Table 2). Maximum plant height was observed in plastic pots, P_1 (26.32 cm) whereas; it was minimum in coir pots (P_2). Among different types of substrates used, maximum plant height (31.22 cm) was observed in S_1 (Red soil + sand + FYM), followed by S_3 (AFC + vermicompost) and minimum (21.30 cm) was recorded in S_2 - AFC alone. Among different nutrient levels used, maximum plant height was observed in application of 96:18:108 ppm of N:P:K - N_3 (26.45cm) and minimum was in Jeevamrutha - N_4 (23.33 cm). Among the interaction effects, plants grown in plastic pots using red soil + FYM + sand media with the application of 96:18:108 ppm NPK

Table 1 : Influence of type of pot, substrate and nutrients on growth and yield parameters of China aster var. Arka Archana potted plant at flowering (pooled mean of three seasons)

Treatment	Plant Height (cm)	No. of primary branches	No. of secondary branches	Av. plant spread area (cm^2)	Internodal length (cm)	Leaf area (cm^2)	Flower size (cm)	No. of flowers/ pt	Duration of flowering (days)
Type of pots									
P_1 - Plastic pot	26.32	6.96	2.96	251.61	2.58	15.59	4.68	16.24	32.40
P_2 - Coir pot	25.26	5.82	2.54	238.42	2.22	14.06	4.54	13.40	29.08
SEm±	0.40	0.20	0.12	8.53	0.1	0.39	0.77	0.26	0.62
CD@5%	1.05	0.52	0.31	20.90	NS	0.95	0.19	0.68	1.50
Type of substrate									
S_1 - Red soil +FYM + Sand (1:1:1 v/v)	31.22	8.37	3.10	295.78	2.46	15.80	4.95	17.46	32.75
S_2 - Arka Fermented cocopeat (AFC)	21.30	4.49	2.47	202.56	2.11	14.09	4.29	11.57	29.05
S_3 - AFC + Vermicompost (1:1 v/v)	24.84	6.30	2.67	236.72	2.63	14.60	4.58	15.44	30.41
SEm±	0.50	0.25	0.25	9.84	0.16	0.26	0.10	0.32	0.71
CD@5%	1.29	0.64	0.66	25.59	NS	0.67	0.24	0.83	1.73
Nutrient concentration									
N_1 - 160:30:180 ppm	24.07	6.21	2.67	236.12	2.31	14.45	4.58	14.88	30.07
N_2 - 128:24:144 ppm	26.77	6.30	2.64	266.76	2.2	14.94	4.65	15.03	30.22
N_3 - 96:18:108 ppm	26.45	6.84	2.87	246.55	2.79	15.38	4.72	15.26	31.92
N_4 - Jeevamrutha @ 3% weekly drenching	25.86	6.21	2.80	230.65	2.31	14.54	4.49	14.13	30.75
SEm±	0.57	0.28	0.29	11.37	0.19	0.22	0.12	0.37	0.79
CD@5%	1.49	0.74	0.76	29.55	NS	0.57	0.28	0.96	1.93

($P_1S_1N_3$) recorded maximum plant height (37.11 cm) and it was minimum in plants grown in plastic pot using red soil + FYM + sand media with the application of Jeevamrutha drenching@ 3% ($P_1S_1N_4$) and pot using AFC media with the application of nutrient combination of 160:30:180 ($P_1S_2N_1$).

Number of primary and secondary branches was significant for individual factors (Table 1) and their interaction (Table 2). Maximum number of primary and secondary branches was observed in plastic pots, P_1 (6.96 and 2.92, respectively), red soil + FYM + sand medium - S_1 (8.37 and 3.10, respectively) and nutrient combination of 96:18:108 ppm of N:P:K – N_3 (6.84 and 2.87, respectively). Among the interaction effect, significantly highest number of primary branches was obtained using plastic pots using red soil + FYM + sand media with the application of nutrient combination of 160:30:180 ppm of N:P:K $P_1S_1N_1$ (12.73). The number of secondary branches was found maximum (3.8) in plastic pots, red soil + FYM + sand medium with the application of 96:18:108 ppm of N:P:K ($P_1S_1N_3$).

Plant spread area was significant for individual factors (Table 1) and their interaction (Table 2). Maximum plant spread was recorded in plastic pots - P_1 (251.61 cm^2) substrate containing red soil + sand + FYM - S_1 (295.78 cm^2) and supplied with nutrients, 96:18:108 ppm of N: P: K - N_3 (266.76 cm^2). Among the interaction effect, maximum plant spread was in plastic pots, red soil + FYM + sand medium with the application of 96:18:108 ppm of N:P:K $P_1S_1N_3$ (380.05 cm^2). Internodal length of pooled mean showed non-significant differences for all the three factors and their interaction (Table 1 & Fig 3). Leaf area was found to be significantly different among the treatment combinations (Table 3). Maximum leaf area was observed in P_1 (15.59 cm^2). In substrate combination, red soil + FYM + sand media (S_1) reported maximum leaf area of 15.85 cm^2. Application of nutrient 96:18:108 ppm of N:P:K (N_3) resulted in maximum leaf area of 15.38 cm^2. Among interaction effect, $P_1S_1N_3$ recorded maximum leaf area of 16.79 cm^2 (Table 1 and 3).

In potted plant production plant growth and appearance plays an important role, not only to increase its marketing value by improving its appearance but also to improve the floriferousness. In this study, plant growth parameters like plant height, number of primary and secondary branches and plant spread was found ideal in plants grown in plastic pots using substrate containing red soil + sand + FYM along with the application of the nutrients, 96:18:108 ppm of N: P: K. This increment of growth parameters could be attributed to the great input of nutrients provided by the media combination and balanced fertilizer dose which supplied the required quantity of major, secondary and micronutrients. The importance of potting media and nutrient application for ornamental plant production has been highlighted by Jayasinghe (2012) and our findings are also in line with his findings.

Floral parameters: Number of flowers per plant was found to be significant for all the three factors (Table 1) and their interaction (Table 3). Among factor A, maximum number of flowers per plant for pooled mean of three harvests was observed in plastic pots (P_1) (16.24) compared to coir pots (13.4). Among different substrates used, maximum number of flowers was observed in S_1 (17.46), followed by S_3 (15.44) and minimum was in S_2 (11.57). Among nutrients, application of 96:18:108 ppm of N:P:K (N_3) recorded maximum number of flowers (15.26). Among interaction treatment $P_1S_1N_3$ recorded maximum number of flowers (21.85).

Flower size is an important aspect of potted plant production. Bigger the bloom indicates better display quality and attractiveness. Flower size was significant for two individual factors (Table 1) and interaction of three factors (Table 3). Maximum flower size was observed in plastic pots (4.68 cm) grown in red soil + FYM + sand media (4.95 cm) with the application of 96:18:108 N:P:K - N_3 (4.72 cm). Among interaction effect, treatment combination of plastic pot+ red soil + FYM + sand along with application of 96:18:108 ($P_1S_1N_3$) recorded maximum flower size of 5.26 cm.

Flowering duration plays an important role in analyzing the display life of the pot plant. In the present study, the flowering duration showed significant differences for individual factors and their interaction (Table 1 & 3). Flowering duration was maximum in plants grown in plastic pots (32.4 days), consist of red soil + FYM + sand media (32.75 days) and with the application of nutrient of 96:18:108 ppm of N:P:K (31.92 days). Among interaction effect, interaction of all these factors (plastic pots, red soil + FYM + sand media and nutrient of 96:18:108 ppm

Fig. 1 : General view of China aster var. Arka Archana potted plant experiment

Fig. 2 : Best performing treatments of the China aster pot plant experiment

Table 2 : Interaction of type of pot, substrate and nutrients on plant growth parameters in pot plant of China aster var. Arka Archana (pooled mean of for three seasons)

Treatment	Plant height (cm)						Number of primary branches						No. of secondary branches						Plant spread (cm²)					
	P_1			P_2			P_1			P_2			P_1			P_2			P_1			P_2		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
N_1	27.23	18.22	27.35	26.93	20.45	24.27	8.46	4.12	6.77	7.62	4.51	5.77	2.82	2.20	2.98	2.49	2.33	3.17	307.73	168.44	249.98	250.74	155.62	269.04
N_2	32.55	22.73	25.10	31.96	21.09	25.30	8.22	4.73	6.48	7.29	4.41	6.69	3.18	2.44	2.54	2.60	2.43	2.66	322.82	206.61	221.04	263.74	216.65	248.45
N_3	37.11	23.03	23.24	29.49	22.47	25.25	12.73	4.46	6.97	6.38	5.00	5.52	3.80	3.21	3.07	2.58	1.70	2.85	380.05	240.79	209.83	286.23	242.70	256.07
N_4	35.20	20.75	23.26	29.29	21.67	24.97	9.69	4.76	6.11	6.60	3.96	6.11	3.36	2.87	2.99	2.71	2.57	2.33	289.72	224.14	198.23	248.60	162.43	260.78
	SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)		
N	0.43			1.05			0.21			0.52			0.22			0.54			8.50			20.90		
P	0.52			1.29			0.26			0.64			0.27			0.66			10.40			25.59		
S	0.61			1.49			0.30			0.74			0.47			NS			12.01			29.55		
P X S	0.74			1.82			0.37			0.90			0.38			0.93			14.72			36.20		
P X N	0.85			2.10			0.42			1.04			0.51			NS			19.8			NS		
S X N	1.04			2.57			0.52			1.27			0.54			1.32			20.81			51.19		
P X S X N	1.48			3.64			0.73			1.80			0.76			1.86			29.43			72.39		

Factor A: Type of pots (P_1: Plastic pot; P_2: Coir pots); Factor B: Substrate (S_1: Red soil + FYM + Sand (1:1:1 v/v), S_2: Arka Fermented cocopeat (AFC), S_3: AFC + Vermicompost (1:1 v/v)}; Factor C: Nutrition concentration (ppm) (N_1- 160:30:180, N_2- 128:24:144, N_3 - 96:18:108 N:P:K; N_4 - Organic source (Jeevamrutha @ 3% weekly drenching)

Table 3 : Interaction of type of pot, substrate and nutrients on yield and quality parameters in pot plant of China aster var. Arka Archana (pooled mean of for three seasons)

Treatment	Leaf area (cm²)						No. of flowers/plant						Flower size (cm)						Duration of flowering (days)					
	P_1			P_2			P_1			P_2			P_1			P_2			P_1			P_2		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
N1	16.71	16.73	14.56	15.04	14.53	16.60	17.03	12.17	17.53	17.02	11.95	13.56	4.33	4.85	5.27	5.18	3.75	4.07	34.8	29.5	32.3	30.1	26.3	27.4
N2	16.15	15.51	14.48	11.99	13.84	14.53	17.43	15.28	15.81	16.50	11.07	14.82	4.46	5.19	5.02	4.96	3.84	4.62	33.2	30.2	31.2	30.7	27.6	28.4
N3	16.79	16.72	13.16	12.49	13.80	15.00	21.85	12.29	13.41	16.62	13.28	13.35	5.26	4.56	4.10	5.08	3.93	5.14	35.9	31.5	34	32.4	28.2	29.5
N4	16.90	14.32	15.11	15.07	12.52	13.32	15.47	11.82	12.98	17.91	13.01	13.57	5.08	4.38	3.41	5.06	3.85	4.75	33.7	30.7	31.8	31.2	28.4	28.7
	SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)			SeM±			CD(P= 0.05)		
N	0.38			0.95			0.28			0.68			0.08			0.19			0.62			1.50		
P	0.27			0.67			0.34			0.83			0.10			0.24			0.71			1.73		
S	0.23			0.57			0.39			0.96			0.11			0.28			0.79			1.93		
P X S	0.38			NS			0.48			1.18			0.14			0.34			0.93			2.26		
P X N	0.45			1.12			0.55			1.36			0.16			0.39			1.05			2.55		
S X N	1.09			2.74			0.68			1.67			0.20			0.48			1.24			3.02		
P X S X N	1.23			3.08			0.96			2.36			0.28			0.67			1.68			4.08		

Factor A: Type of pots (P_1: Plastic pot; P_2: Coir pots); Factor B: Substrate (S_1: Red soil + FYM + Sand (1:1:1 v/v), S_2: Arka Fermented cocopeat (AFC), S_3: AFC + Vermicompost (1:1 v/v)}; Factor C: Nutrition concentration (ppm) (N_1- 160:30:180, N_2- 128:24:144, N_3 - 96:18:108 N:P:K; N_4 - Organic source (Jeevamrutha @ 3% weekly drenching)

of N:P:K) recorded maximum display life of 35.90 days. It was minimum in plants grown in coir pots, AFC media with the application of 160:30:180 ppm of N:P:K (26.30 days). In the present study, plant growth parameters (plant height, number of primary branches, plant spread) at flowering and yield parameters (number of flowers/plants, flower size) and uptake of nutrients were maximum in the plants grown in 6" plastic pots compared to coir pots of the same size. The coir pots maintained the shape for 3-4 months, thereafter started degrading. Similar observations were made in Poinsettia potted plants grown in containers made of 100% biodegradable polyester for breakage problems during the handling of the marketing phase (Castronuovo, 2015). The coir pots required frequent irrigation compared to plastic pots which was also reported by Evans *et al*. (2015) in vinca (*Catharanthus roseus*). Beeks and Evans (2013) also analyzed the behavior of nine bio containers in comparison with a traditional petroleum-based plastic containers for the production of cyclamen (*Cyclamen persicum* L.) cv. 'Rainer Purple' and pointed out those most plantable containers are not suitable for this purpose.

Analysis of substrate composition: The substrate AFC had the following characteristics: bulk density 0.16 Mg m^{-3}; porosity 67.8%; pH 6.75; electrical conductivity 0.5 dSm^{-1}; total carbon 36.1%; total N 0.98%; total P 0.07%; total K 2.20% and Na 0.35%. The average concentration of macronutrients was estimated at 0.58% N, 0.26% P_2O_5 and 0.60% K_2O in FYM. Physical and chemical characteristics of the soil were: bulk density 1.28 Mg m^{-3}; porosity 51.3%; pH 6.97, electrical conductivity 0.26 dSm^{-1}; organic carbon 7.8 g kg^{-1}; available N 0.13 g kg^{-1}; 18 mg kg^{-1} Olsen's P, ammonium acetate (CH_3COONH_4) extractable nutrients as follow: 0.90 g Ca kg^{-1}, 0.174 g Mg kg^{-1} and 0.15 g K kg^{-1} and DTPA extractable micronutrients as follow: 10.3 mg kg^{-1} Fe, 5.70 mg kg^{-1} Mn, 2.24 mg kg^{-1} Cu and 1.35 mg kg^{-1} Zn. Plant production in growth media includes materials that contain soil or soilless media (Savvas *et al*., 2013). Plant faces two basic challenges for root growth in the containerized plant production system. The first one is the very shallow root growth area in the container environment which quickly becomes saturated after watering as compared to normal soil profile having limitless area for drainage. The second one is the limited water storage capacity between the irrigation intervals due to small volume of the container (Bunt, 1988). The physical structure must maintain equilibrium between air and water over the entire crop cycle, which is few months for annuals to prolonged time for perennials. The potting substrate physical properties are usually assessed by its particle size and shape, texture and physical organization (Bilderback *et al*., 2005). Selection of an ideal substrate, either soil or soilless is one of the important keys for success of potted plant production. Our studies have revealed that the substrate combination of soil + sand + FYM (1:1:1 v/v) have recorded compact plant growth, yield and quality parameters compared to AFC + vermicompost and AFC alone. Addition of organic matter as compost or manure (green manure, farm yard manure, poultry manure) is a common practice for growing potted plants. In addition to supplying plant nutrients, it provides a favourable physical and biological environment for plant roots in the growth medium (Kumar and Goh, 2000). Therefore, an ideal potting substrate must possess unique physical and chemical characteristics favoring maximum water retention between irrigation while being well drained in order to avoid drought and root asphyxia (Nkongolo and Carol, 2006). Thus, the potting substrate is a pivotal advancement in plant production system providing grower with the full control over air, water and nutrients delivery as well as pathogen free environment to plant roots (Raviv *et al*., 2002). On a commercial scale, there is a need of bulk quantity raw constituents for the production of soilless growing media (Carlile *et al*., 2015). The growing substrate should also be economical and practical enough to be used for commercial purposes.

Nutrient uptake: The plant nutrient uptake as influenced by pot types, substrates and nutrient levels are given in Figure 4. Nutrient uptake was found to be non-significant for type of pots. Among the substrate combinations, red soil + sand + FYM recorded highest plant nutrient uptake for most of the nutrients {N (0.19 g/pt), Ca (0.15 g/pt), Mg (0.06 g/pt), Fe (4.45 mg/pt), Mn (0.67 mg/pt) and Zn (0.55 mg/pt)} followed by AFC + vermicompost and all these parameters were found minimum in pots containing AFC alone. With respect to nutrient levels, the highest nutrient uptake {N (0.21 g/pt), K (0.35 g/pt), Ca (0.14 g/pt), Mg (0.06 g/pt), S (0.01 g/pt), Fe (4.04 mg/pt), Mn (0.68 mg/pt), Zn (0.59 mg/pt), Cu (0.63 mg/pt)} was recorded with application of 96,

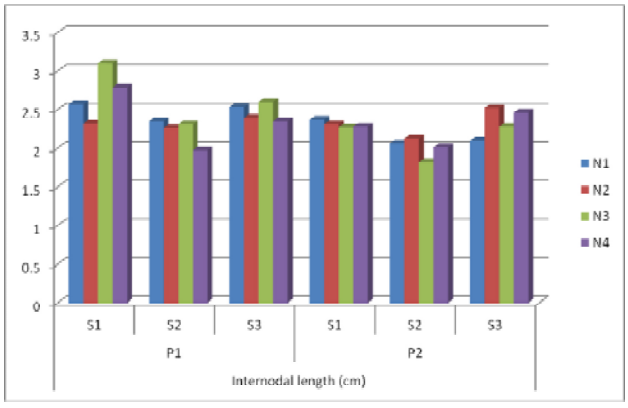

Fig. 3 : Interaction effect of type of pots, substrate and media combination on internodal length of China aster var. Arka Archana

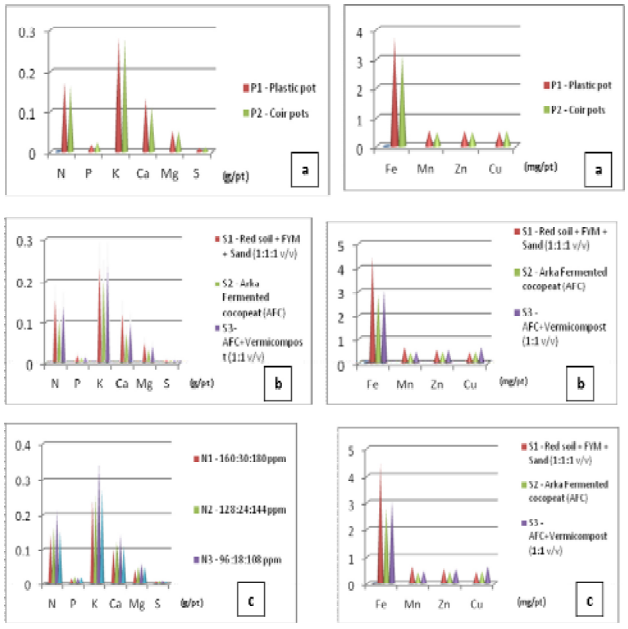

Fig. 4 : Nutrient uptake by China aster Var. arka Archana plants under pot experiment

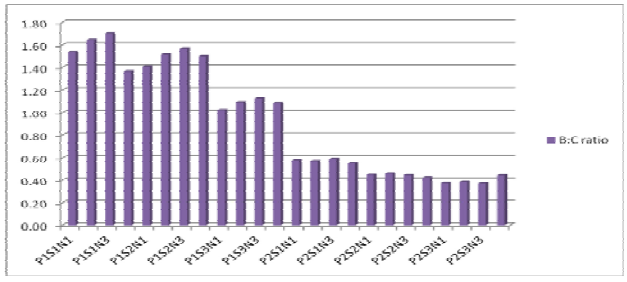

Fig. 5 : B:C ratio of different treatments of China aster Var. Arka Archana pot experiment

Factor A: Type of pots (P_1: Plastic pot; P_2: Coir pots); Factor B: Substrate (S_1: Red soil + FYM + Sand (1:1:1 v/v), S_2: Arka Fermented cocopeat (AFC), S_3: AFC + Vermicompost (1:1 v/v)}; Factor C: Nutrition concentration (ppm) (N_1- 160:30:180, N_2- 128:24:144, N_3 - 96:18:108 N:P:K; N_4 - Organic source (Jeevamrutha @ 3% weekly drenching)

18 and 108 ppm of N:P:K (N_3). This was followed by N_2 (128:24:144 ppm of N:P:K) and found to be on par with Jeevamrutha @ 3% weekly drenching (N_4). The lowest nutrient uptake was recorded with application of higher levels of nutrient application (N_1- 160:30:180 ppm of N: P:K). Plant nutrition plays a pivotal role especially when it is grown in container. In this experiment among three levels of nutrient application and one organic combination - jeevamrutha, weekly application of lower concentrations of nutrient solution of 96:18:108 ppm N:P:K/plant (Arka Sasya Poshak Ras) has given better plant growth, flower yield and quality parameters. Similar studies on *Adenum obesum* 'Red' under low nutrient supply was found to relocate more biomass into roots (McBride, 2014). However, *Tabernaemontana pachysiphon* Staph treated with three levels of Osmocote, two water regimes, and two light intensities indicated that increasing nutrient supply had a positive effect on growth (Hoft *et al*., 1996). Mandevilla Vogue varieties were shown to be moderate feeders, responding best to use of a balanced fertilizer at a rate of 100 to 200 mg L^{-1} and it was recommended that a low to medium rate of a standard slow- release fertilizer should be added at planting (Mart, 2012). *Plumeria rubra* grown in pure silica sand in 4-L containers were treated with a low and high nutrient level (2.4 g and 24.0 g, respectively, of 14N–14P–14K of Osmocote) revealed that more biomass was produced under high nutrient supply, whereas more biomass was allocated to the roots in low nutrient supply (Huante *et al*., 1995). Vinca (*Catharanthus roseus* L.) seedlings benefitted from high concentrations of N (up to 32 mM) in the fertilizer, whereas only low concentrations of phosphorus and potassium (0.25 mM) were needed (Van Iersel *et al*., 1999). The ideal potting substrate must also deliver an appropriate environment for proper plant nutrient availability. Nutrient availability is very much dependent on the chemical properties including pH, cation exchange capacity, electrical conductivity of the substrate etc.

Economic analysis: Economic indicators have been worked out considering the cost of inputs (pots, substrate and nutrients), labour and maintenance cost (Fig 5). The three-season study suggested that pot plant production of China aster var. Arka Archana was profitable in plants grown in plastic pots using red soil + FYM + sand media with the application of

96:18:108 ppm of N:P:K ($P_1S_1N_3$) with a B:C ratio of 1.70. This might be due to the lower cost of production and better display life of the potted plant grown in this treatment. In general plants grown in plastic pots recorded better B:C ratio in the range of 1.02 to 1.70, whereas, coir pots due to higher cost of pots recorded lower B:C ratio range of 0.37 to 0.59. An alternative to the use of plastics in potted plant production could be the use of biodegradable pots instead of plastic pots (Sartore *et al*., 2013). However, the technical performance and suitability for agricultural applications, of these biodegradable materials, that can be easily degraded by naturally occurring microorganisms must be ensured (Lucas *et al*., 2008). The cost of bio pots is too high than traditional ones to make them utilizable by growers on large scale (Brumfield *et al*., 2015) and it is about twice the cost the traditional ones according to Minuto *et al*. (2008). This aspect is even more important for annual crops like potted China aster wherein the production costs should be considered as it is a short duration crop.

CONCLUSION

From the results of the study, it is evident that in pot plant production of China aster var. Arka Archana, plant growth parameters like plant height (33.12 cm), number of primary branches (12.4), plant spread (536.64 cm^2) at flowering and yield parameters like number of flowers/plant (26.47), flower size (5.26 cm) and uptake of nutrients were maximum in the plants grown in 6" plastic pots by using the substrate combination of red soil + sand + FYM (1:1:1 v/v) along with the weekly application of nutrient solution of 96:18:108 ppm NPK/plant (Arka Sasya Poshak Ras). The same treatment is profitable with the highest B:C ratio of 1.70. This technology can be adopted for large-scale commercial potted plant crop production as flowering potted plants are a way to bring in a visually pleasing effect, soften the hard lines of the living spaces that give health benefits.

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Original Research Paper

Effect of chitosan coatings on physico-chemical and enzymatic activities in mango cv Dashehari stored at low temperature

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ABSTRACT

Physico-chemical and enzymatic changes in mango (*Mangifera indica*) cv. Dashehari in response to postharvest application of chitosan (0, 0.5 and 1.0%) were studied during 4 weeks that were stored between 10±1 °C, 90-95% RH. Fruits were evaluated for various quality parameters such as firmness, weight loss, pulp colour, *β*-carotene, soluble solid content (SSC), titratable acidity (TA) and activities of polygalactouronase (PG) and cellulase on 0, 7, 14, 21 and 28 days. Results exhibited that chitosan coatings (1.0%) effectively reduced the weight loss (5.82%) and markedly slowed down the ripening changes as evidenced from their retention of fruit firmness (15.50 N), maintenance of SSC (18.85%) and TA (0.44%) at 21 days of storage. Chitosan coatings also retarded the pulp colour development and lowered activities of PG and cellulase enzymes as compared to non-coated fruits. Overall, chitosan coating at 1.0% was found to be most effective in enhancing the storability and quality of mango fruits at cool storage temperatures.

Keywords: Cellulase, chitosan, fruit quality, mango and polygalactouronase

INTRODUCTION

Mango is the most important tropical fruit cultivated worldwide and is considered one of the choicest fruits due to its attractive colour, sumptuous flavor and high nutritional properties. Despite rapid increase in the mango production and International trade, the marketability of good quality fruit is mainly affected by its short postharvest life. Under the sub-tropical conditions of Punjab (India), the fruit is harvested during the first fortnight of July when high temperature and humidity prevails in the region (Gill *et al*., 2017). Hence under ambient conditions, the fruit becomes highly perishable and reaches climacteric peak after 3 to 4 days of harvest. This peak is usually accompanied by rapid rise in ethylene production which accelerates the fruit softening and ripening process (Singh *et al*., 2013). Shorter shelf-life of mango fruit is associated with various physico-chemical and enzymatic changes including weight loss, textural softening, chlorophyll degradation and starch hydrolysis (Herianus *et al*., 2003) and thus restricting its transportation to distinct markets. To extend the postharvest life of fruits, application of various edible coatings based on lipids, polysaccharides and proteins have been experimented. Among these, polysaccharide-based chitosan coating has demonstrated a positive effects on the maintenance of fruit quality during storage (Xin *et al.,* 2017). Chitosan coating forms a semi-permeable membrane around the fruit surface and modify the internal atmosphere, thereby resulting into a decline of respiration and transpiration rates thus delaying the fruit ripening and senescence.

Chitosan coatings on papaya fruits significantly reduced the weight loss percentage, maintained the fruit firmness, soluble solids concentration and prolonged the storage life of fruits (Ali *et al*., 2011). Furthermore, some studies on the effect of edible coatings on postharvest life and quality parameters in mango fruits have also been reported. Chitosan 2.0 % coating on mango cv. Tainong significantly lowered the respiration rate and maintained the fruit firmness (Zhu *et al*., 2008) and delayed the PG activities in mango cv. Choke Anan fruit during storage (Khaliq *et al*., 2017). However, no reports are available concerning the effect of chitosan on enzymatic activities in the fruits of Dashehari mango stored at low temperature storage; hence, there is a need for further studies to contemplate the efficacy of chitosan

coatings on postharvest life of the most important Indian mango cultivar. The aim of the present work was to elucidate the effect of the surface chitosan coating on quality attributes and enzymatic activities in mango fruit cv. Dashehari under cool storage temperatures.

MATERIALS AND METHODS

Mango fruits (*Mangifera indica* cv. Dashehari) were harvested at mature green stage (specific gravity; 0.98±0.01 and firmness; 94.5±2.5N) from the orchard (30.89 °N and 75.80 °E latitude) of the Department of Fruit Science, Punjab Agricultural University, Ludhiana, India. Healthy fruits without any physical defects were selected for uniformity in shape, size & colour and washed with 100 ppm chlorinated water & then dried in shade. Further, fruits were randomly divided in three groups with each group comprising of 320 fruits. Two groups were coated with chitosan 0.5 % and 1.0 % concentrations using a soft brush, whereas the third group was left uncoated (control). Each treatment comprised of 80 fruits in four replications with 20 fruits under each replicate. The fruits were packed in corrugated fiber board boxes (5 % ventilation) with paper lining and stored under cool storage conditions (10±1 °C, 90-95 % RH). The desired concentration (0.5 % and 1.0 %) of chitosan was prepared as per the method described by Han *et al.* (2004) by dissolving 0.5 and 1.0 g of chitosan in 100 mL of 3 % acetic acid solution and was mixed well using a magnetic stirrer. The pH of chitosan coating solution was maintained at 5.0 with 1 M NaOH.

Fruits were randomly selected from each treatment and analyzed for various physico-chemical attributes on the day of harvest (before treatments) and at 7, 14, 21and 28 days of storage. Weight of fruits after every interval of storage was recorded and per cent weight loss was calculated.Fruit firmness was measured by the destructive method using stand mount penetrometer (Model FT-327, USA) and the values were expressed in Newton (N). SSC was measured using handheld digital refractometer (*ATAGO, PAL -1*, Japan) and expressed in ^{0}Brix. Titratable acidity was measured using 2 mL of strained juice and titrated against 0.1 N NaOH solution using phenolphthalein indicator until the colour changed pink and was expressed as a percentage per 100 g fresh weight. The fruit pulp colour coordinates were recorded using a spectrophotometer (Hunter Lab ColorFlex, Hunter Associates Inc., Reston, VA, USA) as *L*, a** and *b** in Commission International de I'Eclairage (CIE) units with the head of 15 mm diameter to fit fruit surface (Hunter, 1975). Carotenoids were estimated in the form of *β*-carotene from the pulp of fruits as per the methodology followed by (Gill *et al.,* 2017) and the colour intensity of samples were read at 452 nm in a spectrophotometer (Spectronic 20D$^+$ Thermo Fischer Scientific, USA) against petroleum ether used as blank. Polygalacturonase and cellulase enzyme activities were determined as per the method followed by Kaur *et al* (2021) with slight modification.

The data for various parameters were analyzed by two-way analysis (coatings x storage period) of variance in completely randomized design (Factorial) and using the statistical package SAS 9.3 (The SAS system for Windows, Version 9.3, SAS Institute, Cary, NC). Fisher's least significant differences (LSD) were calculated following a significant (P d" 0.05) F-test.

RESULTS AND DISCUSSION

All the fruits irrespective of the coatings exhibited a gradual loss in weight throughout the storage period (Fig. 1A). The uncoated fruits exhibited a maximum weight loss of 11.50 % at 28th day of storage. However, fruits coated with 0.5 and 1.0 % chitosan registered significantly lower weight loss as compared to control. At the end of the storage period, weight loss in uncoated fruits was 24.23 % higher as compared to fruits coated with 1.0% chitosan. It might be due to formation of an effective semi-permeable film on the fruit surface, thus limiting the water loss and exchange of gases and protects fruit against dehydration loses. Results concurred to previous studies that recorded lower weight loss in chitosan coated mango fruits (Abbasi *et al*., 2009).

The firmness of the mango fruits declined progressively throughout the storage period (Fig. 1B). The maximum rate of decline in the coated and uncoated fruits were observed on the 7th day of storage. However, the decrease in fruit firmness was lower in fruits coated with chitosan as compared to uncoated fruits. On 28 days of storage, fruits coated with 1.0 % chitosan retained maximum (7.39 N) firmness, followed by 0.5 % chitosan treatment and the minimum (4.17 N) firmness was recorded in uncoated fruits. This overall retention of fruit firmness in coated fruits might be the due formation of modified

atmospheric condition around the fruit surface by chitosan coating, which reduces the degradation of insoluble proto-pectins into more soluble pectic acid and pectins (Kashappa and Hyun, 2006). These results can be correlated with the findings of Ladaniya (2011) in Nagpur mandarin and in mango (Abbasi *et al*., 2009) where maximum firmness was retained in the wax coated fruits.

The present study showed an increase in SSC in all the coated as well as uncoated fruits throughout the storage period under cool temperature conditions (Fig. 1C). The rate of increase in SSC in uncoated fruits was higher in comparison to chitosan coated fruits. During the entire storage period of 28 days, the increase in SSC in uncoated fruit was 57.02 % as compared to 52.93 % increase in 1.0 % chitosan coated fruits. Hence a gradual increase of SSC was registered in fruits coated with 1.0 % chitosan, which might be due to the formation of semi-permeable film around the fruit which modifies the internal atmosphere of fruit and forms a barrier against oxygen thus reducing the rate of respiration. Similar results were reported in mango (Abbasi *et al*., 2009) fruits where higher SSC were recorded in uncoated fruits as compared to coated fruits.

TA decreased in all the fruits irrespective of the coatings during the entire storage period (Fig. 1D). On the7th day of the storage period, the decline in acidity

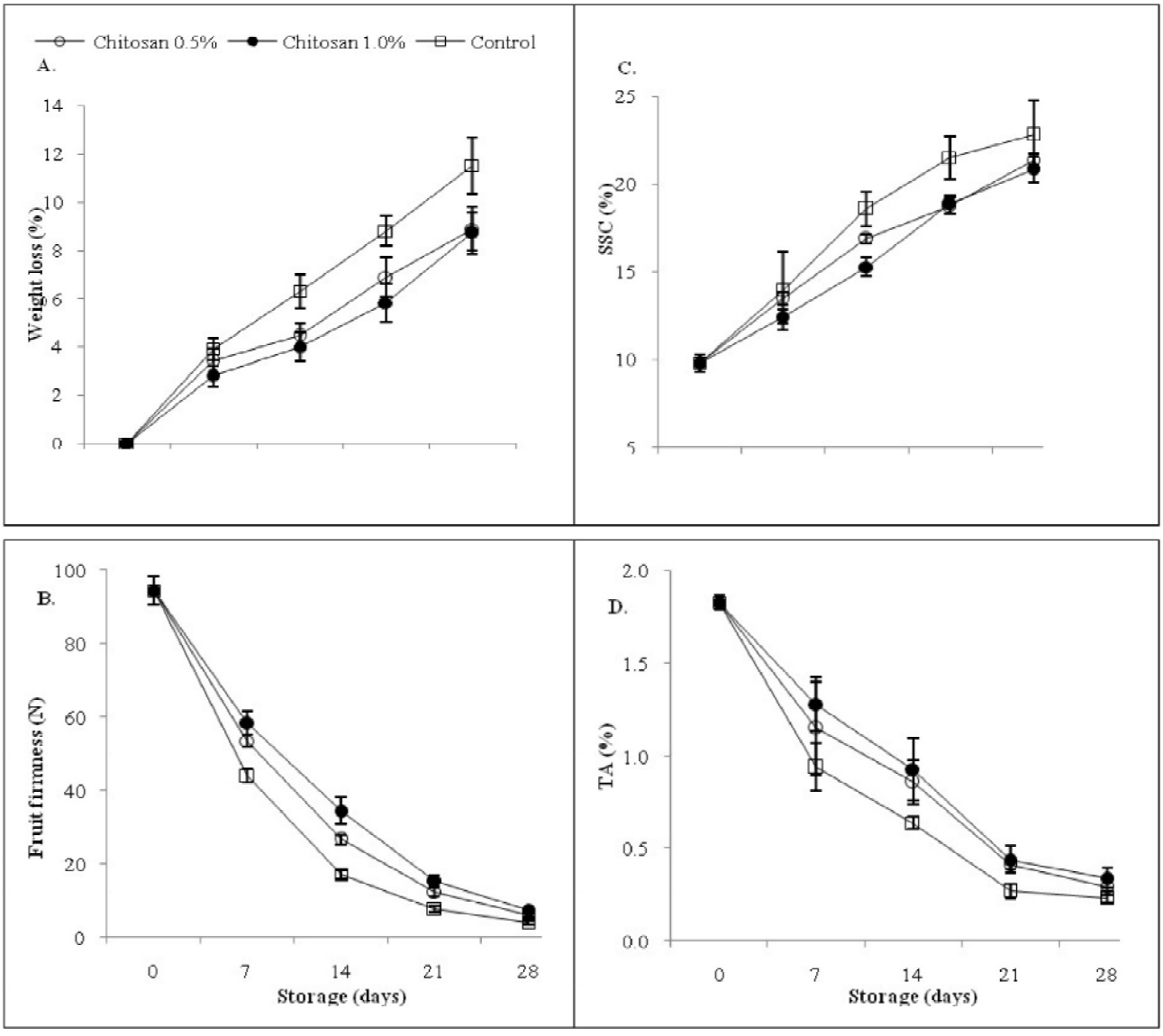

Fig. 1 : Variation in (A) weight loss (B) fruit firmness (C) SSC (D) TA in mango *cv*. Dashehari in relation to chitosan coatings. Vertical bars represent ± Standard error of mean of 4 replicates.

of uncoated fruits was 26.56 % higher as compared to TA in chitosan 1.0 % coated fruits. However, at the end of the storage period, fruits coated with 1.0 % chitosan retained maximum (0.34 %) TA as compared to uncoated fruits, where minimum (0.23 %) TA was recorded. Acidity can be directly correlated with the organic acid's concentration in fruit and their reduction during storage is due to the metabolic alterations involving utilization of these organic acids during respiration for enzymatic reactions (Yaman and Bayoinderli, 2002). In the present study, fruits coated with 1.0 % chitosan were effective in the preservation of organic acids by maintaining highest TA thus indicating its inhibitory role in the oxidation of organic acids. Results were in harmony with the previous findings in mango as reported by Zhu *et al*. (2008).

The colour coordinates *L**, *a**, *b**, chroma and hue angle shown in fig.2 indicates the change in pulp colour of fruit during storage. The *L** value of the pulp colour in all the coated and uncoated fruits decreased with the progression of the storage (Fig. 2A). However, the rate of decrease in *L** value was higher in uncoated fruits as compared to the fruits coated with chitosan. From the day of harvesting to 28 days of storage period, *L** value of pulp in fruits coated with 1.0 % chitosan decreased by 25.62 %, whereas in uncoated fruits L value decreased by 28.66 %. The *a** and *b** value of pulp significantly increased in all the fruits irrespective of the coatings (Fig. 2B and 2C). The fruits coated with 1.0 % chitosan registered minimum *a** and *b** values (27.45 and 61.67, respectively) at the end of storage period, while uncoated fruits recorded maximum *a** and *b** values (28.23 and 64.70, respectively). Results showed that chitosan coated fruits retained the pulp colour index at *L**, *a** and *b**, which indicates the delay of the ripening process. Chitosan treatments effectively slowed down the degeneration of yellow colour of pulp and retained a lighter yellow pulp colour at the end of storage as compared to uncoated fruits.

The chroma value of coated as well as uncoated fruits increased during the study (Fig. 2D). From the day of harvest to 28 days of storage, a lower *C** value (43.75) was observed in fruits coated with chitosan 1.0 % as compared to uncoated fruits, where a rapid change in *C** value (45.15) was recorded. Hue angle of pulp of all the fruits decreased throughout the storage period irrespective of chitosan coatings (Fig. 2E). During 28 days of storage, a comparatively higher (71.80) hue angle was registered in fruits with 1.0 % chitosan coatings as compared to control fruits which recorded minimum (71.26) hue angle. Chitosan coated fruits recorded lesser hue angle and higher chroma values which may be attributed to the slow pulp colour changes and fruit senescence. This proved the effectiveness of chitosan coating in delaying the climacteric peak, which is often associated with colour change in fruit due to the activity of chlorophyllase enzyme as well as accumulation of carotenoids in response to the climacteric rise in respiration rate and ethylene production (Saltveit, 1999). Similar findings were reported in strawberries coated with edible coatings, where a colour change in fruit was significantly delayed (Velickova *et al*., 2013).

A significant progression in β-carotene content was observed in all the fruits irrespective of the coatings throughout the storage period (Fig. 2F). The maximum increase in β-carotene was observed until 25 days in uncoated fruits (94.19 %) as compared to fruits coated with chitosan 1.0 %, which recorded minimum (92.65 %) increase in β -carotene and was found on par with fruits coated with 0.5 % chitosan treatment. However, on 28th day of storage, the β-carotene content of uncoated fruit was 4.9 % higher as compared to fruits coated with 1.0 % chitosan. Carotenoids being the most crucial pigments defining the qualitative characteristic of mango fruit, increases with the progression of the ripening process. β-carotene increased in all the coated as well as uncoated fruits, but their increase was recorded at a slower pace in 1.0 % chitosan coated fruits. The inhibitory effect of chitosan on carotenoid development may be due to the modification of internal atmospheric conditions and suppression of enzymatic activities thus resulting into reduction of chlorophyll degradation and carotenoid biosynthesis (Gol and Rao 2011; Hong *et al.,* 2012).Similar inhibitory effect of wax treatments on carotenoid biosynthesis was reported in mandarin (Ladaniya, 2011).

The study showed a comparable trend in PG and cellulase enzymatic activities (Fig. 3A and 3B). An increase in PG activity was observed in fruits coated with chitosan 1.0 % until 21 days of storage period as compared to chitosan 0.5 % and uncoated fruits where this increase was recorded only until 14 days, followed by a decline. At 28th of the storage period, the maximum (10.89 µg D-galacturonic acid g^{-1} FW min^{-1}) enzymatic activity was observed in 1.0%

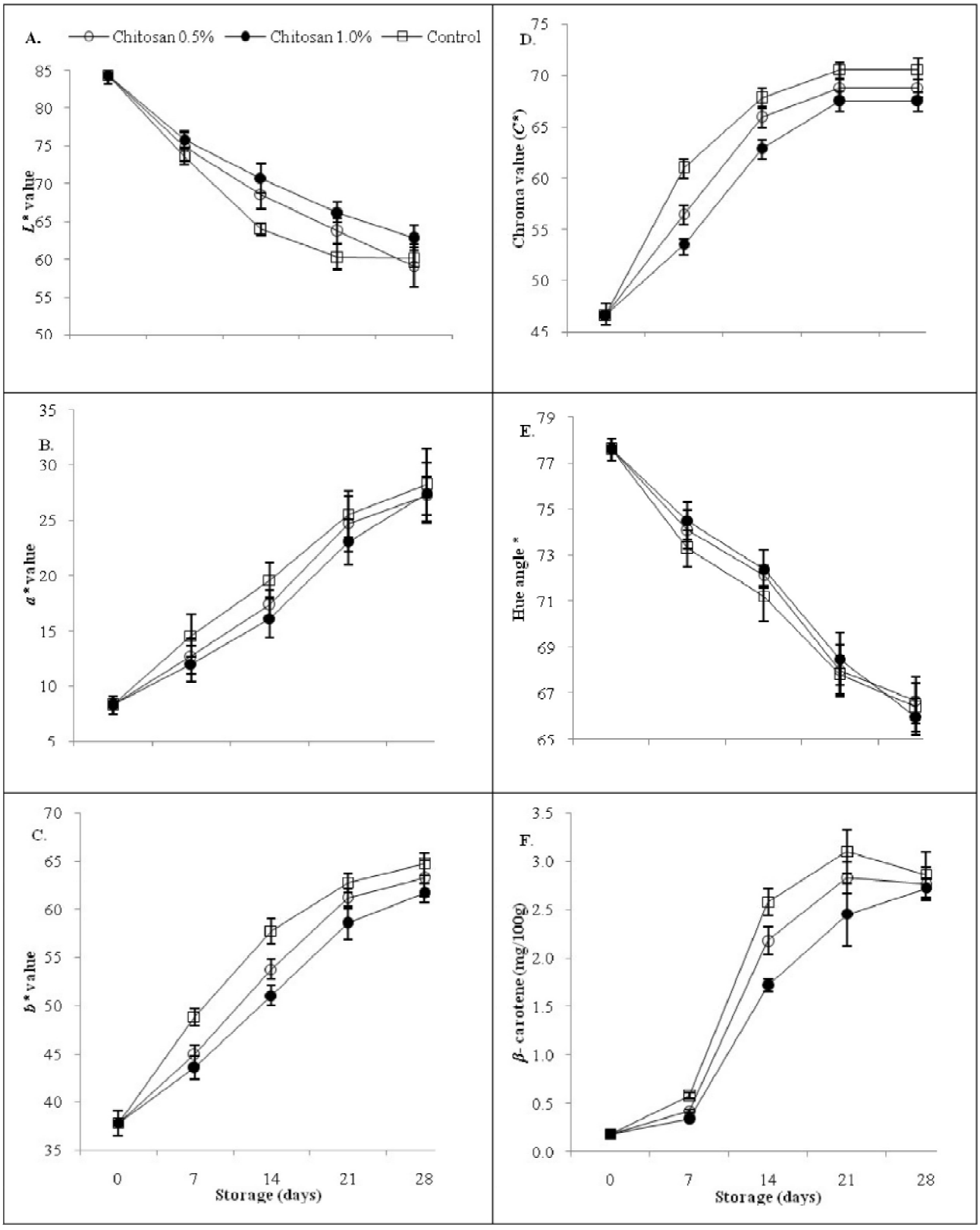

Fig. 2 : Variation in pulp colour (A) *L** (B) *a** (C) *b** (D) chroma *C**, (E) hue value *hR"* and (F) *β*- carotene in mango *cv* Dashehari in relation to chitosan coatings.
Vertical bars represent ± Standard error of mean of 4 replicates.

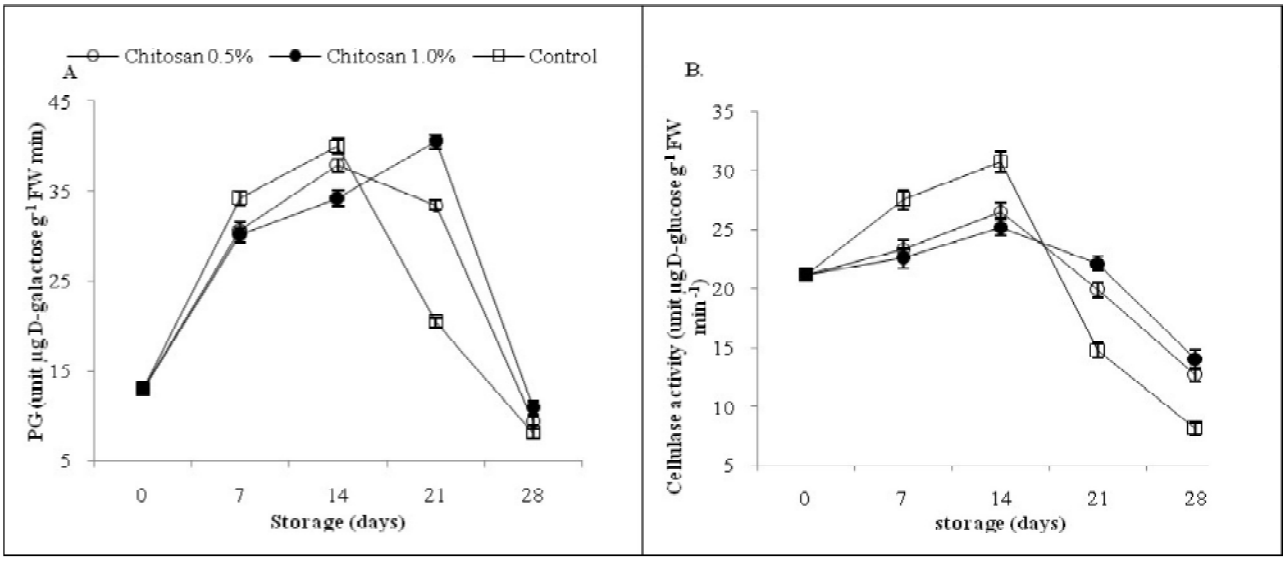

Fig. 3 : Variation in (A) PG, and (B) Cellulase enzyme activities in mango *cv*. Dashehari in relation to chitosan coatings. Vertical bars represent ± Standard error of mean of 4 replicates

chitosan coated fruit as compared to control fruits, which recorded minimum (8.02 µg D-galacturonic acid g^{-1} FW min^{-1}) PG activity. Alteration in the activity of cell wall degrading enzymes is the major concern regarding deterioration in fruit quality. During ripening the latent forms of PG, and cellulase get activated resulting in breakdown of pectic substances, celluloses, and hemicelluloses present in the middle lamella. The rise in the activity of these cell wall degrading enzymes results in the reduction of cohesive forces that bind the cells together weakens of cell wall and cause fruit softening (Wills *et al*., 1998). PG enzymes activity is responsible for the catalysis of depolymerization reactions and hydrolytic cleavage of de-esterified polygalacturnoid chain (Wei *et al*., 2010). Chitosan coatings significantly delayed the enzymatic activity in mango fruit. As per the results, fruits coated with 1.0 % chitosan recorded lower PG activity as compared to uncoated fruits. In uncoated fruits, PG activity increased approximately 3 folds up to 14 days of storage period as compared to 1.0 % chitosan coating where this increase was only 2.6-fold. A similar finding was reported in wax coated 'Manila' mango (Vazquez-Celestino *et al*., 2016).

Similar observations were recorded in cellulase enzyme activity, where all the coated as well as uncoated fruits exhibited an increase in cellulase activity till 14 days of cold storage, followed by a decline. However, at the end of the storage period (28 days) the decline in cellulase activity in uncoated fruit was 42.12 % and 35.91 % higher than the fruits with 1.0 % and 0.5 % chitosan coatings, where PG activity was delayed during storage (Vazquez-Celestino *et al*., 2016). Results indicated that coatings of chitosan inhibited the increase in enzymatic activities, while 1.0 % chitosan coated fruits maintained highest enzymatic activities until 28 days of storage as compared to uncoated fruits which clearly signifies the capability of chitosan in maintaining the cell wall integrity by reducing the breakdown of cell wall components and accumulating higher enzyme substrate levels for a longer period.

CONCLUSION

In conclusions, 1.0 % chitosan coating on mango fruits, effectively maintained the fruit quality over uncoated fruits stored under cool temperature conditions. Chitosan coated fruits exhibited lower weight loss percentage and retained maximum fruit firmness. In addition, these coatings also maintained the SSC and TA and significantly delayed the pulp colour development and enzymatic activities of PG and cellulase in mango fruit during cool temperature storage.

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Original Research Paper

Optimization of freeze drying parameters for moringa (*Moringa oleifera*) flower powder by using response surface methodology and principal component analysis

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ABSTRACT

***Moringa oleifera* Lam. is an incredible plant because of vital nutrients such as minerals, vitamins and phytochemicals. The present work is focused on studying the optimization and quality attributes retention in moringa flowers in a freeze dryer (FD). Because the conventional drying process takes more time and energy which will affect the product quality and safety. Response surface methodology (RSM) was employed to optimize the effect of drying temperature (- 65 to - 45°C), vacuum pressure (0.5 to 2.5 mmHg) and drying time (18 to 24 h.) on the vitamin C, total antioxidant activity(TAA) and hygroscopicity (HS) of moringa flower. The developed model response R^2 values of vitamin C 0.96, total antioxidant activity 0.97 and hygroscopicity0.95. Based on response surface and desirability (0.74) functions, moringa flower was freeze sdried at - 63.75°C for18 hr under 0.55 vacuum pressure had an optimum level of vitamin C 285.84 mg/100g, TAA 453.20 mg/100g and HS 1.57 percent. Freeze dried moringa flower powder at -55°C had maximum drying characteristics with special reference to high powder recovery (98.75%) and excellent flowability.The first principal component, accounting for 52.15 per cent and two 23.02 per cent of the total variance resolved the different drying temperatures.**

Keywords: Dehydration, Freeze drying, moringa flower, nutraceutical and response surface methodology.

INTRODUCTION

Moringa oleifera lam is also known as the "tree of life" because of its crucial importance and belongs to the solitary genus from the family *Moringaceae* and contains 13 known species (Hedhili *et al.,* 2022). Moringa is frequently regarded as a curative for all health issues or diseases. Leaves, flowers and pods of moringa are used in folk medicine to treat many diseases and they have cardiac and circulatory stimulants, anti-tumor, anti-pyretic, anti-epileptic, anti-inflammatory, anti-ulcer, anti-spasmodic, diuretic, cholesterol-lowering, anti-oxidant, anti-diabetic, anti-bacterial and anti-fungal compounds. These health-promoting effects have been attributed to its constituent phytochemicals, such as zeatin, quercetin, -sitosterol and kaempferol (Farooq *et al.,* 2022).

Moringa flower is unique because of its health benefits, medicinal and therapeutic properties. At present, the demand for moringa parts has increased annually due to off-season availability and increased utilization by people. One-fourth of moringa parts produce is spoiled during storage and transport. To prevent postharvest losses in the moringa flower, there is a need for processing, and it will meet the demand of the market throughout the year (Manju *et al.,* 2021)

Dehydration is a traditional system of preservation; done by various means of methods to reduce food moisture and plant materials. During the drying process, a higher temperature can lower the flavour, colour, heat-sensitive nutrients and bioactive compounds. The quality of the dried products can be improved by reducing the process temperature when compared to a higher temperature. (Kinki *et al.,* 2020).

The conventional drying process takes more time and energy than advanced techniques. Due to prolonged processing time and improper handling, higher temperature and pressure will affect the organoleptic characteristics and product quality. Alternatives to conventional drying, advanced dryers specifically freeze or vacuum-drying was used to dry heat-sensitive materials. (Klungboonkrong *et al.,*2018). Response surface methodology (RSM) is a popular and effective statistical tool for the optimization of interactive processes. (Sifat *et al.,* 2021). It is a technique which empowers the experimenter to determine the interrelationships between one or more responses and factors (Chakraborty *et al.,* 2020). RSM is commonly used to map a response surface over a specified region of interest, optimize responses, or choose operating settings to meet target specifications or customer requirements. (Okpe *et al.,* 2018).

Generally, moringa leaves are more focused than moringa flowers because of its high nutritional benefits and availability but the research on moringa flowers is unexplored. The present study aims to optimize the freeze-drying parameters of moringa flower powder using response surface methodology (RSM).

MATERIALS AND METHODS

Processing for the preparation of dried moringa flower powder (MFP): Healthy and disease free moringa flowers were selected. After that, a dehydration process was performed with freeze drying (Silva *et al.,* 2019) used a Liotop® L101 tabletop freeze drier the capacity of drying temperature ranged from -40°C to -80°C. Freeze drying was started at 0.15 mbar after moringa flowers were placed on unheated shelves. The ambient radiation that reached the samples through the clear glass drying chamber provided the sublimation energy.

RSM modeling experimental Design and analysis: Central Composite Rotatable Design (CCRD) is used to optimize the drying temperature of moringa flowers. Drying temperature (- 65°C, - 55°C and - 45°C), vacuum pressure (0.5, 1.5 and 2.5 mm/Hg) and drying time (18, 21 and 24 h.) were taken as the independent variables and vitamin C, total antioxidant activity (TAA) and hygroscopicity (HS) were taken as a dependent variable. The experimental runs for the varied levels of the input variables and 6 number of center points (-55°C, 1.5 mm/Hg and 21 h) were obtained using Design Expert (Version 13.0) software. CCRD as it allows for a larger spread of conditions which helps in prediction where the model involves more complexity the second-order polynomial equation was fitted into each output variable as given in Eqn 1.

$$Y = \beta 0 + \beta 1\,X1 + \beta 2X2 + \beta 3X3 + \beta 11\,X1\,X1 + \beta 22X2\,X2 + \beta 33\,X3\,X3 + \beta 12\,X1\,X2 + \beta 13\,X1\,X3 + \beta 23\,X2\,X3 \ldots \text{Eqn. 1}$$

Where Y is a response factor of the Vitamin C, TAA and HS from freeze dried moringa flower and β_0 is anintercept. Furthermore, β1 and β_2 and $\beta_3$are linear regression coefficients, β_{11}, β_{22} andβ_{33} denote interaction coefficients and $\beta_{12,}$and $\beta_{13}$and β_{23} represent quadratic coefficients. To show the interactive effects of independent factors on a single dependent variable while keeping the other variables constant then three-dimensional surface plots were created for the optimized drying process. For greater precision, an analysis of variance (ANOVA) was used to determine the coefficients of the final equation. Vitamin C, TAA and HS were taken as the dependent variables. Twenty drying experimental runs were obtained from RSM and presented in Table 1.

The dehydration characteristics of MFP were analysed, *viz.,* ascorbic acid, by the methods given by AOAC (1990), total antioxidant activity (Lim *et al.,* 2007), powder recovery, water solubility index (Grabowski *et al*., 2006), flowability, Hausner ratio, Carr index (Seerangurayar *et al,* 2017), bulk and tap density (Chegini and Ghobadian 2005), hygroscopicity (Cai and Corke 2000), water and oil absorption capacity (Rosario and Flores 1981), rehydration ratio and dehydration ratio (Ranganna, 1986).

Statistical analysis

RSM was analyzed by Design Expert (Version 13.0) software. Data analysis was performed in a completely randomized design (CRD) using SPSS 14.0 for Windows (SPSS, 2005). According to the varied drying temperatures, multivariate analysis (Principal Component Analysis - PCA) was performedon the significant variables.

Table 1 : Optimization of process parameters for moringa flower powder (MFP) by RSM

Run	Independent variable			Dependent variable [a]		
	Temperature (ºC)	Pressure (mm/Hg)	Time (min)	Vitamin C (mg)	TAA (mg/100g)	Hygroscopicity (%)
1	- 55	1.5	21	291.64	457.99	1.68
2	- 65	2.5	24	259.05	434.58	1.51
3	- 65	2.5	18	281.2	451.89	1.56
4	- 55	1.5	21	291.64	457.99	1.68
5	- 65	0.5	18	289.18	456.23	1.59
6	- 45	0.5	18	265.29	438.11	1.71
7	- 55	1.5	21	291.64	457.99	1.68
8	- 45	2.5	18	260.77	434.9	1.73
9	- 55	1.5	24	266.5	439.56	1.67
10	- 55	1.5	18	305.81	460.18	1.68
11	- 45	1.5	21	252.46	427.63	1.75
12	- 65	1.5	21	274.24	449.02	1.54
13	- 55	1.5	21	291.64	457.99	1.68
14	- 55	1.5	21	291.64	457.99	1.68
15	- 45	0.5	24	242.28	417.44	1.77
16	- 45	2.5	24	238.53	409.71	1.78
17	- 65	0.5	24	269.12	443.97	1.53
18	- 55	1.5	21	291.64	457.99	1.68
19	- 55	2.5	21	279.33	446.27	1.67
20	- 55	0.5	21	283.12	452.14	1.67

[a]Values observed in the mean value of the three replicates.

RESULTS AND DISCUSSION

RSM model effect of freezedrying on responses of the moringa flower

RSM models for the three response variables statistical summarization (R^2 and ANOVA Estimation) were presented in Table 2 and Eqn 2 to Eqn 4. The model fitting using regression analysis exhibited that the models described the relationship between the input and output variables with regression coefficient (R^2 = Vitamin C - 0.96, TAA - 0.97 and HS - 0.95) indicating that the predicted values were well fitted with the actual values in the experimental conditions. The predicted R^2 values were 0.77, 0.90 and 0.95 and are in reasonable agreement with the adjusted R^2 values of vitamin C - 0.93, TAA - 0.95 and HS - 0.99. The model F value of 29.65 (Vitamin C), 47.99 (TAA) and 360.08 (HS) with p – values < 0.0001 implies that the quadratic model is significant. All response variables were statistically significant based on p - values with 95 per cent level of significance.

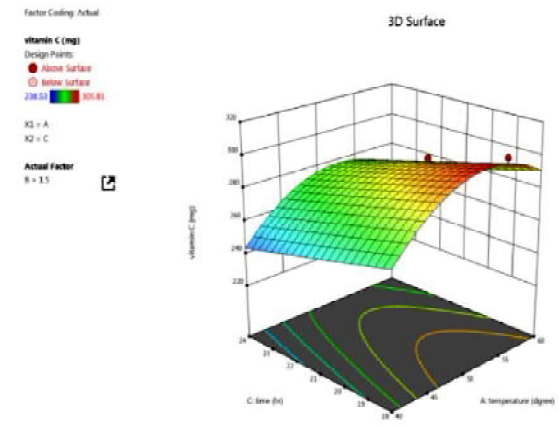
The 3D plots of the group effect of the response variables showed the synergetic effect of drying temperature, vacuum pressure and drying time (Fig.1). The three Independent factors have proven significant combined effects on vitamin C, TAA and HS. In RSM prediction, based on the desirability (0.74), the moringa flower freeze dried at - 63.754°C for 18h under 0.555mm/Hg vacuum pressure had an optimum level of vitamin C - 285.84 mg, TAA - 453.20 mg and HS 1.57 per cent 100 mg, respectively.

Whereas increasing drying temperature (- 45°C), vacuum pressure (2.5mm/Hg) and drying time (24 h)

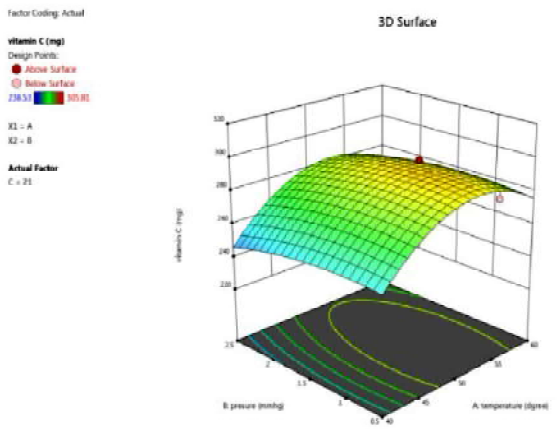
Table 2 : Regression coefficients and ANOVA estimated for response variables of freeze-dried moringa flower

Factor	Vitamin C (mg)				TAA (mg)			Hygroscopicity (%)		
	df	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value	Sum of Squares	F-value	p-value
Model	9	6253.78	29.65	< 0.0001	3983.75	47.99	< 0.0001	0.1165	360.08	< 0.0001
Linear										
A	1	271.76	11.60	0.0067	028.36	03.07	0.1101	0.0368	1024.95	< 0.0001
B	1	099.97	04.27	0.0658	079.41	08.61	0.0149	0.0004	010.54	0.0088
C	1	1188.00	50.70	< 0.0001	561.79	60.90	< 0.0001	0.0017	047.69	< 0.0001
Interaction										
A^2	1	1400.98	59.79	< 0.0001	571.54	61.96	< 0.0001	0.0022	060.37	< 0.0001
B^2	1	060.64	02.59	0.1388	34.39	03.73	0.0823	2.273	0.0006	0.9804
C^2	1	0.1507	0.0064	0.9377	22.67	02.46	0.1480	2.273	0.0006	0.9804
Quadratic										
AB	1	11.96	0.5102	0.4914	0.9730	0.1055	0.7520	0.0009	024.54	0.0006
AC	1	01.16	0.0493	0.8288	33.17	03.60	0.0872	0.0063	174.50	< 0.0001
BC	1	0.2178	0.0093	0.9251	11.45	01.24	0.2913	0.0000	001.13	0.3134
Mean ±SD	275.84 ± 4.84				445.48 ± 3.04					1.67 ± 0.0060
R^2	0.9639				0.9774					0.9969
Adjusted R^2	0.9314				0.9570					0.9942
Predicted R^2	0.7769				0.9042					0.9584
CV	1.75				0.68					0.3598

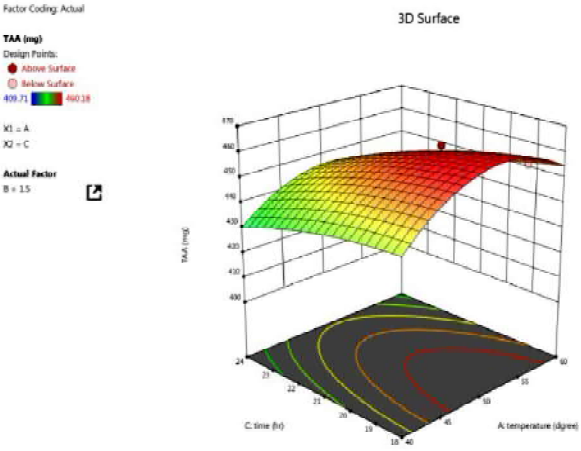
Note: The *p* values indicated that to check the significance level of eachRegression coefficient. A, B and C indicate that drying temperature, pressure and drying time respectively. The pd"0.05.are indicated as a significant effect of an independent factor on the response variable at 5 per cent.(CV, critical value; *df*, degree of freedom; *SD*, standard deviation)



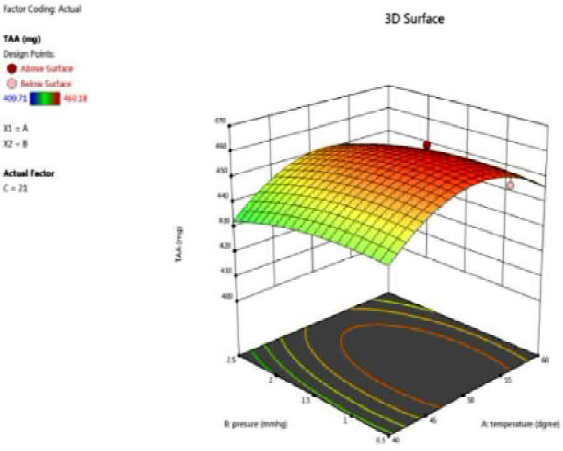
Vitamin C
(Drying temperature Vs drying time)



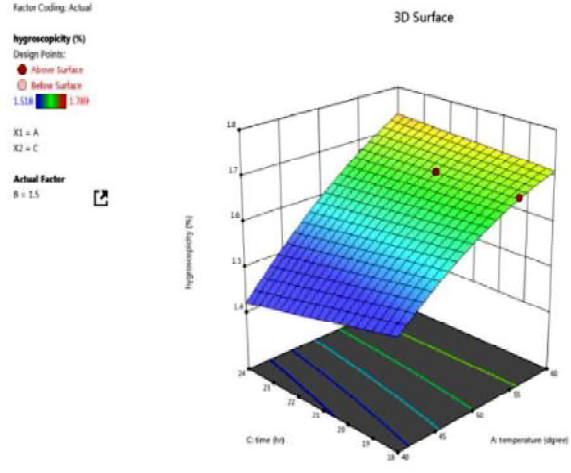
Vitamin C
(Drying temperature Vs Vacuum pressure)



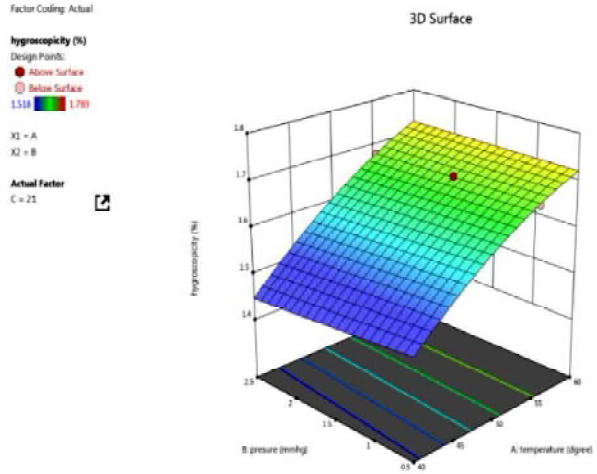
TAA
(Drying temperature Vs drying time)



TAA
(Drying temperature Vs Vacuum pressure)



HS
(Drying temperature Vs drying time)



HS
(Drying temperature Vs Vacuum pressure)

Fig. 1 : RSM 3D plots of the combined effect of independent variables on all the responses in freeze-dried moringa flower

vitamin C (305.81to 238.53 mg) and TAA (460.18 to409.71 mg) were decreased and hygroscopicity (1.51 to 1.78 %) was increased. A similar trend was recorded by Sifat *et al.* (2021) and Ademiluyi *et al.* (2018) in freeze-dried moringa at different temperatures. Due to thermal deterioration and oxidation, raising the drying temperature (- 45°C) resulted in a greater loss of heat sensitivity nutrients such as carotene and ascorbic acids. When drying temperature increase above optimum level, reduction in TAA due to the phenolic compound's high hydrogen atom-donating ability, increased phenolic content was associated with better antioxidant activity at optimum temperature (Ramarao *et al.,* 2022).

$$Y(Vitamin\ C) = +289.38 + 11.22(A) - 3.62(B) - 12.49(C) + 1.22(AB) - 0.3800(AC) - 0.1650(BC) - 22.57(A^2) - 4.70(B^2) + 0.2341(C^2) \ldots \text{Eqn. 2}$$

$$Y(TAA) = +457.68 + 3.63\ (A) - 3.23\ (B) - 8.59\ (C) + 0.3487\ (AB) - 2.04\ (AC) - 1.20\ (BC) - 14.42\ (A^2) - 3.54\ (B^2) - 2.87\ (C^2) \ldots \text{Eqn. 3}$$

$$Y(HS) = +1.62 + 0.1307(A) - 0.007(B) - 0.015(C) + 0.0105(AB) + 0.0280(AC) + 0.0022(BC) - 0.0281(A^2) - 0.0001(B^2) - 0.0001(C^2) \ldots \text{Eqn. 4}$$

Optimization of freezedrying for the production of moringa flower powder by PCA

The results of the principal component analysis (PCA) are presented in Table 3 and Fig. 2 : PCA was applied to describe the relationship between the different dehydration variables and to identify the most important sources of variability *viz.,* different drying temperatures.

The PCA results (75.17 %) clearly showed the variances among A (- 65°C), B (- 55°C) and C (- 45°C) of the different drying temperatures. The first principal component, accounting for 52.15 per cent and PC2 accounted for an additional 23.02 per cent of the total variance resolved the different drying

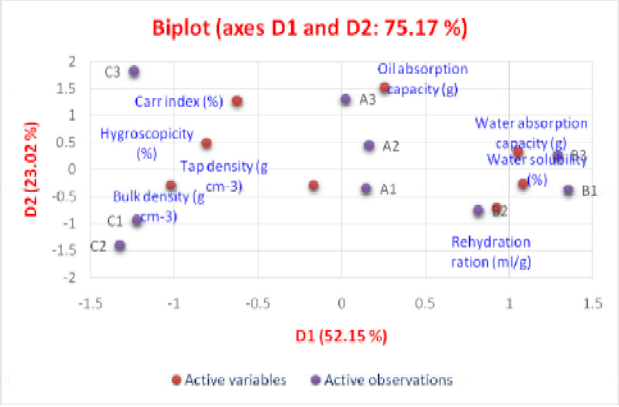


Fig. 2 : Dehydration characteristics variables as a function of first (PC1) and second (PC2) principal components

temperatures according to the dehydration characteristics. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy for the PCA is 0.61. To further investigate the contributors to the principal components, the factor loadings in PC1 and PC2 were compared (Table 4). The eigenvalues for the F_1, F_2 and F_3 (Factor) were 3.56, 1.98 and 0.801. In factor loading F_1, water solubility (0.98), rehydration ratio (0.90) and water absorption capacity (0.86) were positively correlated and bulk density (- 0.87), carr index (- 0.68) and hygroscopicity (- 0.72) were negatively correlated. Similarly, in F_2 and F_3, oil absorption capacity (0.91) and tap density (0.69) had a positive correlation whereas other variances had a negative correlation. These results suggested that the reasonable score range of the principal components could be used for excellent sample selection according to the correlations between the original three variables and these two principal components.

The moringa flowers were freeze-dried at - 55°C (B) and had 98.75% powder recovery, whereas increasing the temperature (- 45°C) led to a lower process yield. The water solubility index of the MFP increased (65.53 to 78.75 %) with increasing drying temperature. The bulk density and tap density of the FD- MFP varied from 0.43 - 0.48 and 0.30 - 0.45g cm^{-3} respectively. During the drying process, a higher drying temperature (- 55°C and - 45°C) will reduce the density of the powder to rapidly remove moisture. The effects of functional characteristics such as water absorption capacity (WAC) and oil absorption capacity (OAC) in dried drumstick powder were analysed. The WAC was 1.96 g at - 65°C (A), 2.09g at - 55°C (B) and 2.22 g at - 45°C(C). The OAC ranged between 4.10 g and 4.67 g at different temperatures. The WAC was higher at 65°C, and the OAC was higher at - 55°C, which may be due to the protein concentration where the binding of the hydrocarbon chains of oil to the non-polar side chains of the amino acids (Wang *et al.,* 2020). The values of WAC (23.2%) and OAC (18.5%) compared favourably with the results of spinach (*Amaranthus hybridus*) by Adeyey and Omolayo (2011).WAC is an important dehydration characteristic that correlates to the function of hydrophilic molecules such as proteins, carbohydrates and dietary fibre. However, the OAC of palmyra palm flours facilitates the improvement of flavour and mouthfeel during food preparation (Abe – Inge *et al.,* 2018).

Table 3 : Dehydration characteristics of freeze-dried moringa flower powder

Particular	Different drying temperature		
	- 65°C	-55°C	-45°C
Powder recovery (%)97.02±2.26[c]	98.75±1.08[a]	98.12±2.23[b]	
Water solubility (%)65.53±1.88[b]	78.75±1.64[a]	72.30±0.32[c]	
Bulk density (g cm^{-3})0.48±0.03[a]	0.43±0.08[a]	0.44±0.02[a]	
Tap density (g cm^{-3})0.45±0.09[a]	0.30±0.04[a]	0.42±0.04[a]	
Dehydration ratio (ml/g)	20.05±0.13[b]	21.69±0.50[c]	19.22±0.02[a]
Rehydration ration (ml/g)	3.92±0.06[a]	4.12±0.04[a]	4.38±0.12[b]
Water absorption capacity (g)	1.96±0.06[a]	2.09±0.01[a]	2.22±0.05[a]
Oil absorption capacity (g)	4.10±0.16[b]	4.52±0.11[b]	4.67±0.16[b]
Hausner's ratio 1.18±0.01[a]	1.28±0.01[a]	1.31±0.02[a]	
Carr index (%) 19.20±0.30[c]	19.51±0.58[c]	19.90±0.17[b]	
Flowability Good	Excellent	Excellent	
Hygroscopicity (%)1.52±0.03[a]	1.68±0.04[a]	1.71±0.02[a]	

Table 4 : Principal Component Analysis, Eigenvalues and factor loadings

Variance	Different factors				
	F_1	F_2	F_3	F_4	F_5
Eigen value	03.56	01.98	0.80	0.53	0.20
Variability (%)	54.21	25.42	9.96	6.74	2.60
Cumulative (%)	54.21	79.63	89.59	96.33	98.94
Factor (F) loadings					
Water solubility (%)	0.98	0.07	-0.03	-0.10	-0.04
Bulk density (g cm^{-3})	-0.87	-0.38	-0.05	0.10	0.27
Tap density (g cm^{-3})	-0.20	-0.65	0.69	0.19	-0.11
Rehydration ration (ml/g)	0.90	-0.18	-0.19	0.30	-0.10
Water absorption capacity (g)	0.86	0.28	0.27	0.23	0.11
Oil absorption capacity (g)	0.05	0.91	0.50	0.11	0.17
Carr index (%)	-0.68	0.63	0.18	-0.18	-0.22
Hygroscopicity (%)	-0.72	0.32	-0.25	0.53	-0.12

The dehydration ratio of FD-MFP ranged between 19.22 and 21.69 ml/g at different drying temperatures. The dehydration ratio was observed to be higher at - 55°C (B) because of the incomplete removal of moisture and lower at - 45°C due to the complete removal of moisture (heat air treatment). Similarly, the rehydration ratio ranged between 3.92 and 4.38 ml/g. The rehydration ratio was observed to be lower at - 65°C (A) due to incomplete reabsorption.

Manju *et al.* (2021) found comparable patterns in freeze-dried moringa, expressing outstanding flowability (Hauser's ratio and carr index) hygroscopicity (1.50 – 1.87), dehydration ratio (17.98 – 23.89) and rehydration ratio (3.37 – 3.72). Potisate *et al.* (2015) also reported the same trend in FD-MFP its having Excellent flowability, hygroscopicity (1.25–2.30) and rehydration ratio

(3.82 – 4.22). As a result, the loss of water and heat causes stress in the product's cell structure, causing the powder to become hygroscopic and dehydration will reduce the dimensions of freeze-dried moringa flower powder.

CONCLUSION

In RSM, the developed model response R^2 values were 0.96 (Vitamin C), 0.97 (TAA) and 0.95(HS) of freeze dried moringa flower powder. Based on response surface and desirability (0.74) functions, moringa flower freeze-dried at - 63.75°C for18 h under 0.55 vacuum pressure had an optimum level of vitamin C 285.84 mg/100g, TAA 453.20 mg/100gand HS 1.57 percent 100 mg. The optimal drying temperature of -55°C results in improved dehydration features and a high powder recovery (98.75%) as well as great flowability. Based on the findings, freeze drying can be considered as one of the best drying techniques to preserve the nutritional quality features of moringa while also being an efficient and effectively utilized for the entire food processing industry**.** Moringa flower powder, which has been freeze dried, can be used to make novel functional foods. Moringa-infused food products will be ideal for commercialization and will help to reduce the nutritional deficit in the community's most vulnerable residents.

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Original Research Paper

Optimization of factors influencing osmotic dehydration of aonla (*Phyllanthus emblica* L.) segments in salt solution using response surface methodology

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ABSTRACT

Optimization of process parameters is a critical requirement in food processing and food product industries for the development of highly acceptable product. Quantification of mass transfer kinetics under different processing conditions is essential step for optimizing the osmotic dehydration process. A Box-Behnken Design (BBD), adopted from response surface methodology (RSM) approach was used for evaluating and quantifying the moisture loss and solids gain kinetics of aonla segments in salt solution during the osmotic dehydration process. The independent variables were fixed at three levels (salt concentration- 2, 4, 6%; process temperature - 45, 50, 55^{O}C and process time - 60, 120, 180 minutes). The process responses were water loss percentage (WL%) and solids gain percentage (SG%). Validation experiments were conducted at optimum conditions to verify predictions and adequacy of the models. The optimum conditions predicted were 5.02% salt concentration, 54.8 ^{O}C temperature and 60.64 minutes process time to attain a desired effect of maximum water loss (6.42%) and minimum solid gain (1.09%) in osmotic dehydration of aonla in salt medium.

Keywords: Aonla, Optimization, Osmotic dehydration, Response Surface Methodology

INTRODUCTION

Phyllanthus emblica L. (aonla), which is indigenous to tropical India and Southeast Asia is one of the most indispensable crops in various traditional and folk systems of medicine. Being rich in ascorbic acid and bioactive constituents (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apigenin, quercetin, corilagin, leutolin, *etc*.), it has been recognized as an excellent source of anti-oxidants to consider as significant dietary source and explored for therapeutic potential against various diseases (Nashine *et al*., 2019; Alsahli *et al*., 2021). The area and production of aonla in India was estimated to be 92 (000 hectares) and 1039 (000 MT) respectively (NHB, 2019). To increase the shelf-life of aonla fruits many methods or combination of methods had been tried that undergo phases of high temperature and time combinations. These significant high temperature processing would impair fresh quality of the food and hence result in the products without original flavor, color and textural attributes after rehydration (Alam *et al*., 2010).

Being highly perishable in nature and highly astringent in taste, it demands processing and value addition for enhanced shelf life to extend its availability throughout the year (Liu *et al*., 2012). Dehydration is found to be a preferred technique to preserve the aonla fruits in the context of producing the high-quality shelf-stable fruit products to enhance the product availability and extend the marketability (Li *et al*., 2019). Since osmotic dehydration is considered as a pre-treatment to aonla dehydration that can improves nutritional, sensorial, and functional characteristics of fruit without altering its integrity, it is exploited effectively (Gantait *et al*., 2021). The process optimization and product quality improvement should be focused well to achieve target specifications. In spite of the numerous researches that have been undertaken on this subject, it is still hard to establish

general rules about the variables that influence the osmotic dehydration. Rate of water loss and solid gain which are the intrinsic aspects of the mass transfer kinetics depends on both operating conditions and kind of cellular microstructure, as well as on the product form in which it was pretreated (Pandiselvam *et al*., 2021). Mass transfer rate upsurges with product surface area and rise in temperatures. In contrast, the ratio of water loss to solid gain depends on the concentration of solute and its molecular weight (Pravitha *et al*., 2022). Furthermore, the use of solutes of high molecular weight enables water loss at the expense of solid gain. The driving force for moisture diffusion is the high osmotic pressure employed by the osmotic medium. The moisture diffusion is accompanied by a concurrent counter-flow diffusion of solutes from the osmotic medium to the fruit matrix. Since the membrane for the osmotic process is not perfectly selective, native solids present in the cells can also be leached into the osmotic solution.

The optimization of the osmotic dehydration process is usually executed to guarantee the rapid processing conditions for development of a product with acceptable quality and achieve a high throughput capacity (Sharma *et al*., 2020). Several studies have been carried out to evaluate the influence of process variables (concentration of the osmotic solution, process temperature, processing time, agitation, food geometry, medium to sample ratio, *etc*.) on the mass transfer kinetics of conventional osmotic dehydration processes (Tiroutchelvame *et al*., 2015). It is important to note that these variables can only be altered over a restricted range, outside of which they adversely affect the quality even though the mass transfer rates may be enhanced (Souraki *et al*., 2012; Herman-Lara *et al*., 2013). Hence, there is a requirement to determine the optimum operating conditions that increases the mass transfer rates without affecting the quality (Garcia-Segovia *et al*., 2010).

With this framework, response surface methodology (RSM) is an important tool in process and product improvement which enables the determination of the relationship between the response and the independent variables. It is characteristically utilized for mapping a response surface over a particular region of interest, optimizing the response, or for selecting operating conditions to achieve target specifications (Box and Draper, 1965; Myers *et al*., 1989; Myers *et al*., 2016; Beegum *et al*., 2018; Pandiselvam *et al*., 2019; Pandiselvam *et al*., 2022).

The objective of the present study was to quantify the effect of salt concentration, process temperature, and contact time on the moisture loss and solid gain during osmotic dehydration of aonla segments using a "Box Behnken Design".

MATERIALS AND METHODS

Procurement of fruits

Freshly harvested aonla fruits of commercial variety "Krishna" of appropriate maturity (large-sized, sound fruits with adequate flavor and gloss, uniformity in color) procured from ICAR-Indian Institute of Horticultural Research, Bengaluru farms. Fresh mature fruits with uniform size, free from injuries, bruises, insect damages and diseases were used in this study.

Optimization process

Based on the literature reviews independent variables for this study were selected as concentration of osmotic solution, process temperature and treatment duration which were found to influence the quality of the final product appreciably (Eren and Kaymak-Ertekin, 2007; Alam *et al*., 2010).The independent osmotic process variables and their levels in the form of coded variables for three-factor three level response surface analyses are given in Table 1.A three-factor three level Box-Behnken design model with three replicates at the central point, which gives 15 experiments was selected to study the influence of processing parameters on mass transfer attributes (Table 2).The levels of processing parameters were chosen as independent variables: Osmotic solution concentration, Treatment temperature, and Process duration; whereby each of these variables was tested at three different coded levels: low (" 1), medium (0), and high (+1). Outcomes of preliminary trials were aided in setting the range of these independent variables. The responses considered attaining optimum condition were water loss (WL) and solid gain (SG) as these are the combination of significant processes or fluxes co-occurring, determining the attainment of equilibrium in osmotic dehydration process as a unit operation and also important for both quantitative modeling and

knowledge of the kinetics of mass transfer in the system.

Osmotic dehydration experimental setup

The desired concentration of osmotic solution of salt (2-6%) was prepared and 150g of aonla segments were immersed in beaker containing osmotic solutions (600 ml) of varied temperatures (45-55 !), fruit to solution ratio (1:4 w/v) in order to ensure that the concentration of osmotic solution did not change significantly during the experiment and contact time of 60-180 min. From the preliminary experimental results obtained from experiments conducted in the laboratory, the process variables and their ranges were selected. The osmotic dehydration process was carried out in a temperature-controlled chamber (Digital Multi Chamber Water Bath (Model-WMB 306, Daihan Scientific Co., Ltd). The experiments were carried out in randomized replicated order to minimize the variability in the observed responses due to extraneous factors. All the experiments were performed in triplicate and the mean value was used for the determination of mass transfer parameters.

Osmotic dehydration process

For each experiment, known weight of aonla segments were put in the glass beakers having calculated volume (as per STFR-Solution to fruit ratio of 1:4) of osmotic solutions of different concentrations pre-set at the desired temperature by water bath at atmospheric pressure. This dehydration process was carried out in a water bath with temperature ranges from 45-55 ^{0}C to make the process effective. At the specified times the aonla segments were removed from the osmotic solutions and rinsed with water to remove surplus solvent adhering to the surfaces. These osmotically dehydrated aonla segments were then spread on the absorbent paper to remove free water present on the surface. A proportion of pre-treated aonla segments (5–8 g) were used for determination of dry matter by oven method (AOAC, 2000). The remaining part of each sample was dried to final moisture content of 10 % (wet basis) using hot air drier (Tray dryer, TD-2A, CM Envirosystems Pvt. Ltd) pre-set at 60°C air temperature.

Determination of mass transfer properties

In osmotic dehydration, both water loss and solid gain take place concurrently. The reduction in weight is attributed to the loss of water from the sample and increase in the weight of the sample due to solute gain from the osmotic solution. The evaluation of mass transfer between the solution and samples during osmotic dehydration process were estimated by using the parameters such as water loss % (WL) and solid gain % (SG) and the parameters were calculated by using the following equations (Sridevi and Genitha, 2012)

$$WL(\%) = \frac{(Wo - Wt)}{Mo} \times 100$$

$$SG(\%) = \frac{St - S0}{Mo} \times 100$$

where, M_0 = initial mass of the sample (g); W_0 = initial water mass of the sample (g); W_t= water mass of the sample (g) after dehydration; S_0 = initial dry mass of the sample (g) and S_t= dry mass of the sample (g) after dehydration

Microstructure analysis

The fresh, and osmotically treated aonla segments were examined using scanning electron microscopy (SEM) in order to determine the effect of osmotic dehydration process on the microstructural changes of the tissue. Samples were cut into cubes with a sharp blade and mounted on aluminium SEM stubs and fixed it. The microstructure of the tissue was examined by a scanning electron microscope (Hitachi, TM3030 plus) and the images were recorded at the magnification of 250X (Mayor *et al*., 2008).

Experimental design

An experimental plan and further statistical analysis of data with regression model fitting for each response were carried out by using response surface analysis with Box-Behnken Design (BBD), SAS Statistical Software package version 9.3 (SAS Institute, Cary NC). Response values were analysed by fitting the data in a second-order polynomial model. The generalized second-order polynomial model proposed for predicting response variables is given as: $Y=B_0+ B_1X_1+ B_2X_2+ B_3X_3+ B_{12}X_1X_2+ B_{13}X_1X_3+ B_{23}X_2X_3+ B_1^2 X_1^2 + B_2^2 X_2^2 + B_3^2 X_3^2$

In this equation, Y represents the dependent variable (the estimated response) and Xi (i = 1–3, 1-C(concentration), 2-T(Temperature), 3-t(time))

represent the independent variables. Coefficients of the polynomial were represented by B_0 (constant term), B_1, B_2 and B_3 (linear coefficients for C, T and t respectively), B_1^2, B_2^2 and B_3^2 (quadratic coefficients), and B_{12}, B_{13} and B_{23} (interactive coefficients). Model adequacy by ANOVA. The fitness of the models was further affirmed based on statistical parameters such as coefficient of determination (R^2), F test value and lack of fit. Interaction effects of independent variables were pictorially represented by 3-D response surface

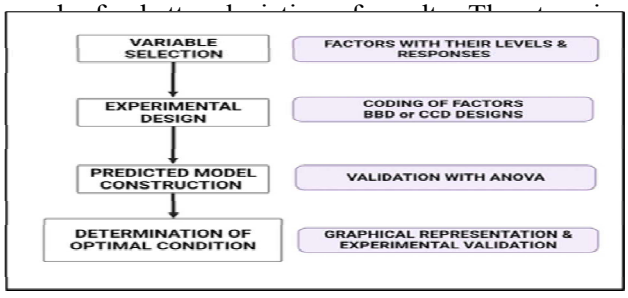

Fig. 1 : Step-wise procedure adopted for response surface methodology analysis

procedure adopted for response surface methodology analysis is given in Fig.1.

Model optimization and validation

Optimization of independent variables was performed by multi-criteria methodology with the analysis of Derringer function or desirability function (Bezerra *et al*., 2008). Further validation of the optimization was performed by comparing predicted and experimental value. The deviation between these two can be assessed by calculating absolute error by using equation as below:

$$\text{Absolute error (\%)} = \frac{\text{Experimental value} - \text{Predicted value}}{\text{Predicted value}} \times 100$$

RESULTS AND DISCUSSION

The observed values of response variables are given in Table 2. The generalized second-order polynomial model proposed for predicting response variables were generated. The data was analyzed employing multiple regression technique to develop a response surface model. A second order model with and without interaction terms were tested for their adequacies to describe the response surface and R^2 values were calculated. The models were compared based on their coefficient of determination (R^2), adjusted coefficient of determination (R^2 -adj), predicted coefficient of determination (R^2 -pred), and the probability (p) of lack of fit. All two models (WL and SG) were tested for their adequacy using ANOVA technique. F-values for the lack of fit were nonsignificant ($p < 0.01$) thereby confirming the validity of the models. The significant terms of the response variable were determined by ANOVA and given in Table 3.

Effect of process variables on responses

The mass transfer parameters i.e., water loss (WL) and solid gain (SG) reflecting as one of the quality attributes of aonla during osmosis. The rate of water loss during osmotic dehydration depends upon the solution temperature, solution concentration, and immersion time (Alam and Amarjit ,2010; Salimi *et al*., 2020; Pinto *et al*., 2021; Zeghbib *et al*., 2022).

Effect of osmotic dehydration process parameters on the water loss (WL %)

WL is a major parameter of mass transfer that shows the efficiency of the osmotic dehydration process. A wide variation in all two responses was observed for different experimental combinations from 1.76 to 6.08 % in WL. The maximum value (6.25%) of water loss was observed for experimental combination of salt concentration of 6%, treatment temperature of 55! and treatment duration of 120 minutes (Table 2). Among the process variables studied, the concentration witnessed maximum effect on water loss. It can be seen that water loss was found to be significantly affected by concentration individually, ($p < 0.01$) and

Table 1 : Coded and uncoded values of process variables and their levels during osmotic dehydration of aonla segments in salt solution.

Independent Variables	Coded Levels	-1	0	+1
Solution concentration, %	X_1	2	4	6
Temperature, °C	X_2	45	50	55
Time, minutes	X_3	60	120	180

X_1, X_2, X_3: Coded independent variables

Table 2 : Outline of the experimental design matrix and observed values of response variables

Treatment run	Independent variables			Responses	
	Concentration (%)	Temperature (°C)	Time (minutes)	WL %	SG %
1	2	45	120	1.85	0.22
2	4	55	180	2.64	0.76
3	2	50	60	1.75	0.25
4	4	45	180	2.08	0.66
5	4	50	120	2.21	0.85
6	6	50	60	6.03	1.00
7	6	55	120	6.25	1.07
8	2	50	180	1.80	0.33
9	4	55	60	6.05	1.22
10	6	50	180	6.08	1.24
11	4	50	120	2.21	0.85
12	4	45	60	2.06	0.74
13	4	50	120	2.21	0.85
14	2	55	120	1.76	0.35
15	6	45	120	5.11	1.56

X_1, X_2, X_3: Coded independent variables; WL: Waterloos; SG: Solid gain

Table 3 : ANOVA of the second order polynomial models of the various responses.

ANOVA	WL %	SG %
Mean of response	3.33	0.79
RMSE	0.79	0.21
R^2	0.94	0.90
Adj- R^2	0.82	0.71
PRESS	50.17	3.56
Model F value	8.45	4.81
P value	0.01^S	0.04^S
Lack of fit	0.93^{NS}	0.96^{NS}

S - Significant at 1-5%; **NS -** Non-significant

in the quadratic and interaction terms on water loss, whereas other factors such as temperature, time and its interactions found non-significant effect on water loss (Table 4).

Second order response surface model resulted in significant ($P<0.06$) estimates for the factor concentration and its interaction as tested by their corresponding student t-test value being significant ($P<0.05$) (Table 4). The RSM solution is minimum for the response WL at 1.16 corresponding to the critical values of 2.38,46.15 and 107.26 for concentration, temperature and time, respectively. All three linear terms, interaction and quadratic terms of solution concentration (C) showed a significant ($p < 0.05$) influence in the model prediction of WL ($R^2 = 0.94$). WL was significantly influenced ($P < 0.05$) by the variation in salt concentration only with insignificant linear, quadratic and interaction terms of temperature and time. Presence of interaction of process variable WL with concentration, temperature and time variables as seen in the plots justified for the choice of RSM with interaction effect as constructed (Fig. 3). The sign and magnitude of the coefficients indicate the effect of the variable on the response. Negative sign of the coefficient means decrease in response when the level of the variable is increased while positive sign indicated increase in the response. Significant interaction suggests that the level of one of the interactive variables can be increased that of other decreased for constant value of the response (Draper and Hunter,1966; Montgomery, 2004). From Table 4 and Fig. 3, levels of salt concentration were in positive correlation with WL in both linear and

interaction terms. The increasing trend of WL with solute concentration could be due to the enhanced osmotic pressure gradients. Comparable results showing the enhancement of the solution concentration resulting in an increase of the osmotic pressure gradients and, hence, higher WL in solutions (Cichowska *et al*., 2018).

Effect of osmotic dehydration process parameters on solid gain (SG %)

A wide variation in all two responses was observed for different experimental combinations from 0.22 to 1.56 % in SG. The maximum value (1.56%) of solid gain was observed for experimental combination of salt concentration of 6%, treatment temperature of 45^0C and treatment duration of 120 minutes. It can be seen from Table 4 that solute gain was found to be significantly affected by concentration individually ($p < 0.01$) whereas in the quadratic and interaction terms showed non-significant effect on solid gain. Studies elucidated similar findings that in osmotic dehydration, the concentration gradient between the intracellular fluid and osmotic solution create a difference of osmotic pressure, which leads to diffusion of water and solid molecules through the semi-permeable membrane of this fruit to achieve osmotic equilibrium, thus increase in solute concentration led to increases in SG (Wiktor *et al*., 2022).

Summary of fit and actual vs predicted plot analysis

RSM is able to capture 93.8% and 89.6% of model response using the experimental runs considered for three response factors each at three level as discussed earlier, which is also supported graphically for WL and SG respectively. In the Actual-by-Predicted Plot (Fig. 2), the mean line falls outside the bounds of the 95% confidence curves (red-dotted lines), which tells you the model is significant. Results of ANOVA table revealed that the model can adequately express the response variable (Prob(F) being <0.05) which is also evident with the results of lack of fit (Prob(F) being >0.05). PRESS (Prediction Residual Error Sum of Squares) statistic value along with low PRESS root mean square error (RMSE) of 1.82 too indicated that least prediction error at this solution and supported that the model fits well for the runs considered (Table 3). As the F-values for the lack of fit were non-significant ($P<0.05$), the model adequacy is well attained.

The prediction formula as elucidated below, were used to construct surface plots individually for WL and SG by considering two independent variables at a time. Cursory look into the various surface plots too revealed that the maximum water loss to the extent of 6.42 % and maximum solute gain of 1.08 %, which corresponds to optimum level of 5.02 %, 54.8^0C and 60.64 minutes for concentration, temperature and time, respectively.

Prediction formula for Water loss (WL %):

$\mathbf{Y_2}$ = 2.21 + 2.03875 * ((C - 4) / 2) + 0.7 * ((T - 50) / 5) + -0.41125 * ((TIME - 120) / 60) + (C - 4) / 2 * (T - 50) / 5 * 0.3075 + (C - 4) / 2 * (TIME - 120) / 60 * - 1.11022302462516e-16 + (T - 50) / 5 * (TIME - 120) / 60 * -0.8575 + (C - 4) / 2 * (C - 4) / 2 * 1.12 + (T - 50) / 5 * (T - 50) / 5 * 0.4125 + (TIME - 120) / 60 * (TIME - 120) / 60 * 0.585

Prediction formula for Solid gain (SG %):

$\mathbf{Y_1}$ = 0.85 + 0.465 * ((C - 4) / 2) + 0.0275 * ((T - 50) / 5) + -0.0275 * ((TIME - 120) /60) + (C - 4) / 2 * (T - 50) / 5 * -0.155 + (C - 4) / 2 * (TIME - 120) / 60 * 0.04 + (T - 50) / 5 * (TIME - 120) / 60 * -0.095 + (C - 4) / 2 * (C - 4) / 2 * -0.095 + (T - 50) / 5 * (T - 50) / 5 * 0.045 + (TIME - 120) / 60 * (TIME - 120) / 60 * -0.05

Where**, Y1:** Solid gain (%); **Y2:** Water loss (%); **C:** Concentration of the salt solution (%); **T:** Temperature of the process (°C)

Optimization of osmo-dehydration process

Only a significant and precise model can supply reliable and essential information for optimizing the results (Ade Omowaye *et al*., 2002; Romero *et al*., 2022). The predictive models are used to generate response surfaces within the experimental range. The response surface plot is the theoretical three-dimensional plot (3D surface) showing the relationship between the response and the independent variables (Bezerra *et al*., 2008; Yuan *et al*., 2018). It is a two-dimensional screen of the surface plot, in which, ranges of constant dependent variables are drawn in the plane of the independent variables (Alam and Singh, 2010; Shameena *et al*., 2019). Fig.4 shows the response surface plot of aonla WL rate under the effects of input parameters of osmotic temperature, time, and solute

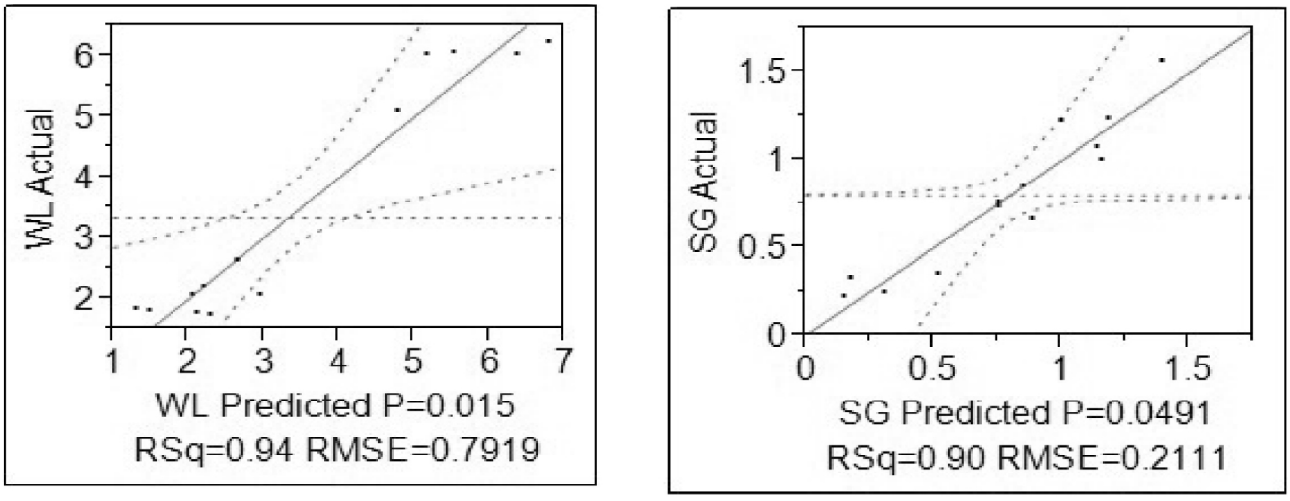

Fig. 2 : Actual vs Predicted plot for WL % and SG %

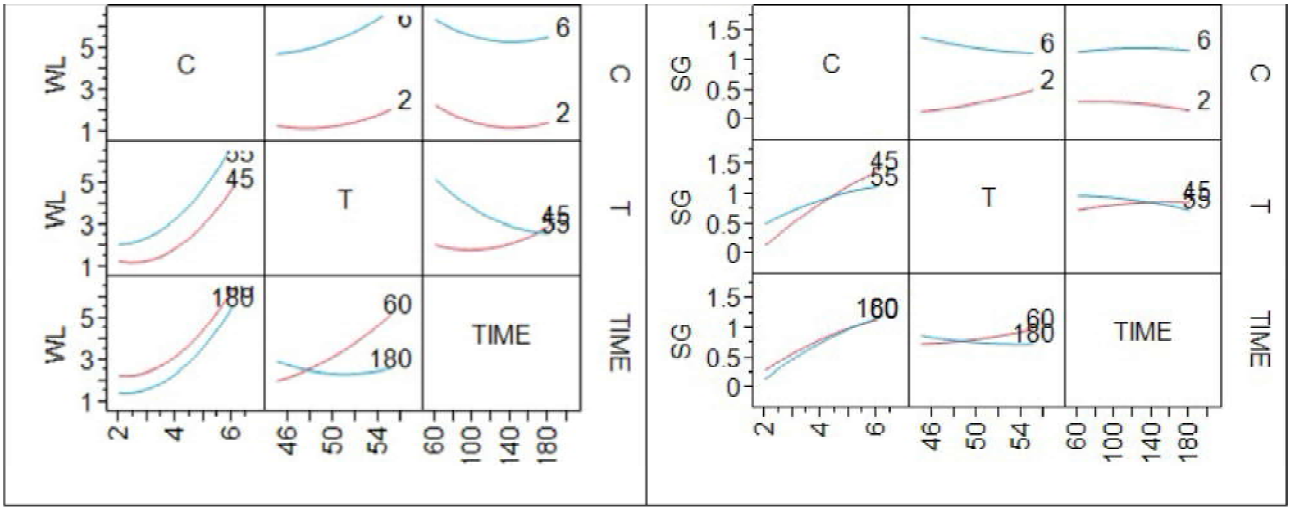

Fig. 3 : Interaction profiles of WL and SG with process variables

Table 4 : Regression coefficients of the quadratic polynomial for salt during optimization

Coefficients	WL %	SG %
Intercept	2.21^{S}	0.85^{S}
C (2,4,6)	2.03875^{S}	0.465^{S}
T (45,50, 55)	0.7^{NS}	0.0275^{NS}
Time (60,120, 180)	-0.41125^{NS}	-0.0275^{NS}
C*T	0.3075^{NS}	-0.155^{NS}
C*Time	-1.11e-16^{NS}	0.04^{NS}
T* Time	-0.8575^{NS}	-0.095^{NS}
C*C	1.12^{S}	-0.095^{NS}
T*T	0.4125^{NS}	0.045^{NS}
Time*Time	0.585^{NS}	-0.05^{NS}

* Significant at 1 and 5% by t-test; S-Significant; NS-Non-significant

concentration, considering the interactive effect of variables. Some profiles for the quadratic response surface plot in the optimization of the two parameters were obtained by keeping the other parameter at zero levels for WL rate in order to visualize the interaction effect of the two factors on the response. The osmotic stress may consequence in disruption of cell membrane and thereby enhanced the cell permeability, enabling the mass transfer between fruit matrix and osmotic medium. Thus, by increasing salt concentration, solute diffusivity can attain a maximum level, with succeeding drop thereafter. In addition, at higher concentrations, the solids could collect faster on surface of product, causing an extra hindrance for mass transfer causing least rate of solid gain (Eren *et al*., 2007; Herman-Lara *et al*., 2013).

Optimization of independent variables was performed by multi-criteria methodology with the analysis of Derringer function or desirability function showed that the prediction profiler constructed with high desirability of 0.82 corresponding to maximum water loss to the extent of 6.42% and minimum solid gain of 1.08% which corresponds to optimum level of 5.02%, 54.8! and 60.64 min. for concentration, temperature and time, respectively (Fig.5). The prediction profiler is a way to interactively change variables and look at the effects on the predicted response.

Validation studies

Osmotic dehydration experiments were conducted at the optimum process conditions (at concentration of 5.02, temperature of 54.58! and process time of 60.64 min.) for testing the adequacy of model equations for predicting the response values. The observed experimental values (mean of 3 experiments) and values predicted by the equations of the model are presented in Table 5. Therefore, it could be concluded from above discussion that model is quite adequate to assess the behavior of the osmotic dehydration of aonla. The fitted values predicted by the models were compared with the experimental data. Under these optimal conditions, the experimental value of WL and SG rate is consistent with the predicted value with 1.09% and 3.66% difference respectively (Liu and Peng, 2017; Pravitha *et al*., 2021; Bchir *et al*., 2020). Perusal of results presented in Table 5 showed minimal deviation between observed and predicted values for both the responses, which further strengthen the conclusion drawn about the suitability of the model developed.

Adequacy of the models

Residual analysis

Even though, the F-values for the lack of fit were non-significant ($p < 0.05$) which clearly shows the model adequacy, residual analysis is done to confirm the developed model to make sure that it gives a sufficient approximation to the actual values residual is the difference among the observed and fitted values (Table 6). Model generated residuals were subjected to detailed residual analysis as sole reliance of R^2 and adjusted R^2 is not enough to ensure the repeatability of the RSM results. Run test statistic Z value in both the cases being less than 1.96, showed that the residuals are randomly distributed. Also, Shapiro-Wilk test statistic value of near to unity for both the response variables too ensured that the residuals are normally distributed. These two conclusions further strengthen the adequacy of RSM models constructed in this study.

Microstructure analysis

The image analysis enabled to show how far the solid penetrates inside the fruit and to estimate the shrinkage factor of the fruit during the osmotic dehydration (Rodrigues and Fernandes, 2007).

Fresh sample: It appeared as a compact structure comprising of swollen cells intimately bonded each other through extensive cell-to-cell attachments (Fig. 6a) with a greater degree of cell compartmentalization and small intracellular spaces of fresh tissue were noticed (Nunes *et al*., 2008).

Osmotically treated sample: It is observed that osmotic dehydration process (at optimal condition) altered the tissue structure compared to the untreated samples (Fig. 6b). The cells were observed shrunk and distorted and their contour seemed irregular and wrinkling probably due to the solubilization of polysaccharides that constitutes the cells walls (Brochier *et al*., 2015). Also, the water loss and the pre-concentration of salt on the surface of the tissue during the process caused shrinkage and tissue collapse. Furthermore, water loss induces the plasmolysis of cells and solid gain gives consistency to the tissues. There are several

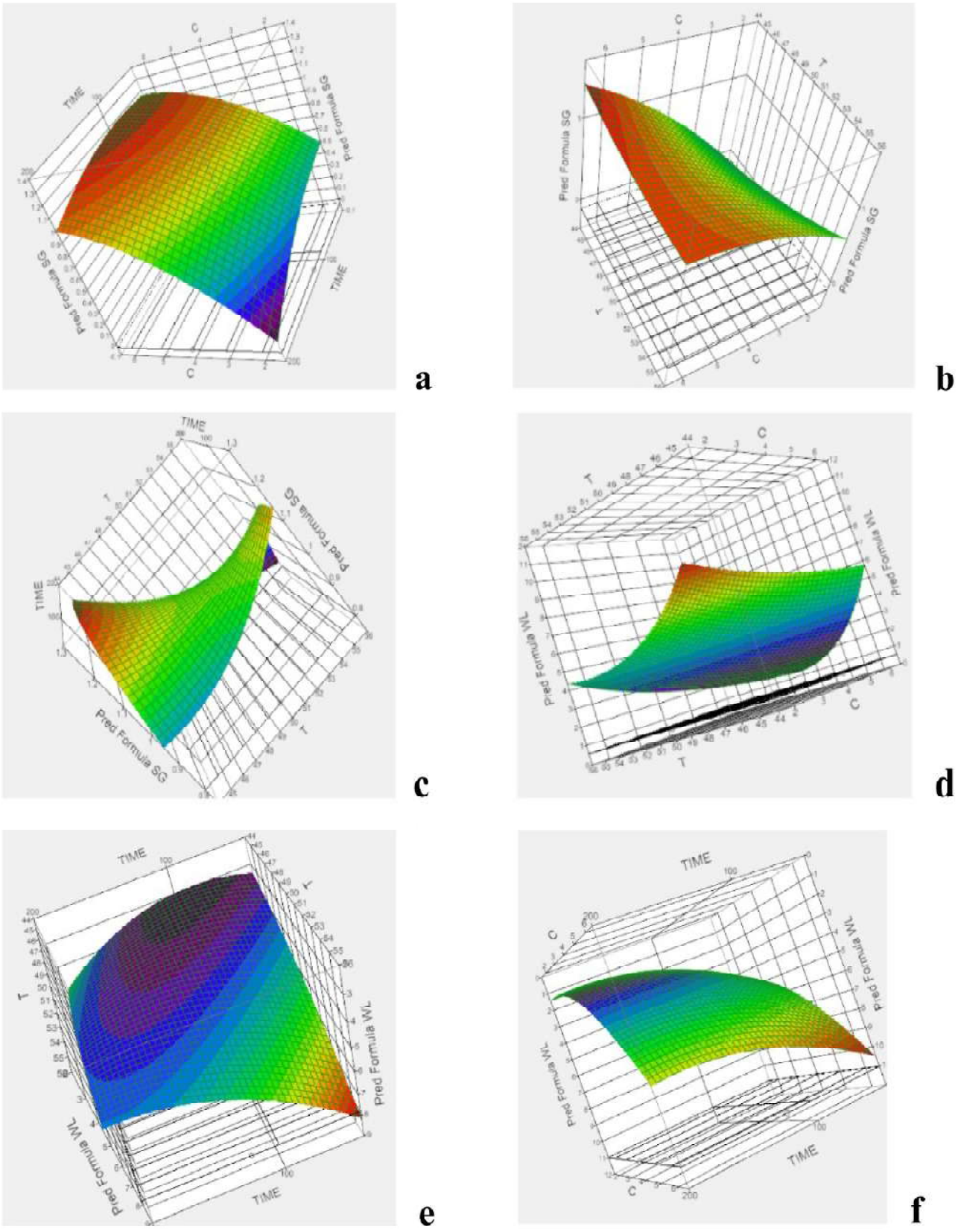

Fig. 4 : Illustration of profile for the quadratic response surface plot in the optimization of two variables for WL and SG

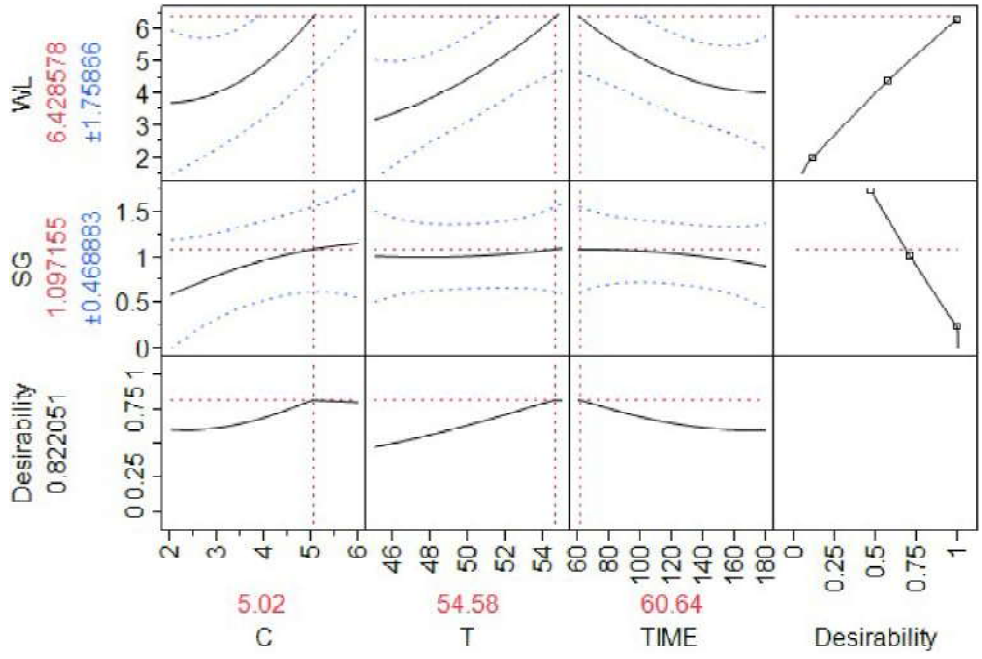

Fig. 5 : Prediction profiler for optimized conditions

Table 5 : Predicted and experimental values of response at optimum process conditions for osmotic dehydration of aonla.

Variance	Predicted value	*Experimental value	Absolute error (%)
Water loss (WL)	6.42	6.35	1.09
Solid gain (SG)	1.09	1.13	3.66

*Experimental values were expressed by average value in triplicate for eliminating the experimental errors.

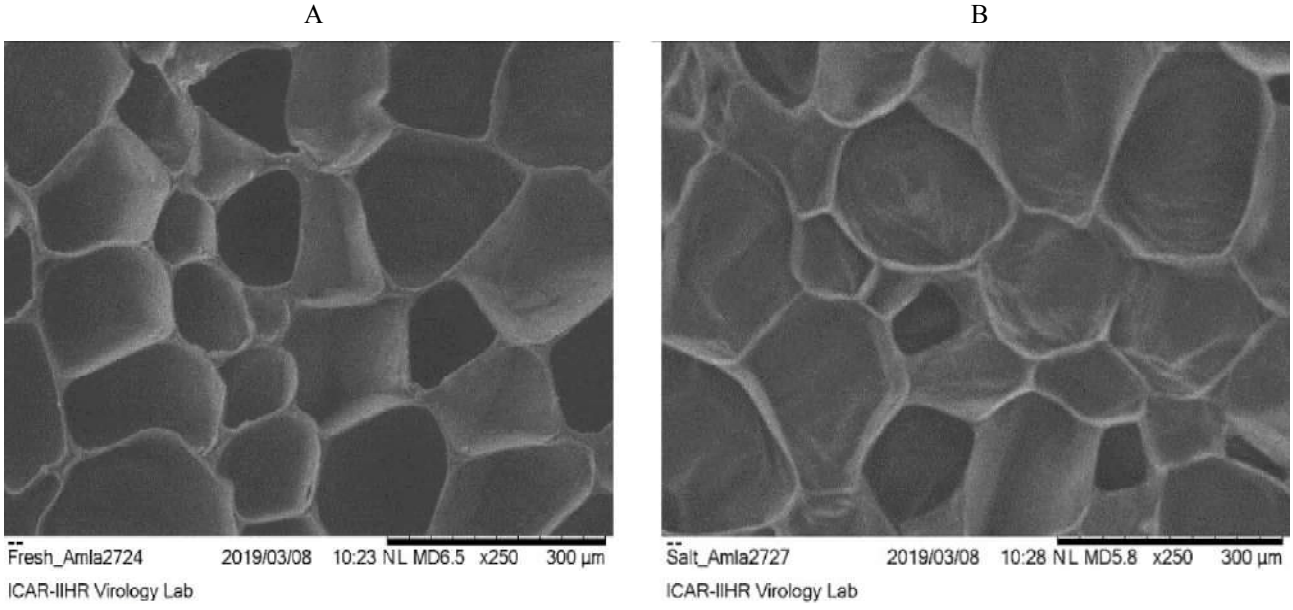

Fig. 6 : a) Fresh aonla b) Osmotically treated (salt) aonla

Table 6 : Predicted and experimental values of responses at optimum conditions for salt medium.

Concentration (%)	Temperature (^{0}C)	Time (Minutes)	WL %	SG %	Predicted SG	RES SG	Predicted WL	RES WL
2	45	120	1.85	0.22	0.15	0.07	1.31	0.54
4	55	180	2.64	0.76	0.75	0.01	2.64	0.00
2	50	60	1.75	0.25	0.31	-0.06	2.29	-0.54
4	45	180	2.08	0.66	0.89	-0.23	2.95	-0.87
4	50	120	2.21	0.85	0.85	0.00	2.21	0.00
6	50	60	6.03	1.00	1.16	-0.16	6.37	-0.34
6	55	120	6.25	1.07	1.14	-0.07	6.79	-0.54
2	50	180	1.80	0.33	0.17	0.16	1.47	0.34
4	55	60	6.05	1.22	1.00	0.23	5.18	0.87
6	50	180	6.08	1.24	1.18	0.06	5.54	0.54
4	50	120	2.21	0.85	0.85	0.00	2.21	0.00
4	45	60	2.06	0.74	0.75	-0.01	2.06	0.00
4	50	120	2.21	0.85	0.85	0.00	2.21	0.00
2	55	120	1.76	0.35	0.52	-0.17	2.10	-0.34
6	45	120	5.11	1.56	1.39	0.17	4.77	0.34

WL: Water loss; SG: Solid gain; RES WL: Residual water loss; RES SG: Residual solid gain

experimental findings in the literature that are consistent with our claims regarding the occurrence of cell structure modification during osmotic processing (Delgado *et al*., 2005).

CONCLUSION

Response surface methodology was found effective for process optimization of parameter using Box-Behnken response surface design was successfully employed in this investigation to estimate and identify the optimal osmotic condition in order to prepare osmotically dehydrated aonla segments using salt solution as an osmotic agent. From the experimental results, second order polynomial models were developed for the responses (water loss and solid gain). The regression equations obtained can be used for optimum conditions for desired responses within the range of conditions applied in this study. The F-values for the lack of fit were non-significant ($p < 0.05$) which clearly shows the model adequacy. Graphical techniques, in connection with RSM, aided in locating optimum operating conditions, which were experimentally verified and proven to be adequately reproducible. The results exhibited that osmotic solution concentration, process temperature and process time have significant effects on the osmotic dehydration process of amla. Response surface methodology was employed to model the effects of the process parameters on the quality attributes of the soaked product and an optimal combination of 5.02% salt concentration, 54.8! temperature and 60.64 min. process time to attain a desired effect of maximum water loss (6.42%) and minimum solid gain (1.09%) in osmotic dehydration of aonla in salt medium.

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Original Research Paper

Tomato late blight yield loss assessment and risk aversion with resistant hybrid

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ABSTRACT

Late blight (*Phytophthora infestans*) is one of the devastating diseases of tomato worldwide. Field trial was carried out in *Kharif* 2019 and 2020 in Hesaraghatta, Bengaluru, Karnataka, India, to estimate yield loss due to late blight and to assess extent of protection in resistant genotype during late blight epiphytotics. Yield loss was calculated as per cent difference in yield between fungicides treated and unprotected plots in three F1 hybrids NS501, Arka Rakshak, both susceptible genotypes and Arka Abhed, a resistant genotype. Over two years, average yield loss due to late blight was 79.47 per cent in NS501, 75.53 per cent in Arka Rakshak and 12.84 per cent in Arka Abhed. With lower mean AUDPC values (147.22 in 2019 and 469.17 in 2020) and with low yield loss, Arka Abhed provided affordable protection against late blight. Our findings indicate late blight as an economically important peril to be considered for tomato yield loss coverage under insurance scheme in Bengaluru region. Arka Abhed hybrid can be cultivated to avert yield loss risk associated with late blight epiphytotics.

Keywords: Arka Abhed, resistance hybrid, tomato late blight and yield loss.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a widely cultivated vegetable crop in India. Karnataka is one of the major tomato producing states in the country. In 2017-18, Karnataka state accounted for 10.54 per cent of the total production of the country (NHB, 2021). Tomato production is limited by several biotic stresses. Among biotic stresses, late blight disease caused by *Phytophthora infestans* (Mont.) de Bary is a devastating disease on tomato in India and worldwide (Fry *et al*., 2015). Tomato late blight has emerged as a major production risk in tomato cultivation in southern hills and plains including Karnataka. Under severe epidemics, crop loss up to 100 per cent has been reported (Chowdappa *et al*., 2013).

In India, crop insurance scheme implies yield insurance. Tomato crop yield loss is covered under Pradhan Mantri Fasal Bima Yojana (PMFBY) and Restructured Weather Based Crop insurance Scheme (RWBCIS). Comprehensive risk insurance is provided to cover yield losses due to non-preventable risks, among other widespread pests and disease attack in standing crop from sowing to harvesting (Anon., 2021).

To consider tomato late blight disease as an important peril under insurance scheme, scientifically validated data on yield loss are required in a particular geography. Previously 100 per cent crop loss due to late blight in tomato due to A2-13 mating type of *Phytophthora infestans* in southern plains and hills has been reported as per rapid roving survey observation (Chowdappa *et al*., 2013; 2015). Currently there are no reports in India with data generated on yield loss assessment due to late blight based on crop cutting experiments. In this context, field trials were undertaken at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, India during *Kharif* 2019 and 2020, with two objectives *viz.*, i) to estimate the magnitude of tomato yield loss due to late blight disease ii) to assess resistant hybrid as risk management option against late blight epiphytotics.

MATERIALS AND METHODS

Estimation of yield loss

Two season field trials were undertaken in Hesaraghatta farm of ICAR-Indian Institute of Horticultural Research, Bengaluru (13.1362° N, 77.4980° E). The trials were conducted in *Kharif* (July-December) 2019 and 2020 under natural epiphytotics of late blight.

Field experiment was laid out in 2 factorial randomized complete block design. Factor 1 was tomato genotypes with three levels. The three tomato F1 hybrids were NS501, Arka Rakshak and Arka Abhed (H-397). The second factor was fungicide protection with two levels, i.e., with and without fungicide protection against late blight as treatments. Each treatment was replicated four times.

These three hybrids were selected as they have relatively different degree of resistance against major diseases of tomato, so that effect of other disease on yield loss estimation is minimal. Among three hybrids, hybrid NS 501 is tolerant to bacterial wilt and TLCV but susceptible to early and late blight. Arka Rakshak has resistance against leaf curl, bacterial wilt and moderate resistance against early blight, but it is susceptible to late blight. Arka Rakshak was chosen to minimize the effect of other diseases on yield loss, which might occur even with pesticide usage. The third hybrid chosen was Arka Abhed (H-397), which has disease resistance to tomato leaf curl disease (*Ty-2+Ty3*), bacterial wilt (Bwr.12), early blight and late blight (*Ph-2* + *Ph-3*) and has field tolerance to bipartite *Tomato leaf curl New Delhi virus* (ToLCNDV) (Kaushal *et al*., 2020). Arka Abhed was included in the experiment, to test the relative efficacy of this hybrid to be adopted as a strategy against this disease risk to get assured yield in conditions of late blight epidemics.

Each plot measured 3m × 3m, with 20 plants were transplanted on raised beds and covered with reflective agriculture mulch film (30µ) at spacing 100 cm × 45 cm. Twenty-five-day old tomato seedlings at 3-4 leaf stage were transplanted on 21st July in both the years. The crop was raised with staking and drip irrigation. Fertilizer application and weed management were made as per package of practices of ICAR-Indian Institute of Horticultural Research, Bengaluru, for open field cultivation of tomato (Sadashiva *et al*., 2018).

Yield loss was calculated by subtracting yield from a plot protected with fungicides and one without fungicide protection. To protect tomato plants from late blight, a total of five sprays of dimethomorph 50% WP (1.2 g/L) + mancozeb 75%WP (2 g/L), fenamidone 10% + mancozeb 50% WG (3 g/L) and famoxadone 16.6% + cymoxanil 22.1% SC (1 ml/L), fosetyl Al 80 WP (80% w/w) (1 g/L), were sprayed at weekly interval until final harvest. All these fungicides have label claim for use on tomato in India (DPPQS, 2021). A control plot without any fungicide protection against late blight was maintained in each hybrid with four replications.

To exclude other pests additional sprays of following pesticides were given; Spinosad 45.00% SC (0.32 ml/ L), indoxacarb 14.50% SC (1.34 ml/L), imidacloprid 17.80% SL(0.5 ml/L), azadirachtin 01.00% EC (10000 ppm)(3 ml/L), streptomycin sulphate 90% + tetracycline hydrochloride 10% SP (500ppm), neem Soap (10 g/L), to manage, bacterial leaf spot, South American tomato pinworm, fruit borer and sucking pests.

Fruit yield data from all the pickings from each plot was pooled and expressed as t ha^{-1}. At each harvest, observations on marketable and non-marketable fruits, incidence of late blight infection on fruits was recorded. In addition, ancillary observations on incidence of early blight, tomato GBNV, and infestation of South American tomato pin worm and tomato fruit borer on fruits were recorded. Yield loss was calculated as the difference between actual yields recorded in plots with fungicide protection and unprotected plots (Cooke *et al.,* 2006) using the formula,

$$Yield\ loss(\%) = ((Yp - Yup)/Yp) \times 100$$

Where *Yp*=yield recorded in protected plot, *Yup*=yield recorded in unprotected plot

Disease assessment

Late blight severity was assessed at weekly intervals from transplanting to final harvest on five randomly selected plants tagged in a plot. Severity on leaves was assessed by using 0-5 scale where, 0=no symptoms, 1=1 to 11% disease (midpoint 6%), 2=12 to 38% disease (midpoint 25%), 3=39 to 61% disease (midpoint 50%), 4=62 to 88% disease (midpoint 75%), 5=89 to 100% disease (midpoint 95%) (Seidl-Johnson *et al*., 2015). Per cent disease index (PDI) was calculated based using the formula,

$$PDI = \frac{\text{Sum of all numerical disease rating}}{\text{Total number of observations} \times \text{Maximum disease grade}} \text{ X } 100$$

From multiple severity assessments made at periodical intervals, Area under disease progress curve for each variety was worked as per equation (Wilcoxson *et al.,* 1975).

$$A = \sum_{i-1}^{K} \frac{1}{2(Si + Si - 1)} \times d$$

Where, Si=disease severity at the end of week i, K = the number of successive evaluations of disease and d=interval between two evaluations.

Statistical Analysis: Disease severity index data was subjected to Arcsine transformation before calculating AUDPC values. The data were subjected to ANOVA at 5 per cent significance level by SPSS software. Yield loss and disease severity data were subjected to ANOVA for statistical significance among different treatments at significance level 5 per cent using SPSS software.

RESULTS AND DISCUSSION

Yield loss assessment

The results on marketable yield and yield loss in two years are presented in Table 1. Significant difference in yield was observed between varieties and level of protection. This may be attributed to inherent yielding potentials of the varieties and efficacy of plant protection schedule applied in both the years. Yield loss in *Kharif* 2019 was less compared to *Kharif* 2020. This may be attributed to higher disease incidence of late blight recorded in 2020 (Table 2).

Over two years, average yield loss due to late blight was 79.47 per cent in NS501, 75.53 per cent in Arka Rakshak and 12.84 per cent in Arka Abhed. In India, severe tomato late blight epidemics have been recorded during 2009-2010 in South Indian plains and hills by Chowdappa *et al*. (2013), during 2014 in eastern and northeastern India (NEI) by Dey *et al*. (2018) and during 2016 in eastern Uttar Pradesh by Tripathi *et al*. (2017). In all these reports there was no yield loss estimation except for reports from South India plains and Hills, where 100% crop loss is reported as per rapid roving survey observation. Our data establishes that late blight is an inevitable risk in *Kharif* cultivation of tomato causing considerable yield loss in Bengaluru region if resistant genotypes are not used. The yield loss data generated will pave way for inclusion of this peril under Pradhan Mantri Fasal Bima Yojana (PMFBY) of India for yield coverage.

Disease assessment

Data on disease severity on three varieties during 2019 and 2020 *Kharif* season are presented in Table 2. Data

Table 1 : Tomato late blight yield loss estimation in *Kharif* 2019 and 2020 at Hesaraghatta, Bengaluru

Treatment	*Kharif* 2019				*Kharif* 2020				Average yield loss (%)
	Marketable yield (t/ha)		Yield loss (%)	Mean (Variety)	Marketable yield (t/ha)		Yield loss (%)	Mean (Variety)	
	P*	UP			P	UP			
Arka Rakshak	70.94	19.98	71.92	45.46	61.47	12.82	79.14	37.15	75.53
NS-501	53.84	13.25	75.39	33.55	48.12	7.92	83.54	28.02	79.47
Arka Abedh	61.25	53.14	13.65	57.20	55.12	48.50	12.02	51.81	12.84
Mean (Protection)	62.01	28.79	-	-	54.90	23.08	-	-	-
Variety (pd≤0.05)	SeM = 1.87, CD = 5.63				SeM = 3.15, CD = 9.51				
Protection (pd≤0.05)	SeM = 1.52, CD = 4.60				SeM = 2.57, CD = 7.76				
Variety* protection (pd≤0.05)	SeM = 2.64, CD = 7.97				SeM = 4.46, CD = 13.45				
CV (%)	11.81				22.48				

*P=protected UP=Unprotected

Table 2 : Tomato late blight severity in *Kharif* 2019 and 2020 under fungicide protected and unprotected conditions

Treatment	*Kharif* 2019 Per cent disease index (PDI)			*Kharif* 2020 Per cent disease index (PDI)		
	P	UP	Mean (Variety)	P	UP	Mean (Variety)
Arka Rakshak	13.89 (21.88)	54.44 (47.55)	34.17 (34.72)	25.83 (30.55)	72.50 (58.37)	49.17 (44.46)
NS-501	18.33 (25.34)	61.11 (51.42)	39.72 (38.38)	27.50 (31.63)	74.17 (59.45)	50.84 (45.54)
Arka Abedh	8.34 (16.78)	12.22 (20.46)	10.28 (18.62)	6.67 (14.96)	17.50 (24.72)	12.09 (19.84)
Mean (Protection)	13.52 (21.33)	42.59 (39.81)	-	20.00 (25.71)	54.72 (47.51)	-
Variety (pd≤0.05)	SeM = 1.42, CD = 4.27			SeM = 1.29, CD = 3.89		
Protection (pd≤0.05)	SeM = 1.16, CD = 3.49			SeM = 1.05, CD = 3.17		
Variety*protection (pd≤0.05)	SeM = 2.01, CD = 6.04			SeM = 1.83, CD = 5.49		
CV (%)	13.12			9.95		

*Value in the parenthesis is arcsine transformed values of per cent disease index. First year peak severity data on September 12, second year peak severity on November 24. P=protected UP=Unprotected

analysis revealed significant effect of varieties and level of protection on severity of late blight. In 2019, significantly lower disease severity was recorded with variety Arka Abhed, which was statistically superior over Arka Rakshak and NS501, which were at par with respect to late blight severity. Similar trend was observed in 2020 except for higher disease severity recorded in second year. The higher incidence in second year may be attributed to build up of soil borne inoculums and prevailing favorable weather conditions. In susceptible varieties, late blight severity ranged from 54.44 to 74.17 over two years. In a trial on four years evaluation of integrated management packages for management of tomato diseases at Hesaraghatta, late blight was recorded as the predominant disease during 2015-18 *Kharif* season (Kumar *et al*., 2020). The current and previous works substantiate that Bengaluru region is a natural hot spot of tomato late blight disease.

In our experimentation, even with protective application of systemic fungicides at 7 days interval, late blight severity values in fungicide protected plots ranged from 8.34 to 18.33 in 2019 and 6.67 to 27.50 in 2020. This is due to prevailing continuous rains that might have reduced the bioefficacy of fungicides applied. This is in conformation with work of Rani *et al*. (2015) that simulated rainfall after spray reduced persistence and bioefficacy of fungicides *viz*., metalaxyl 8%+ mancozeb 64%WP, mancozeb 75%WP, which are widely used against late blight management in tomato. In *Kharif* tomato production, where weather events like continuous rains limits fungicide and protection against late blight. In such situations, Arka Abhed, a resistant F1 hybrid developed at ICAR-IIHR can be used as an effective component to get assured yield with reduction in input costs incurred on usage of protective and curative fungicides.

In two consecutive season's evaluation in Hesaraghatta under high disease pressure, the hybrid Arka Abhed had significantly recorded low AUDPC values (147.22 and 469.17 in 2019 and 2020 respectively) compared to higher AUDPC values of susceptible genotypes *viz*., Arka Rakshak (997.22, 2683.33) and NS501 (1096.68, 2655.83) which were at par with each other in Turkey's test at 5 per cent probability (Fig. 1).

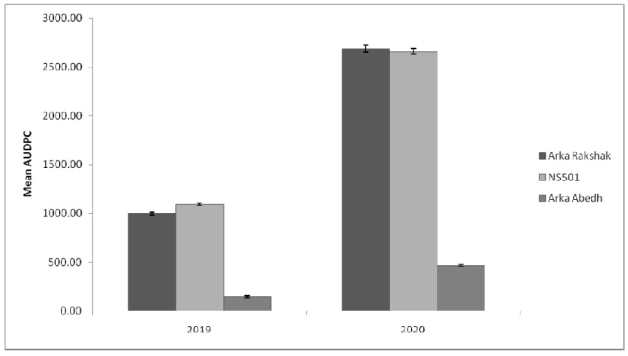

Fig. 1 : Area under disease progress curve (AUDPC) of tomato late blight in three varieties during 2019 and 2020 in unprotected plots under natural epiphytotics. AUDPC values were arrived from seven disease assessments in 2019 and six assessments in 2020.

Higher AUDPC values in 2020 can be attributed to higher late blight severity recorded in second year. Previous study by Hansen *et al*. (2014) suggests that tomato varieties possessing both *Ph-2* and *Ph-3* genes can be used to effectively manage late blight caused by *P. infestans* clonal lineage US-23. In our two years study we have found that Arka Abhed with *Ph-2* and *Ph-3* genes has provided affordable protection against the prevailing late blight population 13_A2 clonal lineage of *P. infestans* in Bengaluru location.

CONCLUSION

The current yield loss assessment validates late blight as a major production constraint causing considerable yield loss in *Kharif* cultivation of tomato in Bengaluru region. Hence, late blight disease has to be considered as an important peril and yield loss arising out of it has to be covered under national crop insurance programme. Based on disease prevalence data it is clear that Bengaluru area is hot spot for late blight disease. Tomato breeders and pathologist should evaluate their material in Bengaluru area for identification of resistant germplasm and testing field efficacy of management measures evolved against this disease. In consecutive two years, two season evaluation, we have found that Arka Abhed is a risk aversion technology with assured yield under late blight epiphytotics.

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Original Research Paper

Epidemiology of ChiVMV and loss assessment in capsicum (*Capsicum annum* var. *grossum* Sendt)

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ABSTRACT

The survey was conducted during *rabi* season (2021) to determine the incidence of mosaic disease of capsicum in major capsicum growing districts namely, Chikkaballapura, Kolar, Bengaluru rural and Ramanagar. The per cent incidence of mosaic disease based on symptoms in field was recorded, highest in Ramanagar (54.85%) and the least incidence of mosaic disease was observed in Chikkaballapura (26.85%). Transmission and host range studies under glasshouse conditions revealed that ChiVMV is transmitted mechanically. Among 16 host plants tested, 7 plant species (*Nicotiana tabacum* cv. Samsun, *N. glutinosa, N. occidentalis, Datura metel, Physalis floridana, S. nigrum, Capsicum annum*) were infected with the Chilli veinal mottle virus disease and the symptom could be seen in 20-25 days. The per cent transmission of ChiVMV by aphid *Aphis gossypii* was studied. The results showed that ChiVMV can be transmitted by *A. gossypii*. However, five aphids per plant showed highest per cent transmission (100%). The effect of different dates of inoculation on different plant growth parameters was also studied, the highest per cent disease transmission was observed in $T_{1:}$ Inoculation 15 days after sowing (100.00%).

Keywords: *Aphis gossypii,* Capsicum, ChiVMV, mosaic

INTRODUCTION

Capsicum (*Capsicum annum* var. grossum sendt) also called as bell pepper is an important vegetable crop. It is known for its nutritional aspects and also for nation's foreign exchange. India contributes one fourth of the world production of capsicum with an average annual production of 1.9 mt from an area of 1.82 mha with the productivity of 1.28 t/ha. Karnataka stands second in area with 89 thousand ha and production of 158 thousand tons (Anon., 2015).

Among the various biotic constraints in the production of bell pepper, viral diseases play a major role. Bell pepper is highly susceptible to natural infection by a large number of viruses in addition to being susceptible to several other diseases. Out of 42 viruses so far reported in bell pepper, 22 are found to occur naturally, while the rest are known to infect on artificial inoculation. Among these, potyviruses *viz.,* potato virus Y (PVY), pepper veinal mottle virus (PVMV), pepper vein banding virus (PVBV), chilli veinal mottle virus (ChiVMV), pepper mottle virus (PMV), tobacco etch virus (TEV) are more prevalent (Caranta *et al.,*1996).

Among these, Chilli veinal mottle virus (ChiVMV) is the major prevalent virus with the incidence of 50 per cent that reduce yield by 50 per cent worldwide (Hussain *et al*., 2008). Further, the ChiVMV is transmitted mechanically and also through aphid vector (*Aphis gossypii*) and found to infect several plant species and induces characteristic systemic mottling symptoms within 7 to 14 days of inoculation.

Several abiotic and biotic stresses affect the productivity of chilli pepper crop worldwide. More than 45-65 viruses have been reported infecting the crop worldwide (Green and Kim, 1994; Anon., 2001). Among pathogenic diseases, viruses are the most devastating agents of chilli pepper, causing serious losses by reducing both fruit quality and quantity (Kang *et al*., 1973; Villalon, 1975; Ong *et al.*, 1980; Yoon *et al.,* 1989; Chew and Ong, 1990). Viruses

produce various types of disease syndrome like mosaic, mottling, leaf distortion, vein etching, yellowing, stunting and narrowing of leaves (Green, 1991; Hameed *et al.*, 1995; Anon., 2001). *Chilli veinal mottle virus* (ChiVMV) is the major virus infecting chilli pepper reducing yield losses up to 50% (Joshi and Dubey, 1973; Ong *et al*., 1980).

MATERIALS AND METHODS

Survey

A roving survey was conducted during rabis season to determine the incidence of mosaic disease in major capsicum growing districts of Southern Karnataka (Chikkaballapura, Kolar, Bengaluru rural and Ramnagar). Plants were observed for the typical symptoms *viz.,* yellowing, mosaic symptoms, mottling *etc.* During the survey, type of symptoms was recorded at different fields and samples were collected. For each one acre of field five sites were randomly selected (10m x 10m) and the average disease incidence was calculated using the following formula.

Per cent disease incidence (PDI) =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Serological survey

The samples brought from the field were subjected to serological assay using CMV and ChiVMV antiserum adopting the DAC-ELISA procedure (Hobes *et al*., 1987).

Host range

To identify the natural reservoirs of the virus different hosts *viz.,* tomato, brinjal, chilli, potato, *Nicotiana tabacum cv. Samsun, Nicotiana glutinosa, Nicotiana occidentalis, Solanum nigrum,* and others like *Chenopodium quinoa, Datura metel, D. stromanium, Physalis minima, Physalis floridana* and *Gomphrena globosa* and also the other weed hosts were sown in polythene bags of 3 X 6" size and seedlings were raised with standard agronomic practices and seedlings of 25-30 days old were used for sap inoculation. The host plants were inoculated by following the procedure described by (Noordam, 1973) and the inoculated plants were observed for symptom expression under insect proof cages for upto 30 days.

Vector transmission

The experiment was carried out to know the aphid transmissibility of ChiVMV using *Aphis gossypii*, as per the procedure explained by *Damiri et al.* (2013). The healthy aphid (*A. gossypii*) colony was first raised on the cotton host plant under greenhouse conditions (25-27OC). The vector aphids were carefully collected in plastic Petri plates and starved for 60 min. in Petri plates lined with black paper on both sides. Later allowed for 5 min. acquisition feeding on ChiVMV infected capsicum leaves, followed by brief inoculation feeding period of 1-3 min. on healthy capsicum plants. After that aphids were killed by spraying with systemic insecticide and the plants were then placed in insect proof conditions in greenhouse at 25-27°C and observed for symptom expression upto 30 days and a set of uninoculated plants were maintained as control.

Loss estimation

To know the impact of ChiVMV on per cent transmission, plant growth and yield. The experiment on loss estimation was carried out using the susceptible capsicum var. Indra. The experiment was conducted in green house conditions using CRD design with nine treatments and three replications with standard agronomic practices using the pots of 9 x 12" cement pots. The artificial sap inoculation was done at fifteen days intervals *viz.,* $T_{1:}$ Inoculation 15 days after sowing, T_2: Inoculation immediately after planting, T_3: Inoculation 15 days after planting, T_4: Inoculation 30 days after planting, T_5: Inoculation 45 days after planting, T_6: Inoculation 60 days after planting, T_7: Inoculation 75 days after planting, T_8: Inoculation 90 days after planting, T_9: Control. The observations on per cent transmission growth and yield parameters *viz.,* plant height (cm), number of branches, number of fruits, fruit weight and per cent disease transmission were recorded at the time of harvest, the data was analyzed statistically.

RESULTS AND DISCUSSION

Survey

In random survey carried out in south Karnataka**,** in Kolar district of 32.99 average per cent disease incidence was recorded, and it ranged from 14.85 to 47.42 per cent. In Chikkaballapura district the average per cent disease incidence was 20.25 and it ranged from 7.99 to 26.85 per cent and in Ramanagar district the average per cent disease incidence was 27.42 and

it ranged from 26.28 to 54.85 per cent and in Bengaluru rural district the average per cent disease incidence was 29.24 and it ranged from 27.42 to 36.56 (Table 1). This difference may be attributed to different climatic factors, vectors activity, different cultivars and different cultivation practices followed. It may also be due to variation in plant protection practices followed by the farmers, low quality seeds (Hameed *et al*., 1995), and similar work carried Laxminarayana Reddy (2006), conducted survey and reported the ChiVMV incidence ranged from 5.3 to 81.5 per cent in Karnataka, 7.6 to 31.7 per cent in Andhra Pradesh, 5.7 to 47.6 per cent in Tamil Nadu, 5.9 to 25.3 per cent in Kerala and 7.5 to 37.8 per cent in Maharashtra. Therefore, the natural incidence of Chilli veinal mottle virus disease would vary from field to field in the surveyed area.

Host range

To identify the natural reservoirs and those susceptible to virus, the host range study of the virus was conducted. Out of sixteen different plant species used in the study (Table 2). Seven plant species *viz., Nicotiana tabacum* cv. Samsun*, Nicotiana glutinosa, Nicotiana occidentalis, Daturametel, Physalis floridana, Solanum nigrum, Capsicum annum* were infected with the ChiVMV and the symptoms could be seen in 20-25 days (Table 2). The infection was confirmed by DAC-ELISA. Similar work was conducted by Siriwong *et al*. (1995) reported that host range of ChiVMV is restricted to Solanaceae family. The present results are in accordance to those reported by Moury *et al*. (2005) *i.e.,* three isolates of ChiVMV induced systemic mosaic symptoms on *N. occidentalis*, *N. glutinosa* but none infected *Solanum melongena*. Brunt *et al*. (1996) reported that *N. glutinosa* is diagnostically not a susceptible host but our findings show that this host species was susceptible and developed mosaic symptoms and was found positive in DAC-ELISA. Similar results have also been reported by Ong *et al*. (1979). Brunt (1996) reported that *Gomphrena globosa* and *Nicotiana glutinosa* is diagnostically not a susceptible host but in our case *Nicotiana glutinosa* became susceptible and developed mosaic symptoms and was DAC-ELISA positive.

Vector transmission

To find out the vector transmissibility and per cent transmission of ChiVMV by aphid *A. gossypii* was used for the transmission of Chilli veinal mottle virus using susceptible capsicum cultivar Indra. The results showed that ChiVMV could be transmitted by *A. gossypii*. Further, five aphids per plant showed highest per cent transmission (100 %) followed by four aphids per plant (80 %), three and two aphids per plant (60 %) and one aphid per plant (40 %) (Table 3). The chilli veinal mottle virus was readily transmitted by sap inoculation and also by aphid vector namely *A. gossypii*, which resembled potyvirus, reported by Mariyappan *et al*. (1973) and Bidari (1982). Jeyarajan and Ramkrishnan (1969) reported *A. gossypii*as the sole vector of potyvirus on bell pepper and chilli. This virus, on young leaves of capsicum produced green vein-banding, leaves are smaller and distorted, stunted and have dark-green streaks on their stems and branches. The symptoms were similar to those produced by potyvirus on chilli and bell pepper as reported by earlier workers (Prasad Rao, 1979; Bidari, 1982 and Pandurangegowda, 1989). The ChiVMV was readily transmitted by sap inoculation to chilli and other herbaceous hosts. The virus was also transmitted in a non persistent manner by the aphids namely, *A. gossypii*, *A. craccivora* and *Myzus persicae* and no seed transmission was observed (Satyaprakash and Singh, 2006).

Table 1 : Average per cent disease incidence of capsicum mosaic disease in different districts in Southern Karnataka

District	Per cent disease incidence	
	Average	Range
Kolar	32.99	14.85-47.42
Chikkaballapura	20.25	7.99-26.85
Ramanagar	27.42	26.28-54.85
Bengaluru rural	29.24	27.42-36.56

Table 2 : Host range of mosaic disease caused by ChiVMV under laboratory conditions

Host	No of plants inoculated	Symptoms	ELISA Absorbance	ELISA reaction (+/-)
Nicotiana tobacum cv. Samsun	5	Necrotic lesion	3.08	+
Nicotiana glutinosa	5	Severe Mosaic	3.40	+
Nicotiana occidentalis	5	Mild Mosaic & vein banding	2.87	+
Datura metel	5	Severe Mottling & rat tail	3.51	+
Physalis floridana	5	Sever Mottling	3.02	+
Solanum nigrum	5	Mild Mosaic	2.45	+
Capsicum annum	5	Mild mosaic	2.32	+
Solanum melongina	5	Nil	0.42	-
Solanum tuberosum	5	Nil	0.38	-
Solanum lycopercicum	5	Nil	0.23	-
Chenopodium quinoa	5	Nil	0.25	-
Datura stromonium	5	Nil	0.52	-
Physalis minima	5	Nil	0.34	-
Gomphrena globosa	5	Nil	0.65	-
Stachy terpeta	5	Nil	0.42	-
Passiflora foetida	5	Nil	0.53	-
ChiVMV (Positive check)	-	-	1.53	-
Healthy	-	-	0.56	-

Table 3 : Vector transmission of ChiVMV by using the aphid- *Aphis gossypii*

No. of aphids per plant	No. of plants inoculated	No. of plants infected	Per cent transmission	Days required for symptom expression
1	10	4	40	20-21
2	10	6	60	19-20
3	10	6	60	19-20
4	10	8	80	19-20
5	10	10	100	19-20
Control (uninoculated)	10	0	0	0

Loss estimation

To know the impact of stage of inoculation on per cent transmission and on plant growth and yield, the plants were inoculated artificially as explained in the material and methods. It revealed that the dates of inoculation on plant growth parameters such as plant height and number of branches and per cent transmission differed significantly over different dates of inoculation (Table 4). The maximum reduction of plant height was observed in T_1 (22.06 cm) and maximum height was

Table 4 : Loss estimation in capsicum due to ChiVMV under polyhouse conditions

Treatment	Plant height (cm)	No. of branches/ plants	No. of fruits/ plants	Average fruit weight (g)	Per cent disease incidence
T_1- 15DAS	22.06	0.66	0.00	0.00	100.00
T_2- 30DAS	25.83	1.73	1.66	7.93	99.00
T_3- 15 DAT	33.56	2.60	1.86	22.93	99.00
T_4- 30 DAT	51.10	3.46	2.37	45.83	98.66
T_5- 45 DAT	49.87	3.60	2.60	61.63	91.33
T_6- 60 DAT	50.37	3.40	4.13	79.67	72.00
T_7- 75 DAT	52.64	3.53	4.27	100.43	71.33
T_8- 90 DAT	54.06	4.13	6.06	101.26	44.66
T_9- Uninoculated (Control)	55.22	4.23	8.04	133.13	0
S.Em ±	0.74	0.08	0.16	0.62	1.05
CD @ 5%	2.21	0.26	0.48	1.86	3.14

Note: DAS- Days after sowing, DAT- Days after transplanting

found in T_9 (55.22). Similarly, maximum reduction in number of branches found in T_1 (0.66) maximum number of branches was found in T_9 (4.23) (Table 4 and Fig.1).

There was significant difference with respect to number of fruits per plant observed among the treatments (Table 4). Maximum reduction of fruits per plant were noticed in T_1 (0.00) and maximum number of fruits per plant was found in T_9 (8.04) (Table 4 and Fig.1). Data pertaining to average fruit weight differed significantly over different dates of inoculation and similar trend was observed in T_9 (133.13) (Table 4 and Fig.1).

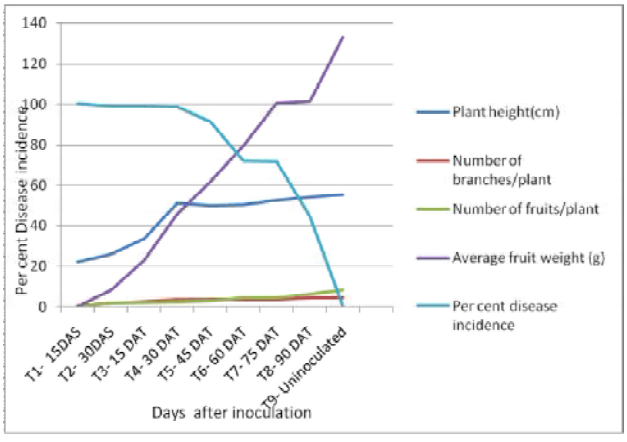

Fig. 1 : Effect of different dates of inoculation on growth and other characters

Per cent transmission

Highest per cent disease transmission was observed in T_1 (100.00 per cent) followed by T_2 and T_3 (99 per cent each), T_4 (98.66 per cent), T_5 (91.33 per cent), T_6 (72 per cent), T_7 (71.33 per cent) and T_8 (44.66 per cent) and the rate of transmission and the impact was decreased with the increase in age of the plant and they differ significantly (Fig.1).

The infection occurs at later stages, the extent of reduction in yield and plant height was less. Sastry and Singh (1976) reported that ToLCV infected plants produced very few fruits when infected within 20 days after planting and resulting up to 92.30 per cent yield loss. While plants infected at 35 and 50 days after transplanting resulted in 82.9 and 74.0 per cent yield loss, respectively. Similar results were reported by Reddy *et al.* (2010).

CONCLUSION

It is concluded that since the infected plants cannot be cured and the early infection leads to severe reduction both in yield and quality, early-stage protection of the crop both in nursery and in the main field is important in order to reap the better yields.

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Original Research Paper

Effectiveness of the field application of UV-C for cucumber downy mildew control

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ABSTRACT

There is growing interest in the application of ultraviolet (UV-C) energy to control crop pathogens. In the present study, the efficacies of UV-C treatments for controlling cucumber downy mildew (*Pseudoperonospora cubensis*) were investigated on a commercial farm in eastern Massachusetts, USA. Controlled doses of UV-C, delivered by a tractor-mounted array of sources, between 120 and 480 J·m^{-2} were applied and compared to conventional fungicide treatments as well as to untreated controls, for each of two consecutive years (2020 and 2021). Visual assessments of foliar disease severity in the trial plots were made several times from planting through the end of productive life. In contrast to the successful control of powdery mildew, the UV-C treatments for controlling cucumber downy mildew were not as successful as conventional fungicides. None of the UV-C treatments affected the overall progression rate of downy mildew once the disease became apparent, although disease onset was delayed slightly compared to untreated controls. This delay may have been due to UV-C induced resistance to infection by the host. Unlike powdery mildews, downy mildew spores from *P. cubensis* are darkly pigmented, possibly decreasing the efficacy of the UV-C treatments for controlling the disease. DM spores may also be only susceptible to UV exposure prior to encysting in the leaves of the host, thereby perhaps limiting the window of opportunity when UV-C treatments can be effective. Although not the primary focus of this study, the use of reflective mulch appeared to delay disease onset relative to black mulch in fields with significant sunlight exposure, perhaps due to lowering plant stress by maintaining a lower soil temperature.

Keywords : Crop pathogens, cucurbits, downy mildew and ultraviolet energy

INTRODUCTION

Pseudoperonospora cubensis is the oomycete pathogen responsible for cucurbit downy mildew It can infect many cucurbits such as cantaloupe, pumpkin, watermelon, and squash, but cucumber is particularly susceptible. In the United States, use of resistant cucurbit varieties was an effective means of control of downy mildew until 2004. Since then, effective control has depended upon chemical fungicides in addition to planting resistant varieties (Savory *et al*., 2011).

P. cubensis is spread by means of aerially dispersed sporangia. When viable sporangia land on a host leaf, they germinate in moisture on the leaf's surface producing biflagellate zoospores that encyst in stoma where a germ tube is formed that penetrates the leaf's surface through the stoma. Hyphae form in the mesophyll layer and produce clavate-branched haustoria in the host's cells. When sporulation is triggered, sporangiophores emerge through stomates bearing sporangia at their tips. When released, these sporangia are carried by the wind and the life cycle repeats at the next site.

Both visible light and ultraviolet (UV) energy have been reported to reduce the viability of fungal spores (Rotem *et al*., 1985; Kanetis *et al*., 2010). UV has been particularly effective for controlling powdery mildew (Patel *et al*., 2020; Skinner *et al*., 2020; Onofre *et al*., 2021). Unlike powdery mildew however, downy mildew spores are darkly pigmented with melanin (Lee *et al*., 2021), a strong absorber of

UV (Meredith and Sarna, 2006), which reduces the potential for damage from natural UV solar energy (Cordero and Casadevall, 2017).

UV energy can be classified into three bands, UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm). Sunlight reaching the surface of the earth is limited to the longer UV wavelengths, and the amount and spectrum depends upon weather, latitude, and season. UV energy in all three bands can be produced by electric sources. Today, low pressure discharge sources are the most common; however, LED sources will undoubtedly displace them in the next few years.

UV energy can inactivate viruses, bacteria, and fungi through several different mechanisms (Nelson *et al.,* 2018). UV-C in the range of 250 to 270 nm acts directly on nucleic acids (RNA and DNA) within the cell (Nelson *et al.,* 2018). When a UV photon is absorbed, the molecular strands become broken and dimers (molecular lesions) are formed. These dimers, typically thymine dimers, prevent cellular replication unless repaired (Kneuttinger *et al.,* 2013). In fact, every organism has nucleic acid repair mechanisms. UV-C can also induce secondary reactions within the cell when a photon is absorbed by an endogenous chromophore and subsequently produces free radicals which, in turn, breakdown one or more vital cellular functions. UV-B and UV-A can also induce these secondary reactions. Generally, the longer the UV wavelength, the higher the dose needed to induce secondary endogenous photocatalytic inactivation. Secondary inactivation can also occur through exogenous photocatalysis. Photocatalysts like TiO_2, with moisture, will also produce free radicals that can kill bacteria and fungi by damaging cell walls, thus disrupting their permeability (Ramesh *et al.,* 2016).

Most plants and their obligate parasites have evolved mechanisms that limit cellular damage from natural UV (Sancar, 1994; Cordero and Casadevall, 2017). Pigmentation that absorbs UV energy and then dissipates it as heat is one protective mechanism, minimizing damage to DNA. Pigmentation can also reduce the number of photons available for absorption by chromophores within the cell that might initiate lethal, secondary reactions. Melanin is the most widely recognized putative protective pigment in fungi (Cordero and Casadevall, 2017), with high absorption throughout the UV spectrum (Meredith and Sarna, 2006). Unlike powdery mildew fungi that are devoid of melanin (Suthaparan *et al.,* 2012), some downy mildew sporangia such as *P. cubensis* (Lee *et al.,* 2021) have high concentrations of protective melanin. PM incorporates a different protective mechanism that entails upregulating a photoactivated (short visible wavelength) repair mechanisms for UV-induced cellular damage (Sancar, 1994).

UV treatments have been particularly successful for mitigating obligate powdery mildew in a variety of crops (rose, strawberry, cucumber, etc.). Of particular note, nighttime applications of UV have been shown to be more efficient than daytime applications at similar doses (Suthaparan *et al.,* 2012). As noted above, powdery mildew has a short-wavelength sensitive repair mechanism which works quite well for pathogen survival because visible light is always in the same solar spectrum as UV. By providing only nighttime UV-C exposure, the powdery mildew pathogen has no access to its short-wavelength repair mechanisms. Through field trials, it has been shown that UV-C doses at night between 100 $J \cdot m^{-2}$ and 200 $J \cdot m^{-2}$ can be more effective and less expensive than conventional fungicides for limiting the proliferation of powdery mildew (Onofre *et al.,* 2021).

In the present study we wanted to learn more about the effects of UV-C on mitigating cucurbit downy mildew. Rotem *et al.* (1985) exposed three different types of spores with increasing pigment density to short wavelength UV energy. Not surprisingly, they found that the spores with higher pigment density were less sensitive to the effects of UV exposure. Based on these findings, we expected that UV-C doses higher than those applied to control powdery mildew would likely be needed to be effective because of high concentrations of protective pigment in downy mildew spores. Of some concern, we wanted to determine whether UV-C doses higher than those previously used might reduce yield from the host plant. To further our understanding of the potential for UV-C to control downy mildew, we added a UV-reflecting mulch to the study. The reflective mulch would not only increase the effective dose but would also redistribute the UV-C to surfaces otherwise in shadow. In addition to better understanding the direct effects of UV-C on controlling downy mildew, we also wanted to see if there was evidence for UV-induced resistance to infection by the host (Kunz *et al.,* 2008; Paul *et al.,* 2012), the idea being UV-C exposure prior to the presence of downy mildew in the field might reduce either the severity or

delay the onset time of infection. Additionally, since *P. cubensis* sporangia may only be susceptible to UV treatment prior to encysting in host leaves, both once- and twice-weekly treatments using different UV doses were investigated.

MATERIALS AND METHODS

UV-C Treatment Device

A tractor three-point hitch mounted UV-C treatment attachment (aka the "Dragon") was designed to treat one crop row at a time. The unit consisted of an array of six 300 W UV-C fixtures [four UV-C lamps (TUV75W/HO, Philips) per fixture, each fixture powered by 2 two-lamp ballasts (Pure VOLT IUV-2S60-M4-LD, Philips/Advance)], which were arranged in a hemi-cylindrical manner arching over the row of plants (Fig. 1). The power to operate the light fixtures was provided by an on-board gasoline powered inverter generator (iGen 2600, Westinghouse Outdoor Power Equipment). Vinyl curtains with a UV reflective foil tape (76145A62, McMaster-Carr) applied to the inner side were installed on both ends of the enclosure to help contain the UV-C within the treatment attachment and to redirect it back into the unit.

Since the output of the UV-C array is fixed, prescribed dose levels were obtained by varying the speed of the tractor over the crop. The speeds required to achieve the specific doses were verified by driving the UV-C attachment over an integrating UV-C logger.A ground speed of approximately 4.0 $km \cdot hr^{-1}$(2.5 MPH) was required to achieve 120 $J \cdot m^{-2}$ and 2.0 $km \cdot hr^{-1}$(1.25 MPH) to achieve 240 $J \cdot m^{-2}$. The 480 $J \cdot m^{-2}$ dose level was achieved by making two passes at the 240 $J \cdot m^{-2}$ ground speed. The UV-C logger used was designed and built by the research team. It consisted of a UV-C detector and a microcomputer housed in a weather resistant housing with the detector located under a UV transparent window. The microcomputer recorded the output of the UV-C detector to a memory chip once every 10 ms. The duration and amount of UV-C irradiance incident on the detector was used to compute dose.

Plastic Mulch

Black (BioTelo, Heartnut Grove Inc.) and reflective mulch (Brookdale Farm Supplies) were used to make the beds in the study plots. The reflectance of both mulch types was measured at 254 nm and 436 nm to determine their reflectance of UV-C and a short visible wavelength (Table 1).

Table 1 : Reflectance at two wavelengths of the black and the reflective mulches

Mulch Type	Reflectance	
	254 nm	436 nm
Reflective	66%	75%
Black	5%	1%

Year 1 Field Trials

The focus of the first-year field trial was to identify a combination of UV dose and frequency of application that was effective for controlling cucurbit downy mildew without negatively affecting yield. In addition, we wished to evaluate the efficacy of UV dosing for mitigating downy mildew with UV-reflective mulch relative to black mulch.

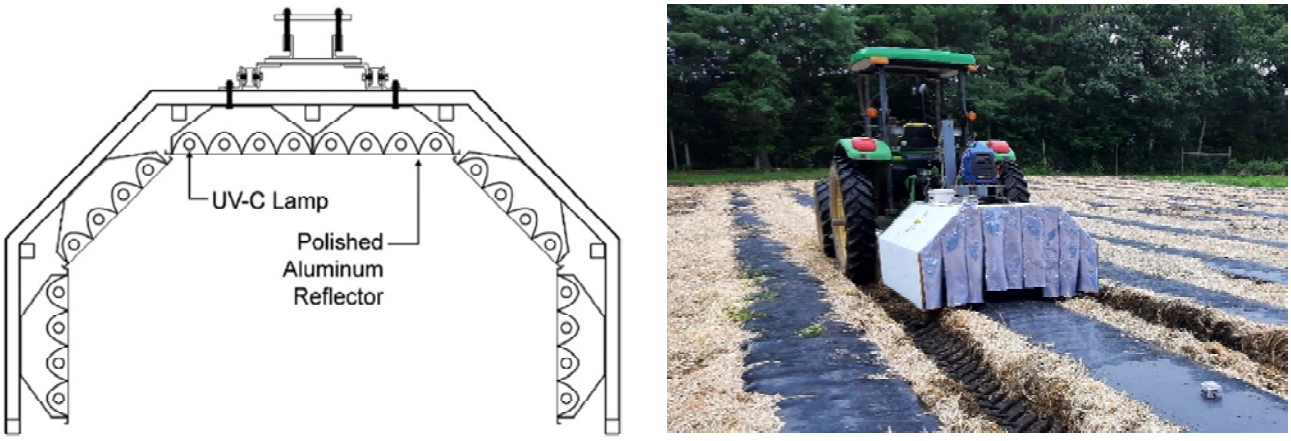

Fig. 1 : (Left) Cutaway end view of the UV-C enclosure showing placement of the fixtures. (Right) UV-C treatment attachment mounted to a tractor and shown positioned over an unplanted row.

Three levels of UV dose (120 J·m^{-2}, 240 J·m^{-2}, and 480 J·m^{-2}) were selected and each of these doses were applied once or twice weekly (i.e., on Mondays and Thursdays). All UV treatments and untreated control plots were duplicated on standard black plastic mulch and UV reflective plastic mulch, according to the field study layout in Fig. S1 (supplimentary data). Additionally, plots treated with the farm's conventional fungicide program were included in the study to benchmark the performance of UV-C only treatments. The conventional fungicide treatments for downy mildew applied during the year 1 trial are summarized in Table 2. The various dose/frequency combinations were distributed throughout the black and reflective mulch blocks such that no combination was replicated in adjacent rows. The cucumber variety Raider F1 (Harris Seed) was used for the first year's field trial, since it is not a downy mildew resistant variety and was the choice of the producer. Raider F1 does have resistance to scab, and intermediate resistance to angular leaf spot and cucumber mosaic virus.

A cooperative extension agent performed the downy mildew severity ratings by visually assessing the percentage of leaf area covered in downy mildew lesions (evidenced by yellowing, necrosis, sporulation) within the whole plot. Individual leaf inspections were then conducted on 10 leaves per plot by inspecting both the upper and lower leaf surfaces, to ensure the extension agent was looking carefully at leaf symptoms and not attributing leaf yellowing to downy mildew without evidence of sporulation.

Year 2 Field Trials

The focus of the second-year trial was to compare the downy mildew control efficacy of the best UV-C only condition from the year 1 field study, the grower's conventional fungicide program, and a combination of both treatment types. The treatments used were: (1) UV-C only (480 J·m^{-2}) twice weekly, (2) weekly conventional fungicide, (3) fungicide weekly plus UV-C twice weekly and (4) fungicide every other week plus UV-C twice weekly. The third treatment was included to determine if the addition of UV-C to the conventional weekly fungicide program would offer additional control beyond the conventional fungicide program alone. The fourth condition, added at the suggestion of the participating extension agent, was included to determine if downy mildew control could be maintained by adding UV-C treatments while reducing the amount of conventional fungicide applications.The conventional fungicide treatments for downy mildew applied during year 2 are summarized in Table 3. Each condition was replicated in two rows on both black and UV reflective mulch, for a total of four replications for each treatment. The last 4.5 m (15 feet) of one row was devoted to a control condition with no fungicide or UV-C treatment. The layout for this field study is shown in Fig. S2 (supplimentary data) The cucumber variety Raider F1 (Harris Seeds) was used again for the second year of the trial.

Table 2 : Summary of conventional fungicide applications for DM during the year 1 trial; products listed as DM / PM are labeled for treatment of both downy and powdery mildew.

Date	Product	Rate		Purpose
07/13/2020	Rampart	3.27	L·Ha^{-1}	DM
	Initiate	2.34	L·Ha^{-1}	DM/PM
07/22/2020	Curzate	0.51	L·Ha^{-1}	DM
07/29/2020	Rampart	3.27	L·Ha^{-1}	DM
08/04/2020	Omega 500F	1.32	L·Ha^{-1}	DM
08/13/2020	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	2.34	L·Ha^{-1}	DM/PM
08/09/2020	Ranman	0.18	L·Ha^{-1}	DM
08/06/2020	Omega 500F	1.32	L·Ha^{-1}	DM
09/01/2020	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	2.34	L·Ha^{-1}	DM/PM
09/08/2020	Orondis Ultra	0.58	L·Ha^{-1}	DM
	OxiDate 5.0	1.40	L·Ha^{-1}	DM/PM
09/16/2020	Ranman	0.18	L·Ha^{-1}	DM
	Rampart	3.27	L·Ha^{-1}	DM
	OxiDate 5.0	1.40	L·Ha^{-1}	DM/PM

Each row was divided into ten sections (Fig. S2) and assessments of percentage foliar downy mildew severity were made within each of the 10 sections to increase the sample size within each row. Assessments were performed visually within a square quadrat with 61 cm (24 inch) sides, placed randomly within each of the ten row sections using the same methodology used in the first year. The top and bottom sides of leaves within the quadrat were inspected to verify that the symptoms were consistent with downy mildew in the same manner as year 1. Assessments were performed by a farm staff member trained to scout

Table 3 : Summary of conventional fungicide applications made during the year 2 trial; dates marked with an asterisk (*) indicate the products listed were not applied to the plots that received conventional fungicide every other week (Products listed as DM / PM are labeled for treatment of both downy and powdery mildew)

Date	Product	Rate		Purpose
07/21/2021	Ranman	0.18	$L \cdot Ha^{-1}$	DM
	Initiate 720	2.34	$L \cdot Ha^{-1}$	DM/PM
07/26/2021*	Microthiol	6.73	$kg \cdot Ha^{-1}$	DM/PM
	Kocide 3000	1.12	$kg \cdot Ha^{-1}$	DM/PM
08/02/2021	Previcur Flex	1.40	$L \cdot Ha^{-1}$	DM
08/10/2021*	Omega 500F	1.17	$L \cdot Ha^{-1}$	DM
08/17/2021	Ranman	0.18	$L \cdot Ha^{-1}$	DM
08/23/2021*	Previcur Flex	1.40	$L \cdot Ha^{-1}$	DM
08/31/2021*	Nordox 75WG	1.23	$kg \cdot Ha^{-1}$	DM/PM
09/06/2021	Tanos	0.73	$L \cdot Ha^{-1}$	DM
09/13/2021*	Rampart	2.34	$L \cdot Ha^{-1}$	DM
	OxiDate 5.0	2.34	$L \cdot Ha^{-1}$	DM/PM

cucurbit downy mildew by cooperative extension agents.

RESULTS AND DISCUSSION

The sets of data from the year 1 and year 2 field trials were analyzed in two ways. First, the area under the disease progress stairs (AUDPS) method (Simko and Piepho, 2012) was used to provide a composite index of the relative impact of each treatment and control condition on disease progression throughout the assessment period in each year. Second, the instantaneous foliar disease severity values (in percent) from each assessment interval were compared among the treatment and control conditions and fitted with mathematical power functions to model disease progression under each condition.

The time reference for the disease progress modeling used in this study is based on an assumed date of initial infection of the cucumber plants in the test plots, based on the average duration of 4 to 12 days between the initial infection and the first observed symptoms in *P. cubensis* (Salcedo *et al.,* 2020). In the year 1 field trials, the first observations of disease occurred on August 18th, when the foliar disease severity for untreated crops ranged from 5% to 12.5%. In the year 2 trials, the initial observations of disease occurred earlier in the year, on August 3rd, when disease severity values ranged from 0.1% to 1%. During the next set of observations on August 11th, disease severity values ranged from 1.5% to 12%; similar to the initial disease observations in year 1. Since these two observations in year 2 occurred 8 days apart and since Salcedo *et al.* (2020) reported a range of 8 days during which initial disease observations could be made following infection, August 18th in year 1 and August 11th in year 2 were defined as 12 days after infection, and August 3rd in year 2 was defined as 4 days after infection.

Year 1 Field Data

Fig. 2 shows the observed foliar disease severity values for each treatment and control condition, when black mulch was used, and Fig. 3 shows the corresponding data for reflective mulch. Each point in Figs. 2 and 3 is a single observation for the once-weekly doses or the average of two observations for the twice-weekly doses. The conventional fungicide program in year 1 was only applied with the black mulch, so that condition is omitted from Fig. 3.

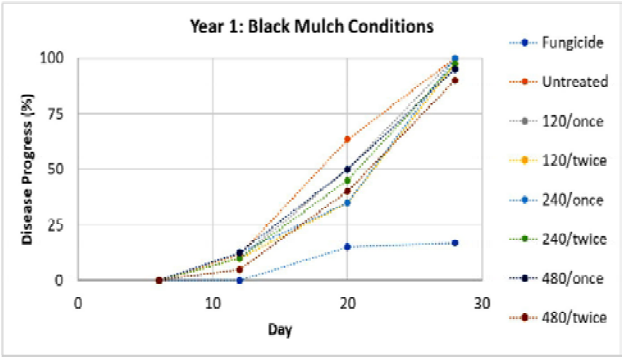

Fig. 2 : Disease progress curves for year 1 under each condition using black mulch.

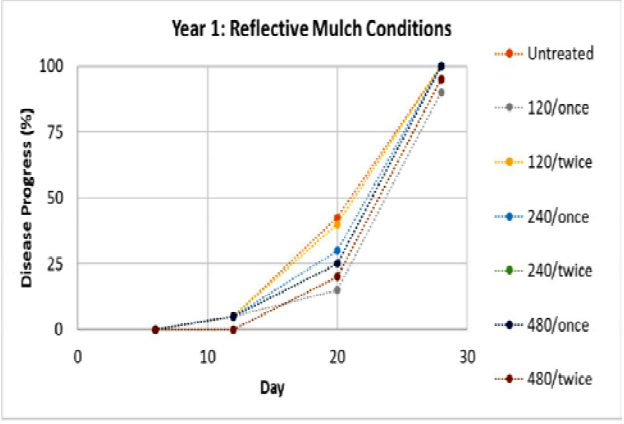

Fig. 3 : Disease progress curves for year 1 under each condition using reflective mulch.

A four-way analysis of variance (ANOVA) was performed on the foliar disease severity data comprising a balanced experimental design with the type of mulch, the UV-C dose, the dosing frequency, and the date of assessment as independent factors. The mulch type had a statistically significant effect ($F_{1,45}$=15.3, $p<0.05$) on disease severity, as did the date of assessment ($F_{5,45}$=1184, $p<0.05$). There was also a statistically significant interaction ($F_{5,45}$=8.89, $p<0.05$) between the mulch type and the date of assessment on disease severity. This can be observed from the fact that the disease severity values for the two mulch types were similar for the earliest and latest assessment dates but differed around day 20.

Qualitatively, the curves in Fig. 2 also illustrate the large difference found in year 1 between the conventional fungicide treatment conditions and the control and UV-C treatment conditions. Disease severity remained under 20% under the fungicide condition for all observation periods, whereas it approached 90%-100% for all other conditions by the last observation period. Generally, the differences among the control and UV-C treatment conditions were small, although the untreated control condition tended to have greater disease severity values than the UV-C conditions.

AUDPS values (Simko and Piepho, 2012) were calculated for each condition representing each treatment type (or control), the frequency of application (for the UV-C treatment conditions) and type of mulch. These values are shown in Fig. 4. Qualitatively, Fig. 4 shows the much lower AUDPS value for the conventional fungicide condition than for all other conditions. It can also be seen that the AUDPS values are usually (with one exception for 120 $J \cdot m^{-2}$ applied twice weekly) lower for the reflective than for the black mulch.

A one-way ANOVA for each treatment condition in Fig. 4 was performed showing that there were statistically significant differences among the treatment conditions ($F_{14,10}$=16.2, $p<0.05$). Tukey's post hoc tests were carried out among each treatment to identify which conditions differed from the others. It was found that the conventional fungicide treatment (with black mulch) was statistically significantly (t=5.07 to 13.2, $p<0.05$) different from all other conditions. No other conditions differed from one another after adjustment of Type I errors for multiple pairwise comparisons.

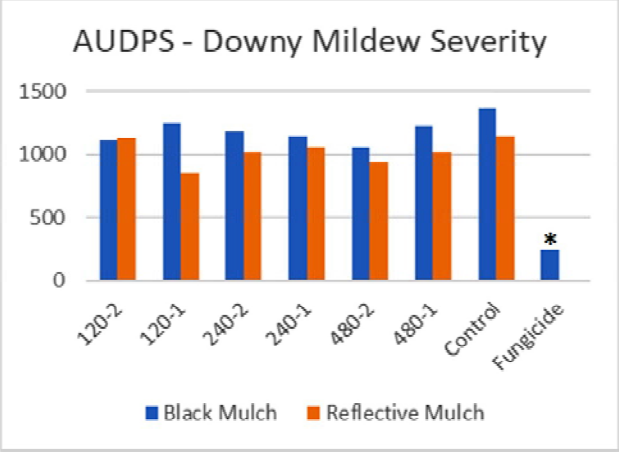

Fig. 4 : AUDPS values (Simko and Peipho, 2012) for each treatment and control condition in year 1. The asterisk (*) for the fungicide condition indicates that this condition was statistically significantly ($p<0.05$) different from all other conditions. Vertical error bars for the conditions are not shown because each value is either a single measurement or the average of two measurement values.

Considering only the UV treatment groups, the AUDPS values could be analyzed using a three-way ANOVA with the UV-C dose, the dosing frequency and the type of mulch as independent variables. This ANOVA revealed a statistically significant main effect of mulch type ($F_{1,7}$=9.80, $p<0.05$), but no other main effects nor interactions among the variables. Because the AUDPS values collapse across the date of assessment, the result of this analysis is consistent with the ANOVA on the disease severity values.

To identify whether and to what extent the treatment types affected the course of disease progression, the data in Fig. 2 and 3 were replotted in Fig.5 and 6, for black and reflective mulch respectively, using logarithmic axes for the abscissa and the ordinate. (Values of zero were omitted as they could not be plotted along a logarithmic axis.) Visual observation suggested that the data for each condition on the log-log plots in Fig. 5 and 6 fell approximately along straight lines, which are represented by power functions of the form $y = ax^b$. The best-fitting power functions to the data (excluding the conventional fungicide condition) had exponent (b) values ranging from 2.42 to 4.63, with an average of 3.19. (The exponent for the best-fitting power function to the fungicide condition was 0.31.) Assuming the disease progression was similar among the UV treatment

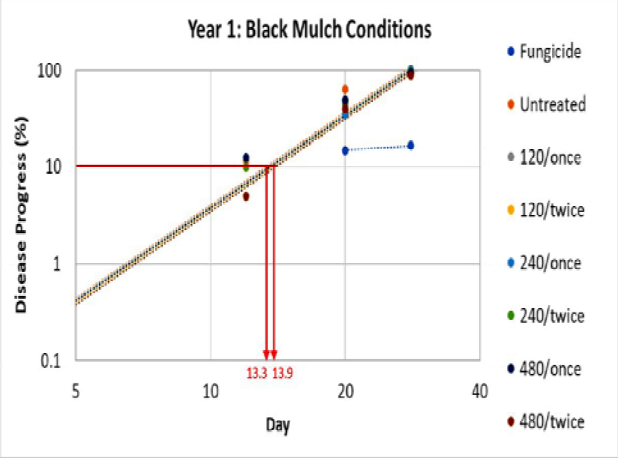

Fig. 5 : Disease progression values (non-zero only) for each condition and using black mulch. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

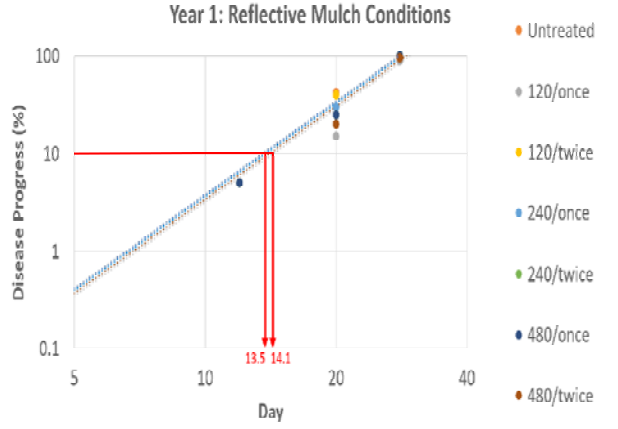

Fig. 6 : Disease progression values (non-zero only) for each condition and using reflective mulch. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

conditions, a fixed exponent value of 3.19 was used and best-fitting power functions to each set of data were determined having the form: $y = ax^{3.19}$, and these are also shown in Fig. 5 and 6. Goodness of fit (r^2) values for each function ranged from 0.88 to 0.998.

These modeled power functions are nearly coincident with each other, suggesting that disease progressions for the control and for all UV-C treatment conditions were essentially the same. Even the sets of curves for each type of mulch differed very little from each other, despite the statistically significant effect of mulch type in the three-way ANOVA. Indeed, taking an arbitrary disease progression value of 10% to represent a threshold for disease in these conditions, less than a single day separates the time after initial infection at which this observable threshold would be met between the control and all UV-C treatment conditions (Fig. 5 and 6).

A limitation of all analyses from year 1 is the small sample size. Only a single observation, or sometimes two observations, were made for the control and treatment conditions in year 1 and this may have limited the ability to achieve statistical significance among those conditions. With or without statistical significance, however, the UV-C applications employed in year 1 were not much of an improvement over the control condition for mitigating DM disease progression.

Year 2 Field Data

As mentioned previously, subsequent field trials in year 2 were carried out to validate the year 1 findings using what would be expected to be the most effective UV-C treatment, 480 $J \cdot m^{-2}$ applied twice weekly. Although the 120 $J \cdot m^{-2}$ dose applied once weekly (with reflective mulch) was empirically the most effective treatment, the same treatment was not as effective with black mulch, and collapsing across mulch type, 480 $J \cdot m^{-2}$ had slightly (albeit not statistically significantly) higher effectiveness than the other doses, and application frequency of twice weekly was slightly more effective than once weekly. Combinations of fungicide (using the producer's usual weekly application schedule or a reduced application frequency of every other week [EOW]) and UV-C treatments were included in year 2 to identify whether UV-C could enhance the effectiveness of fungicide or permit fewer fungicides to be used while providing protection against downy mildew disease. As also stated previously, multiple sections of each treatment row were evaluated for disease to increase sample sizes and statistical power.

Fig. 7 and 8 show the progression of disease for each of the control and/or treatment conditions as a function of time (day after assumed infection as described previously). There are two primary qualitative differences between the data in these figures for year 2 and the corresponding data in Fig. 2 and 3 for year 1. First, there appears to be a greater separation among the conditions in terms of the days that the disease begins to take hold in the plants, especially between the untreated control condition (which

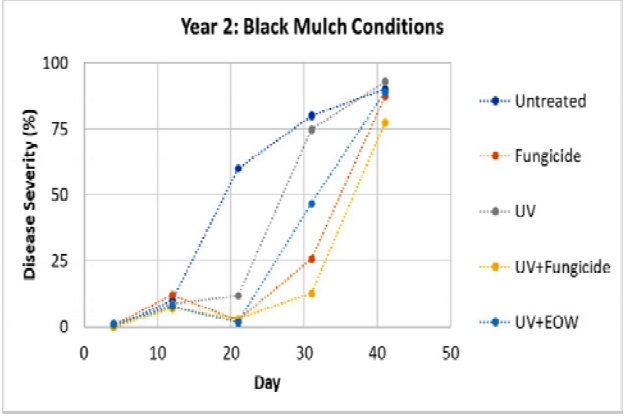

Fig. 7 : Foliar disease progression curves for year 2 under each condition using black mulch.

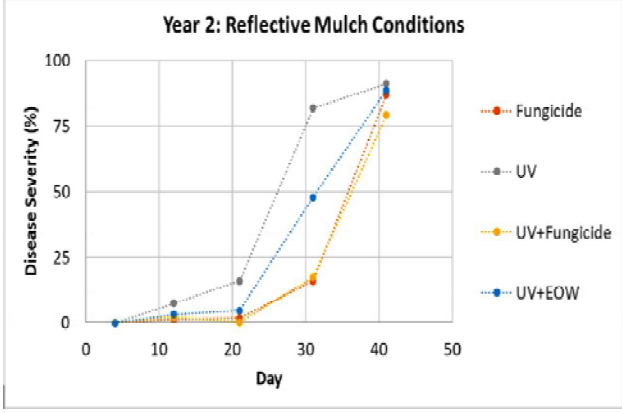

Fig. 8 : Foliar disease progression curves for year 2 under each condition using reflective mulch.

exhibited greater than 50% foliar disease severity by day 21, and the other conditions which exhibited less than 20% disease severity on the same day. Second, the disease severity for the fungicide treatment conditions approached 80% by the end of data collection where as in year 1, disease severity was held to less than 20% with the application of fungicide. (Possibly, disease severity in year 1 for the fungicide treatment condition would have eventually increased to nearly 100%.)

For the four treatment conditions (i.e., fungicide, UV, UV plus fungicide, and UV plus EOW fungicide) for which both types of mulch were used, a three-way ANOVA was performed on the disease severity values, with treatment, mulch type and date of assessment as independent factors. The section number of each row was included in the analysis as a covariate factor to identify whether there were any systematic differences within each row; there were not. The treatment ($F_{3,761}$=118, $p<0.05$) and the date of assessment ($F_{4,761}$=1958, $p<0.05$) had statistically significant main effects on disease severity, and there was also a statistically significant interaction between treatment and assessment date ($F_{12,761}$=52.5, $p<0.05$). This can be observed in Fig. 7 and 8 where the disease severity was similar across all treatments for the first and last treatment dates, with the most variation among treatments for the intermediate dates. Unlike year 1, the type of mulch did not have a statistically significant ($F_{1,761}$=0.98, $p>0.05$) effect on disease progression.

Mean AUDPS values (Simko and Piepho, 2012) for each treatment and mulch condition were calculated and are shown in Fig. 9. A one-way ANOVA was performed to assess differences among the conditions, which were statistically significant ($F_{8,149}$=45.2, $p<0.05$), with Tukey's tests to assess pairwise comparisons while controlling for Type I errors (Supplementary data Table S1). In general, there were no significant differences ($p>0.05$) in AUDPS between mulch types for the same condition. All conditions except for the UV-only conditions differed significantly ($p<0.05$) from the untreated control condition (which only used black mulch). The combination of fungicide and UV-C treatment with the black mulch was statistically significantly different ($p<0.05$) from the fungicide-only treatment with the same mulch type, suggesting a small impact of UV-C treatment in conjunction with fungicide.

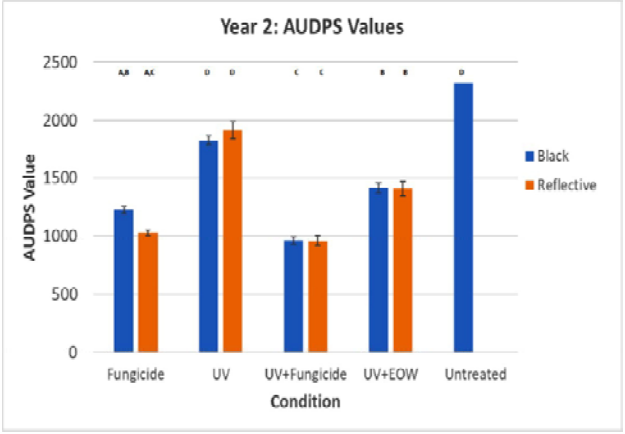

Fig. 9 : AUDPS values (Simko and Piepho, 2012) for each treatment (UV: ultraviolet; EOW: every-other-week fungicide application) and mulch condition in year 2. Letters above each bar indicate non-statistically significant differences among conditions with a common letter.

Excluding the untreated control condition, a two-way ANOVA was performed to assess how the treatment condition and mulch type, and the interaction between them, affected AUDPS. Section from 1 to 10, was included in this analysis as a covariate to identify whether there were any systematic differences across each of the treatment rows; there were not. There was a statistically significant ($F_{3,149}$=110, $p<0.05$) main effect of treatment, but the mulch type did not exhibit a statistically significant main effect ($p>0.05$). There was a significant interaction ($F_{3,149}$=2.72, $p<0.05$) between treatment condition and mulch type on AUDPS; this is seen in Fig. 9 where the black mulch resulted in somewhat higher AUDPS for the fungicide treatment condition, but lower for the UV-only treatment. Aside from the two-way interaction between the treatment and mulch type, this analysis of the AUDPS values was consistent with the ANOVA on the disease severity values in identifying significant differences among the treatments but not between the two types of mulch in year 2.

Using the same analytical procedure as for the year 1 data, the year 2 data for each type of mulch were plotted on log-log axes (excluding zero values) and are shown in Fig. 10 and 11. Similar to data from year 1, these data also seem to fall along straight lines. Using the average exponent value (b=3.19) from the year 1 data, best-fitting power functions of the form $y = ax^{3.19}$ were determined for each condition and these functions are also plotted in Fig. 10 and 11. Goodness of fit (r^2) values for the best-fitting functions ranged from 0.70 to 0.97 with the exception of the untreated (with black mulch) condition, which exhibited a somewhat different shape of its disease progression curve compared to the treatment conditions, as illustrated in Fig. 7 and 8. The goodness of fit value for the untreated data was 0.024.

In general, there are two observations from these figures in comparison to Fig. 5 and 6, which show the corresponding model functions for year 1. First, there was no obvious plateauing effect for the fungicide conditions in year 2 like there seemed to be in year 1. Disease progression for the fungicide conditions in year 2 seemed to follow a similar progression overall as all other conditions, including the untreated control (albeit delayed somewhat). Second, there is somewhat more dispersion among the modeled power functions for year 2 than there was in year 1. For example, using 10% disease severity as a threshold for observable disease (Fig. 10 and 11), the difference in reaching this criterion between the worst (untreated control) and best (UV+fungicide) conditions were 3.8 days for the black mulch, compared to less than 1 day among all non-fungicide conditions in year 1 (Fig. 5 and 6). Depending upon exactly when during the disease progression that the data were collected, the fitted power functions in these figures may have reflected ranges of days closer to the beginning (for year 1) or end (for year 2) of time when disease symptoms were progressing. These differences, as well as the limited sample sizes underlying Fig. 5 and 6, might explain the lack of difference among the fitted curves for year 1.

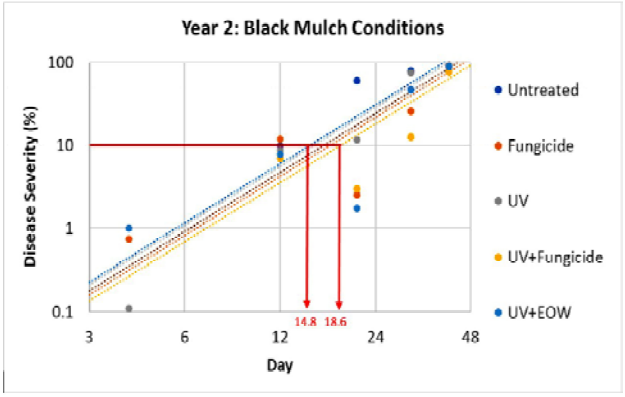

Fig. 10 : Disease severity values (non-zero only) for each condition using black mulch, in year 2. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

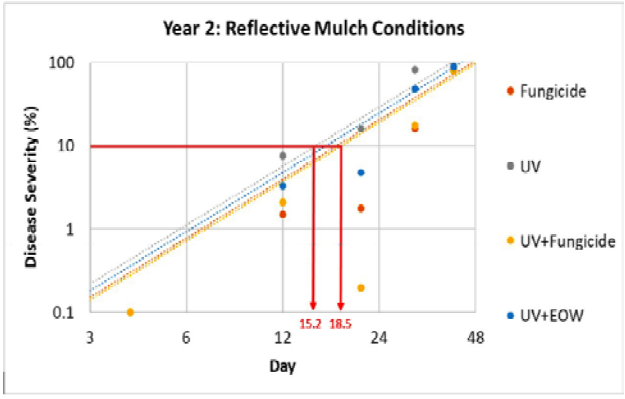

Fig. 11 : Disease severity values (non-zero only) for each condition using reflective mulch, in year 2. Also shown are best-fitting power functions having the form $y = ax^{3.19}$. The range of days at which disease progression reached 10% is also indicated by the red arrows.

The results for the field studies in each of years 1 and 2 exhibited some consistencies and some differences. Overall, the results suggest that, for the doses examined (up to 480 $J \cdot m^{-2}$), UV by itself is not an effective treatment for downy mildew (*P. cubensis*) control in cucumbers in the field, especially in comparison to the fungicide regimens used during this study. Nor were there any obvious monotonic trends in year 1 for the 120 to 480 $J \cdot m^{-2}$ doses as one might expect if the lowest dose were consistently worse than the highest dose. Possibly, higher UV doses (e.g., 1000 $J \cdot m^{-2}$) might have been more effective at reducing the severity of disease. However, higher doses than those used in the present study might be damaging to plants, given the reductions in cucumber leaf area observed by Patel *et al.* (2020) in laboratory studies caused by a UV-C dose from an electric light source of only 70 $J \cdot m^{-2}$. It would also have been challenging to deliver larger doses with the present apparatus without making time-consuming multiple tractor passes over the crops, another practical limitation.

Nonetheless, the application of UV in conjunction with fungicide treatment did show a statistically significantly lower level of disease severity for the black mulch conditions, corresponding to reduced progression of downy mildew (although a significant difference was not observed with reflective mulch). It may be possible to reduce fungicide treatment in conjunction with UV treatment and achieve a level of disease control that is consistent with current conventional practices for fungicide application, but identifying the dosing required to do so is not possible from the present data. In addition, fungicidal products exist that reduce concentrations of melanin pigment in the treated fungi such as tolprocarb (Hamada *et al.,* 2014). If the presence of pigments is a factor in the modest effectiveness of UV found in this study, it is possible to speculate that a combination of melanin-reducing fungicides plus UV might be more effective than the combination of UV and fungicides used in the present study.

Given the parallel disease severity progression curves in Fig. 5, 6, 10 and 11, it would appear that none of the treatments investigated in this study altered the course of disease progression once it was established (shown by the slopes of the curves), but rather that they sometimes delayed it (shown by the horizontal offsets among the curves). This is illustrated by the reasonably good fits of the disease severity progression data to power functions having the same exponent of 3.19. With respect to the UV-C treatments investigated in this study, the observed delays in onset of downy mildew may be attributed to increased resistance to downy mildew induced by the treatments prior to infection (Bonomelli *et al.,* 2004). Even if this could be substantiated in further experiments, however, the observed effect was relatively small.

Yield was not assessed precisely in the first- or second-year trials. However, observations of yield by the grower revealed that none of the treatments (UV at any dose, fungicide, or combination thereof) resulted in an observable reduction in yield relative to untreated crops. The lack of yield reduction suggests that the UV doses applied were below levels would result in significant phytotoxicity in cucumber plants, even though lower doses of 70 J m^{-2} could result in visible leaf damage under laboratory conditions (Patel *et al.,* 2020). It may be possible to increase UV dose to better control *P. cubensis* while still maintaining satisfactory yield, but it seems clear that the thresholds for yield-reducing damage to cucumber plants by UV are not well defined. This is important, however, because as described above, pigmented fungal spores are more resistant to damage from UV than unpigmented spores (Rotem *et al.,* 1985), and *P. cubensis* spores contain melanin pigment as they mature (Lee *et al.,* 2021). Identifying an upper limit for UV doses that do not damage the cucumber plants would be a useful next step in maximizing the potential beneficial impacts of UV-C treatment for downy mildew control. Such investigations should include precise field assessment of crop yields as well as further laboratory studies to identify optimal dosing parameters.

There were two main areas of inconsistency in the field test results between years 1 and 2. First, the disease severity for the fungicide treatment condition in year 1 did not exceed 17% whereas disease severity in year 2 for the fungicide treatment condition exceeded 85%. However, it should be noted that disease assessment was carried out for a greater number of days past the assumed infection date in year 2 (41 days) than in year 1 (28 days). Indeed, on day 31 in year 2, the fungicide treatment conditions had only exhibited 16%-26% disease severity, not much higher than the 15%-17% exhibited on day 28 in year 1.

The second main inconsistency between the results for years 1 and 2 was the impact of mulch types. In year 1, there were larger and more consistent reductions (or delays) in disease progression with the reflective mulch than in year 2. One possible post hoc explanation for this comes from the locations of the fields where the year 1 and 2 trials occurred. In year 1, the test field was in an open area with greater exposure to sunlight, and in year 2, the field was partially shaded by nearby trees. Although the reflective mulch had a much higher UV-C reflectance (66% at 254 nm) than the black mulch (5% at 254 nm), which would be expected to help increase the UV treatment efficacy, this did not seem to be the case in year 2. As one might expect, the reflective mulch also had a substantially higher visible reflectance (75% at 436 nm) than the black mulch (1% at 436 nm), and this could have resulted in soil temperatures being substantially higher with the black mulch because of much higher sunlight absorption compared to the reflective mulch. While not the primary focus of the present study, this suggests that if higher soil temperatures lead to decreased resistance to *P. cubensis*, reflective mulch may have some benefit because of its solar reflectivity.

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Original Research Paper

Morphological and molecular diversity of *Ganoderma* spp. causing basal stem rot of coconut in Southern dry tracts of Karnataka

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ABSTRACT

Morphological and molecular diversity of *Ganoderma* species causing basal stem rot of coconut in Southern dry tracts of Karnataka, India was carried out during 2016-17. A total of 20 isolates were isolated from Chitradurga, Chikamagalore, Hassan and Tumkur districts of Karnataka and were identified based on morphological and molecular characteristics. Sporocarps and diseased root bits were found as good source for isolation of *Ganoderma*. In all the isolates there were high variability in cultural, morphological and molecular characteristics. The dendrogram generated from the cultural and morphological characteristics showed clear variations among *Ganoderma* isolates and formed two main clusters, one cluster consisted of 13 isolates and another cluster consisted of 7 isolates. Several isolates showed 100 per cent similarity in the morphological characters regardless of their geographical origin. All the *Ganoderma* isolates amplified a fragment of 650 bp with fungal universal primers (ITS1 and ITS4). The ITS gene sequences of five isolates *viz*., CG_1 (MK 681870), CG_7 (MK681871), CG_{11} (MK681872), CG_{14} (MK681873) and CG_{20} (MK681874) were deposited in NCBI gene bank. Taxonomic comparison of the isolates with NCBI database proved that the isolates were genetically related to *Ganoderma* spp. with 80-100 per cent identity. However, all the tested isolates could not amplify *G. lucidum* species specific markers which indicate its absence in the region. The phylogenetic analysis of the *Ganoderma* isolates (ITS1 and ITS4) of coconut with other known species of *Ganoderma* from GenBank emphasized the close relationship with India, China and Sri Lanka isolates. The isolate CG_1 grouped with *Ganoderma carnosum* (KR 733545.1) with 98.97 per cent identity which is isolated from Sri Lanka and CG_{14} and CG_{20} grouped with *G. applanatum* (MF 072395.1) and *G. gibbosum* (OM 350473.1) with 98 to 99 per cent identity and CG_7 and CG_{11} isolates of coconut grouped into distinct sub cluster and clearly indicated the species diversity in *Ganoderma* infecting coconut in Southern Karnataka.

Keywords: Coconut, DNA sequence, *ganoderma* wilt, ITS, phylogeny and variability

INTRODUCTION

Coconut (*Cocos nucifera* L.) belonging to family Arecaceae is an important plantation crops of India providing livelihood to a substantial number of farm families. The versatile palm popularly known as 'King of Palms', 'Tree of Heaven', 'Tree of life', 'Tree of Abundance', as well as 'God's gift to mankind', is grown in more than 93 countries within an area of 12.8 million hectares and production of 10.9 m MT (copra equivalent) in 2001. The total area and the production in Asian Pacific Coconut Committee (APCC) countries are estimated at 11.4 mha and 9.2 m MT respectively, which is 90 and 84 per cent of world area and production (Rethinam and Taufikkurahman 2002). In India, coconut palms are grown in an area of 2.17 million hectares with a production of 20,308.70 million nuts and a productivity of 9345 nuts/ha annually (CDB, 2019-20). Kerala ranks first in terms of area and production followed by Tamil Nadu, Karnataka and Andhra Pradesh, while, Tamil Nadu ranks first in the productivity followed by Andhra Pradesh and Kerala.

Coconut palms are normally affected by various biotic and abiotic stresses resulting in drastic reduction in yields. Among the various biotic stresses that affect coconut production in India, Basal Stem Rot (BSR) or *Ganoderma* wilt caused by *Ganoderma applanatum* Pers and *G. lucidum* (*Leys*). Karst. is a major constraint in coconut production, especially in dry tracts of Southern Karnataka. The disease is reported from various places all over the tropical world *viz*., India, Sri Lanka, West Indies, Seycheles, Guam etc., Though the disease was first recorded by Dr. Butler in the beginning of 20th century and later by Venkatanarayan (1936) from Karnataka, a severe outbreak occurred in 1652 in Thanjavur district of Tamil Nadu and hence named as Thanjavur wilt. The disease is also reported from Andhra Pradesh, Kerala, Maharashtra, Gujarat and Orissa (Bhaskaran, 1994; Wilson *et al,* 1987). *Ganoderma* species are important wood decaying fungi occurring throughout the world. They are diverse in the tropics affecting plantation crops such as coconut, arecanut and oil palm by causing basal stem rot (Flood *et al.,* 2000 and Pilotti, 2005) and they also affect ornamental and forest trees in tropical and temperate areas causing disease and wood rots of timber (Lee, 2000).

The taxonomy of basidiomycetes has traditionally been based on the morphological features of the basidiocarps. Identification based on the basidiocarp features, however, is prone to problems such as absence of basidiocarps during certain times of the year, their morphological plasticity and presence of cryptic species (Moncalvo and Ryvarden, 1997; Gottlieb and Wright, 1999). However, studies had shown that *Ganoderma* species were genetically heterogeneous since wide range of genetic variation were reported and caused by out crossing over generations and different geographical origins (Miller *et al.*, 1999; Pilotti *et al.*, 2003). This leads to variation in their morphological characteristics even within same species (Hong *et al.*, 2001). For these reasons, contemporary taxonomists employ morphological studies, mating tests, analyses of biochemical and DNA sequence information or combinations of these for identification of the pathogen. Recently, molecular approach has been adapted to identify *Ganoderma* species such as through multiplex polymerase chain reaction (PCR) which is a more rapid and precise approach (Idris *et al.*, 2010; Wong *et al.*, 2012). Disease management is an important aspect to sustain the palm industry. Accurate identification of the pathogen is pre requisite for designing management strategies. Hence, the present study was undertaken to investigate the diversity of *Ganoderma* species isolated from BSR infected coconut palms in terms of their molecular and morphological characteristics.

MATERIALS AND METHODS

Collection of diseased root samples/stem bit and sporocarps of coconut from different places of Southern Karnataka

Different parts of the coconut palms such as diseased root bits/stem bits affected by *Ganoderma* wilt showing typical symptoms and sporocarps were collected from infected palms from various places of Southern Karnataka (Table 1). The samples were labeled and packed in polythene bags for the purpose of isolation of the causal organism.

Isolation and designation of the causal organism isolates

Infected roots/ stem bits collected from infected palms were washed thoroughly with sterile water and cut into small bits/pieces and were surface sterilized in 1 per cent sodium hypochlorite solution for 30 seconds and rinsed with sterile distilled water thrice serially to remove the traces of sodium hypochlorite. After surface sterilization, diseased specimens were kept in sterilized bags along with wet cotton under room temperature for about 8 to 10 days. After 8 to 10 days of incubation period, slight mycelial growth was observed and that was transferred into potato dextrose agar (PDA) medium. The inoculated plates were incubated at room temperature (28 °C ± 2 °C) for 3-5 days to facilitate growth of the fungus. Later, the bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by following hyphal tip culture technique under aseptic conditions. The isolated *Ganoderma* isolates of coconut were designated as CG_1, CG_2, CG_3, CG_4, CG_5, CG_6, CG_7, CG_8, CG_9, CG_{10}, CG_{11}, CG_{12}, CG_{13}, CG_{14}, CG_{15}, CG_{16}, CG_{17}, CG_{18}, CG_{19} and CG_{20}.

Table 1 : Identity and designation of *Ganoderma* isolates of coconut and their source of collection

Source for isolation	Place of collection	Designation of *Ganoderma* Isolates
Sporocarp	Karekodihally, Arsikere Tq. Hassan Dist.	CG_1
Root sample	Haranahally, ArsikereTq. Hassan Dist.	CG_2
Sporocarp	Vittalapura, ArsikereTq. Hassan Dist.	CG_3
Sporocarp	Nagenakoppalu, CR Pattana Tq. Hassan Dist.	CG_4
Root sample	Badarahally, Channarayapattana Tq. Hassan Dist.	CG_5
Root sample	Belagralli, Tiptur Tq. Tumkur Dist.	CG_6
Sporocarp	Hindiskere, Tiptur Tq. Tumkur Dist	CG_7
Sporocarp	Thimmanahali, C.N.Halli Tq. Tumkur Dist.	CG_8
Sporocarp	Anesidri, Hiriyur Tq. Tumkur Dist.	CG_9
Root sample	Dharmapura(H), Hiriyur Tq. Chitradurga Dist.	CG_{10}
Root sample	Venglapura, Hosdurga Tq. Chitradurga Dist.	CG_{11}
Sporocarp	Shettihalli, Hosdurga Tq. Chitradurga Dist.	CG_{12}
Root sample	Thirumalapura Holalkere Tq. Chitradurga Dist.	CG_{13}
Sporocarp	Thalakatta, HosdurgaTq. Chitradurga Dist.	CG_{14}
Sporocarp	Vaderahalli, Holalkere Tq. Chitradurga Dist.	CG_{15}
Root sample	Doddanaramangala, Tumkur Tq. Tumkur Dist.	CG_{16}
Root sample	Kodipalya, Tumkur Tq. Tumkur Dist	CG_{17}
Sporocarp	Shettikere, C.N.Halli Tq. Tumkur Dist.	CG_{18}
Sporocarp	Hullekere, Turvekere Tq. Tumkur Dist.	CG_{19}
Sporocarp	Thyagaturu, Gubbi Tq. Tumkur Dist.	CG_{20}

Note: CG-Coconut *Ganoderma*

Maintenance of pure cultures

The isolated fungus was sub-cultured on PDA slants and allowed to grow at 28 °C ± 2°C temperature for 8-10 days. The cultures so obtained were stored in refrigerator at 4°C for further studies and they were cultured periodically once in 2 to 3 months.

Study on variability of *Ganoderma* isolates of coconut

Twenty *Ganoderma* isolates of coconut isolated during course of investigation were used in variability study.

Cultural morphological variability of *Ganoderma* isolates

Growth on potato dextrose agar

Twenty *Ganoderma* isolates [CG_1, CG_2, CG_3, CG_4, CG_5, CG_6, CG_7, CG_8, CG_9, CG_{10}, CG_{11}, CG_{12}, CG_{13}, CG_{14}, CG_{15}, CG_{16}, CG_{17}, CG_{18}, CG_{19} and CG_{20}] of coconut collected from different geographic locations were cultured on PDA. The morphological characters like colony diameter/growth, biomass production, colony colour, colony margin, mycelial density, appearance of zones, reverse pigmentation etc were studied.

Mycelia plug (6 mm) from seven days old active culture was transferred onto the centre of a standard 9 cm PDA plate and incubated for 7 days at an ambient temperature (Idris *et al.,* 2000). The test for all isolates with three replications was run simultaneously to avoid bias due to external factors. The diameter was measured daily and the number of days required for maximum growth of mycelium was also recorded. The colony texture, appearance of zone, reverse pigmentation colour, type of colony margin and mycelial density were recorded after seventh day of incubation.

Growth on liquid media

The flasks containing 100 ml of sterilized potato dextrose broth (PDB) were inoculated with the 0.6 cm mycelial discs of *Ganoderma* isolates of coconut. Three replications were maintained for each treatment. The inoculated flasks were incubated at room temperature (28±2 °C) for 10 days, and then mycelial mat was harvested on a previously weighed Whatman No.4 filter paper and dried at 60 °C in a hot air oven till constant weight was obtained. The dry mycelial weight was recorded and expressed in mg 100 ml^{-1} broth and results were analysed statistically.

Qualitative data of cultural characteristics on solid media and bio mass were transformed into code and a numerical data matrix was generated (Table 2). The data was subjected to cluster analysis using multivariate statistical package (MVSP version 3.13). Similarity matrices were calculated using the simple matching coefficient and a dendrogram was generated using the unweighted pair group method of arithmetic averages (UPGMA) (Pilotti *et al*., 2004).

Table 2 : Cultural morphological characters and their corresponding codes used to describe *Ganoderma* isolates for assessment of cultural morphological characteristics

Character	Description	Code
Days for full plate	< 8	1
	8-9	2
	10-11	3
	> 11	4
Biomass (g/100 ml-1)	< 1	5
	1-1.25	6
	> 1.25	7
Colony colour	White	8
	Creamy white	9
Mycelia texture	Smooth	10
	Leathery	11
	Fluffy	12
Concentric rings	Present	13
	Absent	14
Reverse pigmentation	No pigmentation (White)	15
	Pale yellow	16
	Yellowish	17
	Yellow	18
	Pinkish	19
Mycelia density	Thin	20
	Dense	21
	Thin at center & dense at corner	22
	Dense at center	23
Margin	Filamentous	24
	Even	25
	Undulate	26
	Erose	27
	Lobate	28

Molecular characterization of *Ganoderma*

The isolates of *Ganoderma* species were identified through ITS (Internal Transcribed Spacer) region using universal primers ITS1 and ITS4 amplification.

Reagents and chemicals

All the chemicals were of analytical grade (M/s Sigma Ltd. and M/s Merck Ltd.). The following buffers and solutions were prepared : Extraction buffer (100 mmM Tris-HCl (pH 8); 20 mM EDTA (pH 8); 2 M NaCl; 3 % CTAB (w/v); 1 % PVP; 2 % β-mercaptoethanol (v/v); phenol : chloroform (24:1); potassium acetate 7.5 M; proteinase K, 0.05 mg ml^{-1} ; wash solution [15 mM ammonium acetate in 75 % (v/v) ethanol]; TE buffer [10 mM Tris-HCl (pH 8), 1mM EDTA (pH 8)].

Fungal genomic DNA extraction

Fungal mycelia (100 mg) were ground to fine powder using liquid nitrogen. Pre-warmed extraction buffer (1 ml) was added to the samples and it was ground once more in the buffer. All the samples were transferred to 2.0 ml Eppendorf tubes and 5 µL proteinase K (10 mg ml^{-1}) was added. The tube was incubated in 37 °C for 30 min and then at 65°C for another 30 min with frequent swirling. Samples were centrifuged at 10,000 x g for 10 min at RT and the supernatant was transferred to fresh Eppendorf tube. To the supernatant, 100 µL of 7.5 M potassium acetate was added and incubated at 4°C for 30 min. The samples were centrifuged at 13,000 x g for 10 min at RT; the supernatant was transferred to fresh tube, an equal volume of chloroform: isoamyl alcohol was added and mixed by gentle in version 30-40 times. The samples were centrifuged at 10,000 x g for 10 min at RT. The supernatant was transferred to a fresh tube and precipitated with 2/3 volume of isopropanol. The precipitated nucleic acids were collected and washed twice with the wash solution. The nucleic acid pellet so obtained was air dried until the traces of ethanol was removed and dissolved in an appropriate amount of TE buffer (50-70 µL). The nucleic acid dissolved in TE buffer were treated with ribonuclease (RNase 10 $mgml^{-1}$), incubated at 37 °C for 30 min and stored at -20°C until further use. The experiment was repeated thrice and the results described as the mean of three independent experiments (Sambrook and Russel, 2001).

Qualitative and quantitative analysis of DNA

The quality and quantity of DNA was analyzed by running 2 µL of each sample mixed with 2 µL of 10x loading dye in one per cent agarose gel. The DNA from all the isolates produced clear sharp bands in one per cent agarose gel indicating good quality of the DNA. The DNA has been quantified by comparing with the 1 kb size marker (Genei, Bangalore) and by spectrophotometer (Nanodrop ND 1000).

PCR amplification of internal transcribed spacer (ITS) region

The ribosomal DNA (rDNA) unit contains genetic and non-genetic or spacer region. Each repeat unit consisted of a copy of 18S, 5.8S and 28S like rDNA and its spacer like internal transcribed spacers (ITS) and Inter-Genic Spacers (IGS). The rDNA has been employed to analyze evolutionary events because it is highly conserved whereas ITS rDNA is more variable. Hence, it has been used for investigating the species level relationships. The primers for amplification were custom synthesized at Bangalore Genie Pvt. Ltd., Bangalore and supplied as lyophilized products of desalted oligos. PCR was carried out in poly propylene tubes using universal primers ITS 1 (5' -AACGTTACCAAACTGTTA-3') and ITS 4 (5' -AAGTTCAGCGGGTATTCCT-3') and *G. lucidum* specific primers GSF (5' -CCCTAAACCTCTCAAA GTCA-3') and GSR (5' -TATCGTACAGGTTCT CGTG -3). PCR amplification was performed in 25 µl reaction mixture containing 10× reaction buffer supplied by the manufacturer, 100 ng of fungal DNA, each dNTP at a concentration of 0.5 mM, 20 Pico moles of each primer and 1 U of Taq DNA polymerase (NEB, USA). Thermo cycling conditions were 94° C for 5 min, followed by 30 cycles of 94° C for 30 sec, 56° C for 1min and 72° C for 1min and a final elongation step of 72° C for 5min.

Separation of amplified products by Agarose gel electrophoresis

Agarose gel electrophoresis was performed to resolve the amplified product using 1.4 per cent agarose in 1X TBE (Tris borate EDTA) buffer, 0.5 mg ml^{-1} of ethidium bromide and loading buffer (0.25 % bromophenol blue in 40 % sucrose). Four µL of the loading dye was added to 5 µL of PCR product and loaded to the agarose gel. Electrophoresis was carried at 65 V for 1.5 hrs. The gel was observed under UV light and documented using gel documentation unit.

Sequencing of ITS region: The ITS region was sequenced from isolates of *Ganoderma* species to confirm the organism and to know the variability present in them. Homology search was done using BLAST algorithm (Basic Local Alignment Search Tool).

RESULTS AND DISCUSSION

Cultural and morphological variability/ characteristics of *Ganoderma* isolates of coconut

The results revealed that there were cultural morphological variations between isolates of *Ganoderma* isolated from infected palms of coconut in Southern dry tracts of Karnataka. The colony diameter on 5th, 7th and 9th day after inoculation was significantly varied, where radial growth ranged from 1.87 to 8.53 cm on 5th day after inoculation. Similarly on 7th and 9th day after inoculation it ranged from 2.63 to 9.00 cm and 4.75 to 9.00 cm respectively. The number of days taken to cover full plate ranged from 7 to 18 days and most of the isolates covered entire plate in 7 days as noted in CG_4, CG_7, CG_{10}, CG_{11}, CG_{12}, CG_{13}, CG_{14} and CG_{20}. However, some of isolates taken <10 days to cover entire plate. The bio mass production also varied significantly between different isolates and it ranged from 0.56 to 1.46 g/ 100ml. There were lot of variations observed with respect to colony/ mycelial characteristics *viz*., concentric rings. reverse pigmentation, density of mycelium and colony margin. However, there was not much variations were observed with respect to colour and texture of the colony (Fig.1 & 2 and Table 3)

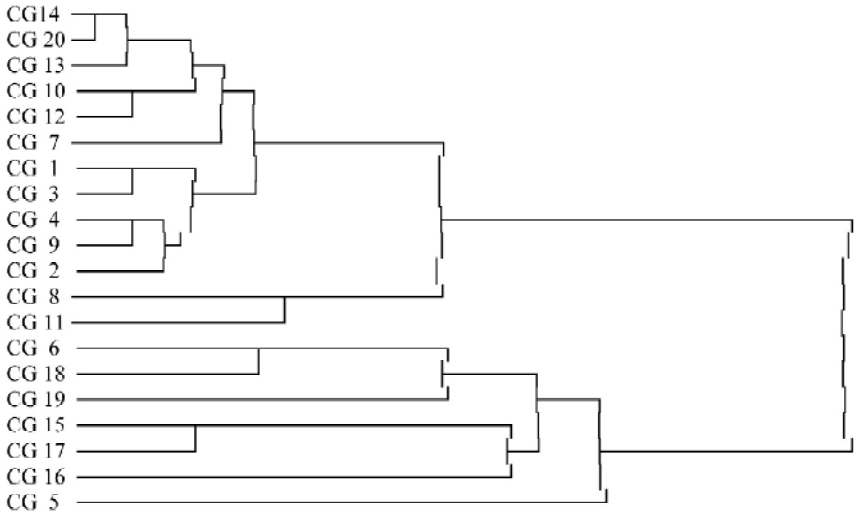


Fig. 1 : Dendrogram showing relationships of *Ganoderma* isolates of coconut based on similarity matrix of cultural/morphological characteristics

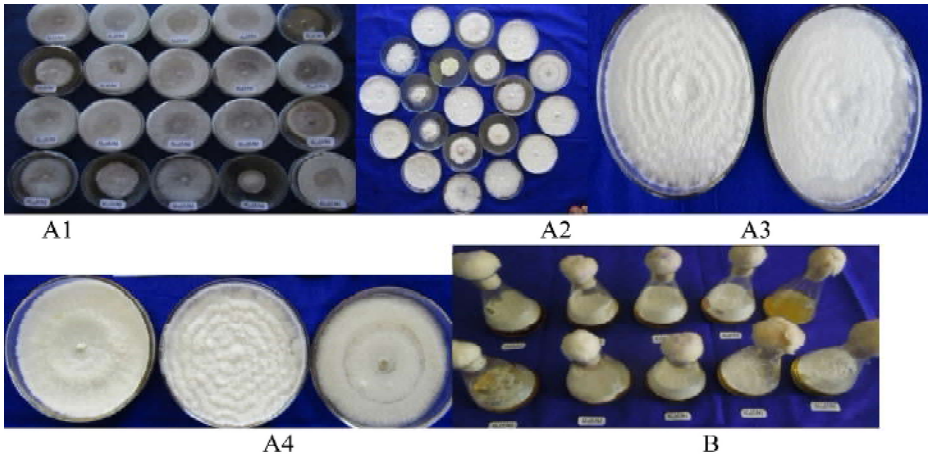


Fig. 2 : Cultural morphological variability *Ganoderma* isolates. A1-A4,) *Ganoderma* isolates on PDA and B) *Ganoderma* isolates on PDB

Table 3 : Cultural and morphological characteristics/variability of *Ganoderma* isolates of coconut

Isolate	Radial growth (cm)			Days taken	Biomass g/100ml	Colony/ mycelial characters			
	5 DAI	7 DAI	9 DAI			Colour/reverse pigmentation	Texture/ Density	Concentric Rings	Margin
CG_1	6.16	7.75	9.00	9	1.27 (6.48)	White /white	Fluffy/dense	-	Filamentous
CG_2	7.08	8.68	9.00	8	1.17 (6.14)	White/white	Fluffy/dense	-	Even
CG_3	7.63	8.95	9.00	8	1.36 (6.69)	White/pale yellow	Fluffy/dense	-	Filamentous
CG_4	8.36	9.00	9.00	7	1.09 (5.98)	White/white	Fluffy/dense	-	Filamentous
CG_5	2.50	4.75	6.25	14	1.01 (5.75)	Creamy white/pale yellow	Smooth/dense	-	Even
CG_6	2.67	4.33	6.00	11	0.77 (5.05)	White/ pale yellow	Fluffy/thin	-	Even
CG_7	8.53	9.00	9.00	7	1.46 (6.94)	White/yellowish	Fluffy/dense	-	Filamentous
CG_8	7.89	8.64	9.00	8	0.87 (5.33)	White/yellowish	Fluffy/dense	-	Filamentous
CG_9	6.58	8.22	9.00	9	1.16 (6.18)	White/White	Fluffy/dense	-	Filamentous
CG_{10}	8.39	9.00	9.00	7	1.14 (6.12)	White/ pale yellow	Fluffy/thin	-	Filamentous
CG_{11}	8.34	9.00	9.00	7	1.20 (6.28)	White/yellow	Fluffy/thin	-	Filamentous
CG_{12}	8.22	9.00	9.00	7	1.07 (5.95)	White/ pale yellow	Fluffy/dense	-	Filamentous
CG_{13}	8.34	9.00	9.00	7	1.05 (5.86)	White/ pale yellow	Fluffy/dense	-	Filamentous
CG_{14}	8.26	9.00	9.00	7	1.32 (6.58)	White/ pale yellow	Fluffy/dense	+	Filamentous
CG_{15}	2.64	5.58	7.00	14	0.95 (5.53)	White/yellow	Leathery/dense	+	Even
CG_{16}	2.47	5.50	7.50	11	1.30 (6.54)	White/ yellowish	Fluffy/thin	-	Undulate
CG_{17}	2.08	4.08	6.25	17	0.71 (4.81)	White/ yellow	Fluffy/dense	-	Undulate
CG_{18}	3.08	6.08	8.00	15	0.87 (5.36)	White/ pale yellow	Fluffy/dense	+	Even
CG_{19}	1.87	2.63	4.75	18	0.56 (4.26)	White/white	Fluffy/thin	-	Erose
CG_{20}	8.08	9.00	9.00	7	1.27 (6.45)	White / pale yellow	Fluffy/dense	+	Filamentous
SEm ±	0.086	0.155	0.014	-	0.226	-	-	-	-
CD (p=0.01)	0.850	1.12	0.395	-	1.348	-	-	-	-
CV (%)	4.998	5.273	1.719	-	7.978	-	-	-	-

Note: + Present; - Absent; DAI-Days after Inoculation *Mean of three replications

The dendrogram generated from the cultural morphological characteristics showed clearly the variations among *Ganoderma* isolates and dendrogram formed two main groups (Fig.1). The isolate CG_{14} and CG_5 are distinct. The complete similarity (100%) was found in several isolates of *Ganoderma* regardless of their geographical origin. All the isolates used under study showed high variability in cultural and morphological characteristics. Rakib *et al*. (2014) who had studied the genetic morphological variability of forty six isolates of *Ganoderma* causing basal stem rot and upper stem rot in oil palm stated that, there were significant variations within and between *Ganoderma* species in terms of their cultural morphology and basidiospore characteristics and they also reported that, cluster analysis of the cultural morphology and scattered plot of basidiospore features indicated that there was no distinct relationship within and between species, disease types or geographical origins of *Ganoderma* species.

The wide range of variation in morphological characteristic can be related to the heterogeneity of *Ganoderma* species. The cultural characteristic that appeared to distinguish *G. zonatum* from *G. boninense* and *G. miniatocinctum* was the strongly wavy characteristic of the colony in *G. zonatum*. However, this characteristic also varied and was not present in all of the *G. zonatum* isolates. Furthermore, the cultural appearances of fungi are also highly dependent on several factors such as type of media, pH and temperature (Adaskaveg and Gilbertson, 1989). Although similar (100 % similarity) cultural morphological features were observed between G_3 and G_4, G_{15} and G_{33}, G_{19} and G_{27}, and G_{30} and G_{31} based on the dendrogram generated, they were still genetically different based on the somatic incompatibility between the isolates. This showed that different genotype in *Ganoderma* species may express similar morphological features (phenotype). The dendrogram also showed same species of *Ganoderma* may be separated by up to 40 per cent dissimilarity, while different species of *Ganoderma* may have up to 92 per cent similarity. This indicates that *Ganoderma* species in an oil palm plantation could not be separated according to their species, disease type or geographical origins based on their cultural morphological features. More precise tool such molecular techniques/tools should be used to identify the *Ganoderma* species accurately.

Molecular characterization of *Ganoderma* isolates

Genomic DNA of different isolates of *Ganoderma* was isolated by CTAB method and the size was determined by resolving on one per cent Agarose gel. The concentration of DNA was determined using nanodrop equipment which was 75µg/µl.

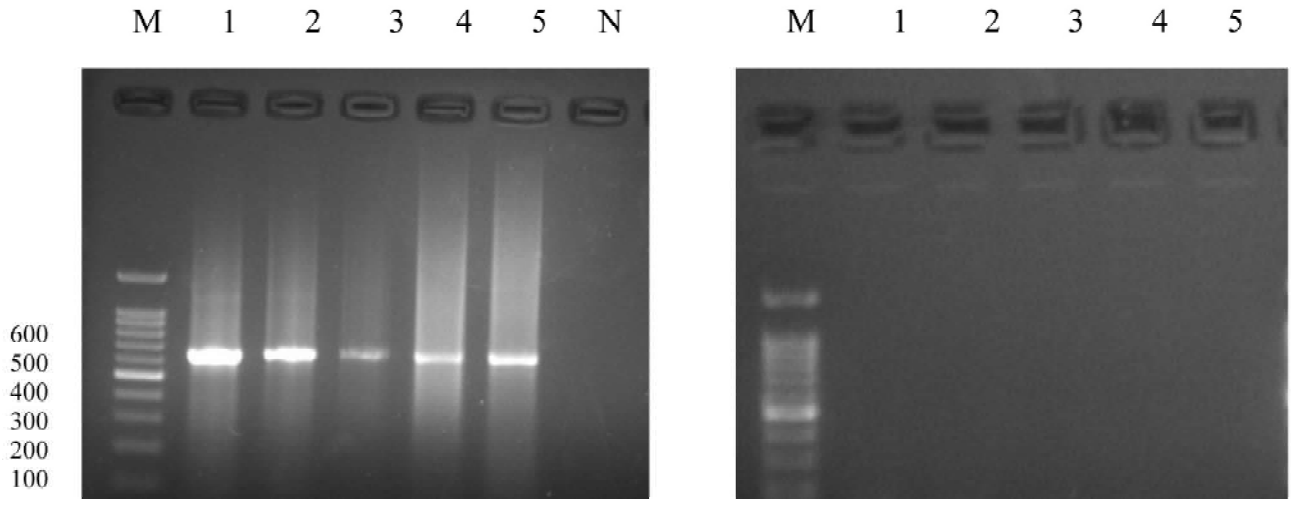
Amplification of ITS1 and ITS4 region of rDNA

The full length ITS rDNA region was amplified with ITS region with fungal universal primers (ITS1 and ITS4) and *G. lucidum* specific primers from the total genomic DNA of all the five isolates of *Ganoderma.*DNA amplicon was 600-650 bp in length in universal primers (Fig.3) and DNA was not amplified with *G. lucidum* specific primers and results revealed that, the *G. lucidum* species was absent in coconut isolates tested. Further, the species identity was confirmed with DNA sequencing.

DNA sequencing and specific amplification of *Ganoderma* isolates

The ITS rDNA fragments of *Ganoderma* isolates sequences were sequenced and DNA amplification from *Ganoderma* was observed at good specificity for the genus *Ganoderma* and approximately 600-650 bp product was exclusively amplified in all the isolates tested with fungal universal primers. DNA sequences of selected isolates of coconut was compared using bioinformatics tool like NCBI (National Centre for Bioinformatics) BLAST programme. Based on the sequence comparison, the identification of *Ganoderma* isolates was confirmed and all the ITS rDNA sequences of the isolates were confirmed as *Ganoderma* sp. with 80-100 per cent identity. The GenBank accession number for the ITS sequences for the isolates CG_1, CG_7, CG_{11}, CG_{14} and CG_{20} were MK681870, MK681871, MK681872, MK681873, MK681874. and Phylogenetic tree of *Ganoderma* constructed with ITS region sequences is shown in Fig. 4.

The phylogeny of the *Ganoderma* isolates of coconut revealed that, the isolate CG_1 grouped with *Ganoderma carnosum* (KR 733545.1) which is originated from Sri Lanka and CG_{14} and CG_{20} grouped with *Ganoderma* sp. (KR154930) and *Ganoderma* sp. (KM229652). These species were originated from India and CG_7 and CG_{11} isolates of coconut grouped into distinct sub cluster and indicated the species



ITS primers *G. lucidum* specific primers

Legend : Lane M = 100bp Ladder; Lane 1-5 = *Ganoderma* isolates of coconut; Lane N = Negative control

Fig. 3 : Gel picture showing PCR amplification of rDNA of *Ganoderma* isolates of coconut with ITS1, ITS4 and *G lucidum* primers

diversity and dissimilarity of *Ganoderma* in Southern Karnataka.

Abundance and uniform distribution of genetic markers in any pathogen is necessary for applications like diversity analysis at various levels. Almost unlimited in number, they are widely and evenly distributed in the genome. Unaffected by other genes and environment, the genotype of any individual of a population with respect to DNA based markers can be determined unequivocally at any stage of the development non-destructively. In addition, it is possible to generate markers to suite specific applications without altering the genotype of the individuals. It is difficult to distinguish these species using traditional morphological and physiological differences. To understand existence of variation among the isolates of pathogens, PCR based technique with *G. lucidum* specific markers and ITS sequence was used in the present investigation.

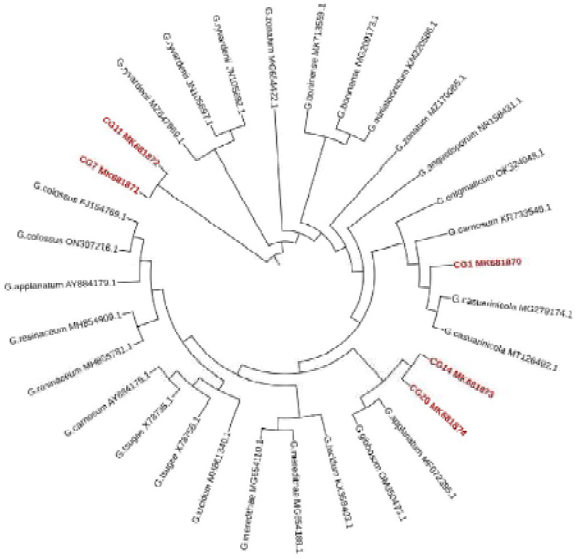


Fig. 4 : Phylogenetic relationships of *Ganoderma* isolates of coconut inferred from the sequences of the ITS region

Variations in morphological characteristics of *Ganoderma* have led many taxonomists to introduce biochemical and molecular methods to differentiate *Ganoderma* species. DNA amplification from *Ganoderma* was observed at good specificity for the genus *Ganoderma* and approximately 600-650 bp product was exclusively amplified in all the isolates tested with fungal universal primers. However, DNA amplification was not amplified with *G. lucidum* specific primers in the isolates tested. The sequencing and phylogenetic analysis of selected isolates *Ganoderma* infecting coconut revealed the *Ganoderma* species diversity in dry tracts of Southern Karnataka.

Nuclear rDNA, including the small and large subunits, 5.8S, and the Internal Transcribed Spacer (ITS) region, proved an ideal target for specific PCR primers, as each sequence is variable at the family,

genus, or species level. Internal Transcribed Spacer (ITS) regions have been successfully used to generate specific primers capable of differentiating closely related fungal species. Amplification of target DNA through PCR with taxon-specific primers is a potentially more sensitive and accurate approach than conventional microscopic techniques. Nucleotide sequences from certain regions of the DNA reflect phylogeny at various taxonomic levels. Such regions need to be evolving at an appropriate rate in order to supply enough consistent differences to separate the taxa into statistically supported monophyletic groups. These regions must be present as a single copy in the genome or evolve as a single copy region in order to avoid comparisons of different copies in different species (paralogous comparisons) if the region exists as multicopy. Also, the region should have the same function in all organisms (Mitchell *et al*., 1995). The ribosomal RNA (rRNA) genes, certain ribosomal elongation factors, and genes from the nuclear and the mitochondrial genomes have been useful for DNA sequence analysis in fungi (Tan and Niessen, 2003; Moreau *et al.,* 2006). Consequently, nucleotide sequence information from relatively conserved genes/ DNA segments such as the ITS (Moncalvo *et al*., 1995a, b; Smith and Sivasithamparam, 2000a), the mitochondrial small subunit (mtSSU) (Hong and Jung, 2004), and nuclear large subunit (LSU) (Lee *et al.,* 2006) rDNA have been widely used in the taxonomy and phylogeny of *Ganoderma* species. This is because the variability of these regions, which is harboured mainly in the introns, provides sufficient resolution at various taxonomic levels.

Gottlieb *et al.* (2000) adopted rDNA analysis (ITS I and II of 5.8S rDNA) to identify South American isolates of *Ganoderma* and *Elfvingia* and found molecular and morphological agreement at the sub generic level, however this relationship was difficult to visualize at the species level. Singh *et al*. (2003) characterized 61 accessions using DNA finger printing technique and RAPD/ AFLP analysis which revealed highly significant genetic variability among *G. lucidum* isolates collected from coconut gardens in Coimbatore. Phylogenetic analysis of the ITS sequence data was used to resolve Australian *Ganoderma* isolates into five terminal clades, and showed that a number of isolates had been misnamed (Smith and Sivasithamparam, 2000a). Based on the phylogenetic analysis of the ITS and 5.8S sequence, Latiffah *et al.* (2002) showed that *Ganoderma* isolates from infected oil palm and coconut stumps belong to the same group as classified by PCR-RFLP. Gottlieb *et al*. (2000) also used ITS-based phylogenetic analysis together with PCR-RFLPs to elucidate the taxonomy of *Ganoderma* species in South America. They reported that molecular and morphological data agree at the subgeneric level, but that it was difficult to determine relationships at the species level.

Earlier studies based on morphological identification asserted that North American *G. lucidum* and European *G. resinaceum* belong to the same biological species (Adaskaveg and Gilbertson, 1986). Based on phylogenetic relationships and nucleotide sequence variations of the ITS (Moncalvo *et al*., 1995a, b) as well as the mtSSU (Hong and Jung, 2004), these two species were shown to be different. The gene phylogeny by Moncalvo *et al*. (1995b) has indicated that isolates that were morphologically identified as *G. lucidum* did not cluster together, neither did those identified as *G. tsugae* or *G. resinaceum*. In the phylogenetic analysis of *Ganoderma* species using mtSSU sequence data by Hong and Jung (2004), *Ganoderma* species were divided into six monophyletic groups (*G. colossus* group, *G. applanatum* group, *G. tsugae* group, Asian *G. lucidum* group, *G. meredithiae* group, and *G. resinaceum* group) that included different species that were identified based on morphological characters. Species that were identified as *G. lucidum* were scattered over three of the groups, the Asian *G. lucidum* group, the *G. resinaceum* group and the *G. tsugae* group. Also, isolates that were identified as *G. oregonense* and *G. oerstedii* did not group together. These two studies indicate that some isolates were misidentified based on morphological characters since isolates that were identified as one thing do not form a monophyletic group.

CONCLUSION

From the preceding discussion it is clear that DNA sequence analysis of the ribosomal DNA region has provided an alternative approach to elucidate the taxonomy of *Ganoderma*. These techniques have played an important role in the taxonomy of *Ganoderma*, and have proved to be more reliable than other techniques that have been used for the same purpose. Misidentification and species synonyms based on morphological identification have been reduced using the molecular techniques. Among 5 isolates sequenced, isolate CG_{14} and CG_{20} are grouped

in same cluster both in morphological and molecular phylogeny. However, other isolates *viz*., CG_7 and CG_{11} which are genetically 100 per cent similar and grouped in same cluster are morphologically different as evidenced by grouping in different clusters in morphological phylogeny. In this study, a combination of cultural/morphological characteristics and molecular techniques allowed identification of groups within *Ganoderma* isolates of coconut and results indicated existence of morphological and molecular variability of *Ganoderma* isolates of coconut causing BSR in dry tracts of Southern Karnataka. Further, molecular characterization with *G. lucidum* species specific markers and fungal universal primers also indicated species diversity in *Ganoderma* causing basal stem rot/ *Ganoderma* wilt in coconut. In the present study based on phylogenetic analysis isolate CG_1 was identified as *G. carnosum* with 98.97 per cent identity and isolates CG_{14} and CG_{20} showed maximum (98.96 to 99.46 %) identity with *G. gibbosum* and *G. applanatum* species and indicating the different species associated with *Ganoderma* wilt of coconut in dry tracts of Southern Karnataka. However, the species identity has to be confirmed by systematic investigation with polyphasic taxonomic approach to unravel the species diversity of *Ganoderma* causing basal stem rot in coconut in Karnataka.

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Original Research Paper

Management of diseases and insect-pests of French bean in Northwestern Indian Himalayan region using integrated approaches

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ABSTRACT

French bean (*Phaseolus vulgaris* L.) production is adversely affected by many pathogens and insect-pests worldwide. In the present investigation, effect of different bio-fortified composts, organic amendments, botanicals and pesticides were evaluated against diseases and insect-pests of french bean. The results showed that seed treatment and drenching with *Trichoderma harzianum* strain 11, followed by soil application of fortified farmyard manure resulted in the lowest root rot incidence, highest germination, vigour and yield in french bean. In another set of experiment, soil incorporation of *Parthenium hysterophorus*, *Urtica dioica* and *Lantana camara* were found to reduce root rot incidence with high germination and pod yield. Among the bioproducts and botanicals tested, foliar spray of cow dung extract (50%) reduced angular leaf spot, rust and bacterial blight severity by 51, 69 and 25 per cent, respectively. Among the fungicides, foliar application of azoxystrobin 23 SC (0.1%) and difenoconazole 25EC (0.025%), also reduced angular leaf spot and rust severity by 93 and 90 per cent, respectively. Among different insect pest management strategies under field conditions, cartap hydrochloride and batain seed extract registered low sucking bug (*Chauliops choprai*) population. Integrated approaches including bio-agents, botanicals along with chemicals for managing these diseases and insect-pests were found appropriate options. Out of six different IPM modules evaluated, seed treatment with carbendazim along with foliar spray of 0.1% azoxystrobin and cartap hydrochloride resulted in lowest root rot, rust, angular leaf spot, bacterial blight and *Chaulopsis choprai* bug population in French bean.

Keywords : Bioagents, botanicals, eco-friendly management, french bean, fungicides and insecticides

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is globally grown in nearly 1.58 million ha with a production of 23.28 million ton (FAO, 2020) of which major production is from developing countries. The increasing incidence of soil-borne and foliar diseases, *viz.,* root rot, rust, bacterial blight and angular leaf spot and insect like sucking bug has become a major constraint for the profitable cultivation of bean since last few years (Joshi *et al*., 2009; Mageshwaran *et al.,* 2012; Jakhar and Chaudhary, 2013). The losses due to bean root rot disease some time exceed 70 per cent (Navarrete-Maya *et al*., 2009). Losses due angular leaf spot caused by *Phaseoisariopsis griseola* and rust caused by *Uromyces appendiculatus* varies up to 80 per cent and 18-100 per cent, respectively. The management practices for diseases and insect-pests rely primarily on application of chemical pesticides. The excessive and indiscriminate uses of pesticides adversely affect soil flora-fauna and have raised serious concern about health and environmental hazards.

There is growing awareness about the use of plant extracts, bio-agents, bio-pesticides and chemicals together that can provide more environmentally sound and economically feasible alternatives for disease and insect-pests management. Washings of vermicompost (biowash) prepared from foliage of Jatropha (*Jatropha curcas*), Annona (*Annona squamosa*) and parthenium (*Parthenium hysterophorus*) were

reported to be effective against *Fusarium oxysporum* f. sp. *ciceri* (wilt of chickpea), *Sclerotium rolfsii* (collar rot of chickpea) and *Macrophomina phaseolina* (Subramaniam *et al*., 2010). Similarly, leaf extracts (20%) of neem and chinaberry were reported to inhibit *Alternaria solani* and *F. oxysporum* f. sp. *lycopersici*, the pathogens of early blight and wilt diseases of tomato (Hassanein *et al*., 2008). Seed treatment with *T. harzianum* isolates CEN287, CEN289 and CEN316 reduces the incidence of *Aspergillus*, *Cladosporium* and *Sclerotium sclerotiorum* in common beans and promoted plant growth and rhizosphere competence (Carvalho *et al*., 2011). Botanicals and bioagents are inexpensive, easily available and biodegradable with less non-target effects besides being effective against plant pathogens (Siddiqui and Gulzar, 2003). Use of bioagents as seed treatment and soil drenching reduces dissemination of pathogens and contribute for good crop establishment (Pomella and Ribeiro, 2009). Application of organic amendments is one of the disease management strategies especially against soil borne plant pathogens. Soil application of paper mill residual amendments suppressed cucumber damping-off and foliar brown spot of snap bean (Stone *et al*., 2003).

Organic matter (OM)-mediated suppression of soil borne diseases in field soils caused by pathogenic species of *Pythium* and *Phytophthora* has been reported for a variety of plant species and organic substrates (Lewis *et al*., 1996; Lourd *et al*., 1987). Soil amendment with bio-agent fortified compost can modify the microbial community composition by enhancing the competition for nutrients or antagonism or mycoparasitism among microbes (Aryantha and Guest, 2006). In addition, beneficial microbes activate the plant to defend themself, a phenomenon termed 'induced systemic resistance' (Conrath *et al.,* 2002; Van-Loon, 2007). Chemical control is an important option in the management of bean diseases and pests because of the widespread occurrence of foliar diseases and the susceptibility of the available cultivars (Emeran *et al.,* 2011). Most of the studies focused on laboratory and greenhouse-based evaluations of inhibition ability, production of antibiotics and suppression of mycelial growth. In addition to right choice of fungicides, more studies are required to identify the time and schedule of sprays. Very few and inadequate studies are available on field assessment of bio-control agents, botanicals in combination with pesticides for their ability to reduce the incidence of diseases and insect-pests of french bean as an IPM module under Northwestern Himalayan region (Joshi *et al*., 2009). Hence, integrated approaches including bio-agents, botanicals along with chemicals for managing bean diseases and insect-pests could be the appropriate options and has been explored in this study.

MATERIALS AND METHODS

All laboratory and field experiments were conducted in completely randomized design (CRD) and randomized block design (RBD), respectively with three replications for each treatment on a susceptible variety, VL Bauni Bean-2.

In vitro studies

Preparation and evaluation of plant extracts

Leaf samples of plants *viz*., *Eucalyptus globulus, Parthenium hysterophorus, Urtica dioica, Quercus leucotrichophora, Lantana camara, Oxalis latifolia* and *Artemisia hirsuta* were collected, air dried at 50°C, powdered and stored at room temperature in desiccator before analysis. Dried samples (5 g) were grinded in a super mill grinder 1500 series (Newport Scientific Pvt. Ltd.) and grinded samples (1.5 g) were extracted by semiautomatic Soxhlet apparatus (Pelican, Socsplus, 2AS, Chennai) in methanol at 100 °C for 1 h and 90 per cent methanol was recovered during recovery phase at 130°C for 30 min, The methanolic extracts were dried at 80 °C, again dissolved in methanol to a concentration of 250 and 500 ppm and stored at 4 °C for further use.

Field studies

Trichoderma isolates (50) isolated from soil samples collected from different locations of Uttarakhand hills of North-western Himalayas were evaluated *in vitro* for their antagonistic activity against french bean pathogens. Based on antagonistic activity, three *T. harzianum* isolates (Th-11, Th-28 and Th-34) were selected and mass multiplied for further testing under field conditions. On the other hand, soil borne pathogens *viz*. *Rhizoctonia solani* and *Fusarium oxysporum* were also identified (Booth, 1985; Domsch *et al*., 1980) and mass produced for field inoculation.

Effect of bioagent fortified compost on root rot incidence

Bioagent fortified composts were evaluated against root rot of french bean at experimental Sick plot ,

Hawalbagh during the year 2008 and 2009. *Trichoderma harzianum* isolates (Th-11, Th-28 and Th-34) having 2 x 10^8 cfu (20 g/kg), were mixed with farm yard manure (FYM) (10t/ha) and poultry manure (PM) (5 t/ha), 15-20 days prior to the soil incorporation. Soil was pre-inoculated with the test pathogens. Bean seeds, treated with different isolates of *T. harzianum* (2 mL of suspension at 2.5x10^8 conidia mL^{-1} per 100 g of seeds) were sown at 15 cm plant to plant space in 3x3 m^2 at ICAR-VPKAS experimental station, Hawalbagh, Almora. The mean average temperature during cropping period was 32°C. Plant emergence was recorded 15-20 days after sowing (DAS). At 20 DAS, four adjacent plants were removed per plot and vigour index was calculated.

Effect of organic amendments on root rot incidence

Field experiments to study the effect of organic amendments on root rot incidence were carried out during *kharif* 2009 and 2010. Sixteen organic amendments *viz.,* neem oil cake @ 5t/ha, mustard cake @ 5t/ha, saw dust @ 2.5 t/ha, poultry litter @ 5t/ha, wheat straw @ 2.5t/ha, *E. globulus* @ 20t/ha, vermicompost @ 5 t/ha, FYM @ 10 t/ha (dry wt basis), *P. hysterophorus* @ 20t/ha, mushroom spent compost @ 5t/ha, *U. parviflora* (or *dioica* @ 10t/ha, *Q. leucotrichophora* @ 20t/ha, *L. camara* @ 20t/ha, *O. latifolia* @ 20t/ha, *A. hirsuta* @ 20t/ha and composted paper @ 10t/ha were incorporated one month before sowing and untreated field plot was kept as control. Soil was inoculated to entire experimental field with test pathogens (*R. solani* and *F. oxysporum* @ 2 x 10^8 cfu) one week before incorporation of organic products. Seedling emergence and root rot incidence was recorded at post-emergence stage as well as 30 DAS and average accumulated disease incidence was calculated.

Evaluation of bio-products and fungicides against foliar diseases

In field conditions, six fungicides *viz.,* azoxystrobin @ 0.1%, difenoconazole @ 0.025%, propiconazole @0.05%, tebuconazole @ 0.05%, chlorothalonil @ 0.2% and mancozeb @ 0.25% were evaluated against rust and angular leaf spot diseases during 2009 and 2010. In another set of field experiments, effect of three fungicides *viz.,* azoxystrobin @ 0.1%, difenoconazole @ 0.025% and propiconazole @ 0.05%, with 1, 2 and 3 fungicide spray against rust, angular leaf spot and bacterial blight diseases of french bean were evaluated during the year 2011 and 2012. In a third set of field experiment, efficacy of 12 different bioproducts *viz.,* batain (*Melia azederach*) seed kernal extract @ 30%, *A. hirsuta*, *P. hysterophorus*, azadirachtin, panchgavya, neem cake extract, cow urine, cow dung extract, *Z. officinale* rhizome, *A. sativum* bulb, *T. domestica* bulb and horticultural mineral oil were evaluated against rust, angular leaf spot and bacterial blight diseases during the year 2009 and 2010 along with mancozeb @ 0.25% spray as positive control and untreated as negative control.

Evaluation of different insecticides against *Chauliops choprai*

Field trials were conducted at experimental farm, Hawalbagh with nine insecticides *viz*, thiamethoxam 25%WG, imidacloprid 17.8 SL, dinotefuran 20SG , cartap hydrochloride 75SG, deltamethrin 2.8 EC, spinosad 45SC, indoxacarb 14.5SC, endosulfan 35EC and profenophos 50EC during 2008-09 to test the efficacy of insecticides against the sucking bug, *C. choprai.* Observations were made on the number of adult insects per randomly selected three leaves in ten plants in each plot before the treatment and 3, 7, 14 and 21 days after spray. The per cent pest control by the treatment with respect to untreated check was calculated using Henderson and Tilton (1955) formula by taking average of the insects present after treatment. The insect count was subjected to statistical analysis adapting RBD with 3 replications using SPSS version 3/93 after converting it to square root values. The mean values of treatments were then subjected to Tukey highly significant difference (HSD) test.

$$\% \text{ reduction} = 100 \times 1 - \frac{(AT \times BC)}{(BT \times AC)}$$

whereas,

AT – No. of bugs present in the treated plants after treatment; BT – No. of bugs present in the treated plants before treatment, AC – No. of bugs present in the control plants after treatment and BC – No. of bugs present in the control plants before treatment

Another set of field trials were carried out using botanical (batain-*Melia azederach*), Bt and insecticide (cartap hydrochloride) to find their efficacy on sucking bug *C. choprai* in french bean for four years. Observations were made as given above on the

number of adult insect in the leaves and per cent reduction with respect to control calculated.

Statistical analysis

The experiments were analyzed separately using analysis of variances. Chi-square test was performed on the variances to test the homogeneity among the repeated experiments. The disease incidence was assessed based on the total number of plants infected over total number of plants observed in square meter area and then expressed in percentage. The data were subjected to analysis of variances and Fisher's protected least significant difference or critical difference was used to separate the treatment means. The data were statistically analyzed by using SAS 9.3 version software. The original data was transformed to arcsine in order to bring the data under normal distribution before analysis.

RESULTS AND DISCUSSION

Evaluation of plant extracts against *Rhizoctonia solani* and *Fusarium oxysporum*

Plant extracts Parthenium hysterophorus, U. dioica and *L. camara* showed significantly higher antifungal activity against *R. solani* and *F. oxysporum* at 500 ppm concentration (Table 1). A reduction of 76.30 per cent *R. solani* mycelial growth in comparison to control was observed for *P. hysterophorus* followed by *U. parviflora* and *L. camara* (73.33%). However, *L. camara* inhibited maximum radial growth of *F. oxysporum* (65.19%) followed by *P. hysterophorus* (58.89 %) and *U. parviflora* (58.15 %). This corroborates the findings of Hadi and Kashefi (2013) and Baraka et al. (2011). Hadi and Kashefi (2013) reported that *Cinnamomum zeylanicum, Mentha piperita, Allium hirtifolium* and *Allium sativum* recorded largest inhibition on the growth rate of *F. oxysporum*. Similarly, Baraka *et al*. (2011) reported *in vitro* efficacy of marjoram, garlic and jojoba against *F. oxysporum, F. moniliforme, F. solani, Thilaviopsis paradoxa, Botryodiplodia theobromae* and *Rhizoctonia solani* and reported the antifungal activity of different plant extracts against *F. oxysporum* and *R. solani*.

Effect of bioagent fortified composts on root rot incidence

The results of two years of field trials to evaluate the effect of composts fortified with biocontrol agents on root rot incidence of french bean are presented in Table 2. Significantly higher seedling emergence (87.02%) and lower root rot incidence (16.07%) was found in case of treatment T2 *i.e.,* seed treatment and drenching with *T. harzianum* strain 11 @ 1% along with soil application of fortified farmyard manure @ 10 t/ha, followed by T7-treated check *i.e*. seed treatment with thiram @ 3g/kg seed along with application of farm yard manure @ 10 t/ha. The results showed 29 to 49 per cent increase in seedling emergence and 13 to 47 per cent root rot incidence reduction in various treatments in comparison to control (Table 2). Maximum vigor index (3312) and yield (12.59t/ha) was found from treated check *i.e*., seed treatment with thiram @ 3g/kg seed along with application of farmyard manure @ 10 t ha^{-1}. The present results agree with Manjunatha *et al.* (2013), who reported that combining soil application through bioagent (*Trichoderma viride* and *Pseudomonas fluorescens)* enriched farm-yard manure, along with seed treated with the bio-control agents resulted in maximum germination, least root rot incidence and highest yields of chickpea plants against root rot pathogen, *Macrophomina phaseolina*.

Effect of organic amendments on root rot incidence

The results of experiments on organic amendments showed that various organic amendments resulted in increase in seedling emergence (26% - 47%), reduction in root rot incidence (32% - 64%) and increase in yield (13 to 111%) as compared to control (Table 3). The soil incorporation of *P. hysterophorus* and *L. camara* @ 20 t ha^{-1} was found to have maximum seedling emergence (83.28 and 81.57% %) and lower root rot incidence (11.58 and 11.86%) respectively. However, maximum yield (7.0 t ha^{-1}) was recorded with amendment of *L. camara* @ 20 t ha^{-1} followed by *U. parviflora* @ 10 t ha^{-1} (6.98 t ha^{-1}) and *P. hysterophorus* (6.85 t ha^{-1}). In this study, soil incorporation of *P. hysterophorus*, *L. camara* and *U. parviflora* resulted in maximum seedling emergence and reduction in root rot incidence which is in accordance with the findings of Angiras (2008) and Subramaniam et al. (2010). Soil amendment is a practice, which favours plant development, improves soil quality as well as having suppressive effect on many soil-borne plant pathogens (Nawar, 2008; Elwakil *et al*., 2009). Organic amendments in addition to disease suppression, improves the aggregation,

Table 1 : Evaluation of plant extracts against *Rhizoctonia solani* and *Fusarium oxysporum* causing root rot of French bean *in vitro*

Treatment	*Rhizoctonia solani* mean mycelial inhibition (%)		*Fusarium oxysporum* mean mycelial inhibition (%)	
	250 ppm	500 ppm	250 ppm	500 ppm
T1- *Eucalyptus globulus*	15.19	59.63	14.81	47.78
T2- *Parthenium hysterophorus*	17.04	76.30	13.70	58.89
T3- *Urtica parviflora*	12.22	73.33	8.52	58.15
T4- *Quercus leucotrichophora*	1.48	19.26	0.00	15.19
T5- *Lantana camara*	15.93	73.33	10.37	65.19
T6- *Oxalis latifolia*	0.74	24.07	0.00	20.74
T7- *Artemesia hirsuta*	4.07	27.41	1.85	19.63
T8- Control	0.00	0.00	0.00	0.00
Turkey HSD (P = 0.05)	0.41	0.57	0.20	0.67

(Mean of three replications)

Table 2 : Effect of bioagent fortified composts on root rot incidence of French bean under field condition

Treatment	Vigour index	Seedling emergence		Root rot incidence		Yield	
		Per cent	increase (%)	Per cent	Reduction (%)	t/ ha	Increase (%)
T1 - ST & drenching with *T. harzianum* (T- 11) @ 1% + SA of fortified PM @ 5 t/ha	2828	83.51[a] (66.44)	43	17.85[a] (24.87)	41	12.75[a]	45
T2 - ST & drenching. with *T. harzianum* (T- 11) @ 1% + SA of fortified FYM @ 10 t/ha	3179	87.02[a] (69.58)	49	16.07[a] (23.44)	47	11.77[a]	33
T3 - ST & drenching with *T. harzianum* (T-28) @ 1% + SA of fortified PM @ 5 t/ha	2627	83.03[a] (66.96)	42	20.63[ab] (26.59)	31	11.67[a]	32
T4 - ST & drenching. with *T. harzianum* (T-28) @ 1% + SA of fortified FYM @ 10 t/ha	2392	75.95[ab] (61.22)	30	24.10[ab] (28.80)	20	11.40[ab]	29
T5 - ST & drenching with *T. harzianum* (T-34) @ 1% + SA of fortified PM @ 5 t/ha	2533	75.51[ab] (61.44)	29	24.27[ab] (29.02)	19	11.57[ab]	31
T6 - ST & drenching with *T. harzianum* (T-34) @ 1% + SA of fortified FYM @ 10 t/ha	2550	79.19[ab] (64.27)	35	26.27[ab] (30.50)	13	11.13[ab]	26
T7 - Treated check (ST with thiram @ 3g/ kg seed with FYM @ 10 t/ha)	3312	84.33[a] (67.00)	44	16.45[a] (24.20)	44	12.59[a]	43
T8 – Control (FYM @ 10 t/ha)	2301	58.57[b] (56.05)	0	30.08[b] (33.08)	0	8.82[b]	0
Turkey HSD(P = 0.05)	241.5	10.93	-	8.14	-	1.98	-

Seed Treatment; Soil Application
Figures in parentheses represent arc sine transformed values
Means in the same column followed by different letters are significantly (P < 0.05) different.

Table 3 : Effect of organic amendments on root rot incidence of french bean

Treatment	Seedling emergence		Root rot incidence		Pod Yield	
	Per cent	Per cent increase	Per cent increase	Per cent increase	t/ha	Per cent increase
T_1 - Neem oil cake @ 5t/ha	74.75(59.84)	32	17.82(21.80)	45	5.88^{abc}	77
T_2 - Mustard cake @ 5t/ha	72.49(58.38)	28	21.11 (25.04)	34	3.83^{cd}	15
T_3 - Saw dust @ 2.5 t/ha	76.30(60.94)^{c}	35	17.78 (21.33)	45	4.96^{abcd}	49‘
T_4- Poultry litter @ 5t/ha	74.77(59.85)	32	17.28 (21.20)	46	5.63^{abcd}	69
T_5 - Wheat straw @ 2.5t/ha	77.03(61.44)^{c}	36	17.65 (20.82)	45	5.71^{abc}	72
T_6- *Eucalyptus globulus* @ 20t/ha	74.33(59.57)	31	19.58 (23.13)	39	5.13^{abcd}	54
T_7- Vermicompost @ 5 t/ha	75.72(60.48)	34	17.09 (21.06)	47	5.69^{abc}	71
T_8- FYM @ 10 t/ha (dry wt basis)	74.03(59.37)	31	15.40 (22.72)	52	5.15^{abcd}	55
T_9 - *Parthenium hysterophorus* @ 20t/ha	83.28(65.94)^{a}	47	11.58 (15.15)^{a}	64	6.85^{ab}	106
T_{10} - Mushroom spent compost @ 5t/ha	75.12(60.08)	33	18.02 (22.00)	44	3.75^{cd}	13
T_{11}- *Urtica parviflora* @ 10t/ha	78.25(62.21)^{b}	38	15.37 (19.35)	52	6.98^{a}	110
T_{12}- *Quercus leucotrichophora* @ 20t/ha	72.93(58.66)	29	20.71 (24.66)	36	4.45^{cd}	34
T_{13}- *Lantana camara* @ 20t/ha	81.57(64.59)^{a}	44	11.86 (15.34)	63	7.00^{a}	111
T_{14}- *Oxalis latifolia* @ 20t/ha	74.07(59.39)	31	19.38 (22.84)	40	4.58^{bcd}	38
T_{15}- *Artemesia hirsuta* @ 20t/ha	74.78(59.86)	32	18.83 (22.25)	42	5.79^{abc}	74
T_{16}- Composted paper @ 10t/ha	71.55(57.79)^{f}	26	22.00 (25.45)	32	4.58^{bcd}	38
T_{17}- Control	56.65(48.82)^{g}	0	27.20 (31.63)^{d}	0	3.32^{d}	-
Turkey HSD(P = 0.05)	-	-	6.34	-	2.87	-

(Mean of three replications)

Figures in parentheses represent arc sine transformed values;
Means in the same column followed by different letters are significantly (P < 0.05) different.

reduce compaction and surface crusting, increase Carbon sequestration and nutrient availability, and enhance infiltration and water holding capacity of soil (Min *et al.,* 2003).

Effect of bioproducts against foliar diseases

Twelve bioproducts along with one fungicidal control and untreated control were evaluated against foliar diseases *viz*., rust, angular leaf spot and bacterial blight of french bean for two years and the data obtained were summarized in Table 4. The results indicated a reduction of 7-78 per cent rust disease, 5-65 per cent angular leaf spot and 25-54 per cent bacterial blight severity and an increase of 1-49 per cent in yield as compared to control. Foliar spray of bioproducts, batain seed kernel extract (30%), cow urine (50%) and cow dung extract (50%), were found at par with fungicidal (mancozeb) spray in reducing rust severity. Similarly, significantly lower angular leaf spot was recorded for foliar spray of *P. hysterophorus* (22.11%), azadirachtin (19.33%), Panchgavya (16.33%), neem cake extract (19.57%), cow urine (26.17%) and mancozeb (11.83%). All the bioproducts reduced bacterial blight severity significantly in comparison to control. The present results agree with Vijayalakshmi and Saranya (2010), who reported antimicrobial activities of cow urine against *Staphylococcus aureus, Escherichia coli, Aspergillus* and *Rhizopus*. Basak *et al.* (2001 and 2002) reported the inhibitory activity of cow urine and cow dung against *F. oxysporum* f. sp. *cucumerinum, F. solani* f. sp. *cucurbitae* and *Sclerotinia sclerotiorum*.

Evaluation of different fungicides against foliar diseases of french bean

The fungicides under study were highly efficacious, completely preventing rust and angular leaf spot infection, when applied thrice at 10 days interval. All the treatments were found significantly effective in reducing rust (25-93%) and angular leaf spot severity (36-93%) and resulted in increasing yield by 64-194

Table 4 : Effect of bioproducts against foliar diseases of French bean under field condition

Treatment	Rust		Angular leaf spot		BB		Pod Yield	
	Per cent	Reduction (%)	Per cent	Reduction (%)	Per cent	reduction (%)	Per cent	increase (%)
T1-BSKE @ 30 % FS	28.02^{abcd} (31.73)	55	27.50^{bcd} (31.57)	19	17.50^{abc} (24.66)	29	4.40^{abc}	25
T2-*A. hirsuta* @ 30 % FS	(34.25)	31.83^{bcd}	49 (31.79)	28.05^{bcd}	17 (24.29)	17.00^{abc}	31	3.77^{bc} 7
T3-*P.hysterophorus* @ 30 % FS	40.02^{defg} (39.13)	36	22.11^{abcd} (27.90)	35	17.00^{abc} (24.06)	31	3.73^{bc}	6
T4-Azadirachtin 0.03 % @ 0.2 % FS	36.00^{cde}	42 (36.82)	19.33^{abc}	43 (25.89)	14.84^{ab}	40 (22.58)	4.05^{bc}	15
T5-Panchgavya @ 3 % FS	53.84^{efgh} (47.25)	14	16.33^{ab} (23.69)	52	18.33^{bc} (25.26)	26	3.84^{bc}	9
T6-Neem cake extract @ 20 % FS	39.16^{cdef} (38.70)	37	19.57^{abc} (26.12)	42	14.55^{ab} (22.28)	41	4.43^{abc}	26
T7-Cow urine @ 50 % FS	22.83^{abc} (28.35)	63	26.17^{bcd} (30.64)	23	18.33^{bc} (25.30)	26	4.42^{abc}	25
T8-Cow dung extract @ 50 % FS	19.52^{ab} (26.00)	69	16.66^{ab} (23.92)	51	18.58^{bc} (25.43)	25	4.64^{ab}	32
T9-*Z.officinale* rhizome @ 5% F	54.26^{fgh} (47.51)	13	25.67^{bcd} (29.91)	24	16.70^{abc} (24.05)	32	3.87^{bc}	10
T10-*A. sativum* bulb @ 5% FS	32.83^{bcd} (34.85)	47	20.05^{abcd} (26.49)	41	14.52^{ab} (22.27)	41	3.96^{bc}	12
T11-*T. domestica* bulb @ 5% FS	43.33^{defg} (41.15)	31	26.00^{bcd} (30.41)	23	17.50^{abc} (24.66)	29	3.76^{bc}	7
T12-Horticultural mineral oil @ 1% FS	57.83^{gh} (49.55)	7	31.99^{cd} (34.25)	5	15.33^{ab} (22.89)	38	3.55^{bc}	1
T13-Mancozeb @ 0.25%	13.85^{a} (21.58)	78	11.83 (19.90)	65	11.33^{a} (19.50)	54	5.24^{a}	49
T14-Control	(52.35)	62.41^{h}	0 (35.46)	33.83^{d}	0 (29.68)	24.67^{c}	0	3.52^{c} 0
Turkey HSD (P = 0.05)	9.51		8.10		4.81		0.64	

Figures in parentheses represent arc sine transformed values;
Means in the same column followed by different letters are significantly different (P < 0.05).

per cent in comparison to control (Fig. 1a), emphasizing the effect of foliar spray of fungicides towards yield enhancement. Amongst all tested fungicides, application of azoxystrobin was found to have lowest rust (2.81%) and angular leaf spot (2.71%) severity with highest yield (11.94 t ha^{-1}) followed by difenoconazole. Similarly, from the repeated fungicidal spray experiment, lowest rust (3.33%), angular leaf spot (4.17%) and highest yield (10.12 t ha^{-1}) was recorded when three sprays of azoxystrobin was given (Fig. 1b), which was at par with two sprays of azoxystrobin.

Fungicide application appears to be a suitable short-term strategy for bean rust control in the absence of resistant cultivars. The differences in efficacy among the tested fungicides were probably related to their fungicidal activity. The present results are in line with the findings of various workers who reported reduced severity of bean diseases by applying different fungicides (Emeran *et al.,* 2011) Azoxystrobin is one of the leading systemic fungicide of strobilurin class developed from naturally occurring wood-decaying mushroom mainly inhibiting mitochondrial respiration by binding to the Q_0 site of cytochrome b, blocking

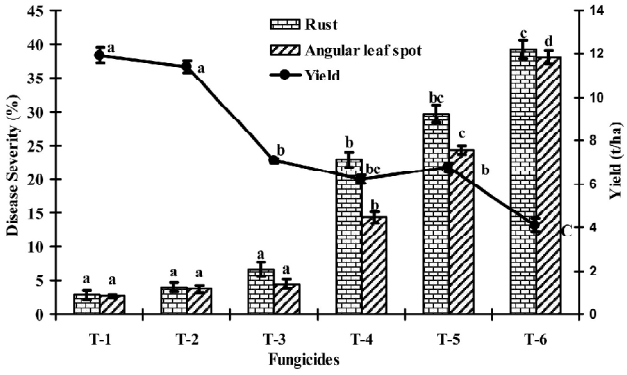

Fig. 1. : Effect of different fungicides against foliar diseases of french bean

T_1-Azoxystrobin @0.1%, T_2-Difenoconazole @ 0.025%, T_3-Propiconazole @0.05%
T_4- Chlorothalonil @0.2%, T_5- Mancozeb @ 0.25%, T_6-Control

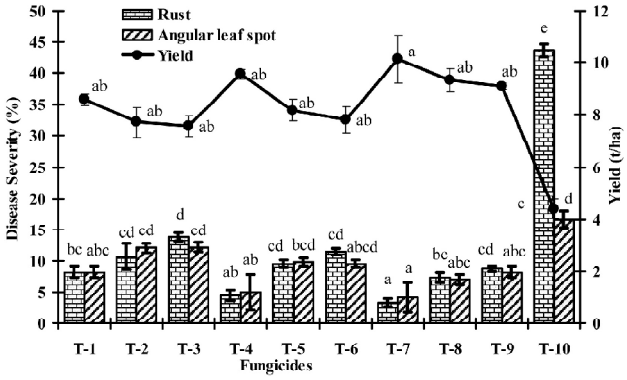

Fig. 2. : Effect of number of fungicide spray on foliar diseases of french bean

$T_{1,}$ $T_{4,}$ T_7-Azoxystrobin one, two and three sprays, $T_{2,,}$ $T_{5,}$ T_8-Difenoconazole one, two and three sprays
$T_{3,}$ $T_{6,}$ T_9-Propiconazole one, two and three sprays, T_{10}- Control

electron transfer and disrupting the production of ATP (Sundravadana *et al.,* 2009) and hence effectively controls many plant diseases (La Mondia, 2012). Difenoconazole are sterol synthesis inhibitors known to have acropetal systemic movement in plants and many have good activity against rust disease (Kuck *et al*., 1995). Similar findings on the impact of fungicides on plant diseases were reported by various workers (Miles *et al.,* 2007).

Bio-efficacy of insecticides on *Chauliops choprai* under field conditions

The results of the field trial on the insecticidal efficacy on *C. choprai* are given in the Table 5. The mean number of bugs in the first trial before treatment ranged from 4.67 – 5.67 three leaves^{-1} plant^{-1}. At three DAS, less number of bugs (0.33 plant^{-1}) was recorded in deltamethrin treated plots. Indoxacarb, cartap, endosulfan and spinosad treated plots were not significantly different with each other in terms of insect population at three days after treatment (DAT). At 14 DAS, 0.83 bugs plant^{-1} was recorded in deltamethrin sprayed plants which is not significantly different with the cartap treatment (0.84 bugs plant^{-1}) and endosulfan treatment (1.50 bugs plant^{-1}). Cartap hydrochloride treatment harbored the least number of bugs (1.0 plant^{-1}) at 21 DAS which is not significantly superior to deltamethrin (1.17 bugs plant^{-1}). The overall per cent reduction registered by deltamethrin and cartap hydrochloride were 82.70 and 79.16, respectively. Phosphamidon, parathion-methyl, endosulfan, quinalphos and fenitrothion are reported as effective insecticides for the management of *C. fallax* in India (Kashyap *et al*., 1980).

Integrated management of *Chauliops choprai* under field conditions

The results of the field trials on Integrated Pest Management (IPM) on *C. choprai* using botanical, *Bt* and insecticide is given in Table 6. The pretreatment count ranged from 9.42 to 10.42 bugs three leaves^{-1} plant^{-1}. At 3 DAT, treatments like, cartap, batain extract and batain + Bt registered low pest population of 0.84, 2.92 and 2.92, respectively which were not significantly different with each other. Batain and cartap hydrochloride was found to reduce the bug population to 1.0 and 1.82 per plant on 14 DAT. The overall reduction with respect to control showed cartap hydrochloride as superior with 89.59% followed by batain 78.82 per cent.

In the present study, more emphasis was given on use of bioagents, biopesticides, botanicals/plant extracts and judicious use of pesticides in compatible or complimentary manner. Gajanana *et al*. (2006) reported that root dipping of seedlings in imidacloprid, soil application of neem/ pongamia cake, spraying of botanicals like pongamia soap and bio-pesticide like Ha NPV has been found effective against both insect and diseases. Similarly, El-Mougy *et al*. (2013) reported that combine treatments of calcium chloride, thyme oil with bio-agents reduced significantly root rot incidence of cucumber, cantaloupe, tomato and pepper plants.

Table 5 : Bio-efficacy of insecticides on *Chauliops choprai* in french bean under field conditions

Treatment	PTC	3 DAT	7 DAT	14 DAT	21 DAT	Reduction*(%)
T1- Thiamethoxam	5.67^{a}	1.50^{abc}	3.84^{b}	2.67^{ab}	1.83^{bc}	58.45
T2- Imidacloprid	4.67^{a}	1.67^{abc}	1.34^{a}	2.00^{ab}	1.34^{bc}	67.46
T3- Dinotefuron	5.34^{a}	2.17^{bc}	2.67^{bc}	3.17^{b}	1.67^{bc}	56.62
T4- Cartap hydrochloride	5.17^{a}	1.17^{abc}	1.50^{a}	0.84^{a}	1.00^{a}	79.16
T5- Deltamethrin	4.83^{a}	0.33^{a}	1.17^{a}	0.83^{a}	1.17^{a}	82.70
T6- Spinosad	5.17^{a}	1.17^{abc}	1.83^{a}	2.00^{ab}	3.00^{b}	62.94
T7- Indoxacarb	4.84^{a}	0.84^{ab}	1.34^{a}	2.17^{ab}	1.84^{bc}	69.44
T8- Endosulfan	4.67^{a}	0.17^{a}	1.17^{a}	1.50^{ab}	1.67^{bc}	76.93
T9- Profenophos	5.00^{a}	2.83^{c}	1.67^{a}	2.67^{b}	2.33^{bc}	54.53
T10- Control	5.17^{a}	7.17^{d}	5.50^{c}	5.00^{c}	6.00^{c}	-
CD$^{\$}$		1.18	0.56	0.95	0.67	

(Mean of three replications)

* Per cent reduction is with respect to control calculated using Henderson Tilton formula
$^{\$}$ Analyzed using SPSS after square root transformation
In a column means followed by a common letter are not significantly different at p=0.05 by LSD

Table 6 : Integrated management of *Chauliops choprai* in the frenchbean under field conditions

Treatment	PTC	3 DAT	7 DAT	14 DAT	21 DAT	Reduction *(%)
Bt	10.17^{a}	9.00^{b}	8.50^{b}	8.17^{b}	9.50^{b}	26.09
Batain	10.25^{a}	2.92^{a}	2.17^{a}	1.83^{a}	3.25^{a}	78.82
Bt+ Batain	10.42^{a}	2.92^{a}	2.17^{a}	2.09^{a}	3.33^{a}	78.46
Cartap hydrochloride	9.42^{a}	0.84^{a}	0.84^{a}	1.00^{a}	1.92^{a}	89.59
Control	10.17^{a}	11.42^{c}	11.50^{c}	12.17^{c}	12.50^{c}	-
CD$^{\$}$	-	2.53	3.11	3.47	3.23	-

(Mean of three replications)

* Per cent reduction is with respect to control calculated using Henderson Tilton formula
$^{\$}$ Analyzed using SPSS after square root transformation
In a column means followed by a common letter are not significantly different at p=0.05 by LSD

Evaluation of different IPM modules against French bean diseases and insect pests

Based on the findings of different sets of experiments, six efficacious treatments against bean disease and insects were selected. Six different IPM modules involving different organic/inorganic substrate/ chemicals/bioagents in different combinations along with pure chemicals and untreated check were tested against french bean diseases and insect pests. Results presented in Table 8 revealed a lowest root rot incidence (8.63%), rust severity (3.50%), angular leaf spot (2.50%), bacterial blight (2.17%) and *Chauliops choprai* bug population (1.50 plant^{-1}) in T7 *i.e.* seed treatment with carbendazim along with foliar spray of azoxystrobin @ 0.1 and cartap hydrochloride, followed by T4 (seed treatment with *T. harzianum* strain 11 and 28 along with soil application of *P. hysterophorus* and foliar spray of azoxystrobin @ 0.1 and cartap hydrochloride). However, other IPM modules comprising of organic substances *viz*., T1, T3 and T5 were also found equally effective in reducing diseases and insect-pests of french bean. An increase of 96.6 per cent yield over control was obtained with treatment T4 (9.66 t/ha) which was at par with treatments T2 (95.5 %) (9.55 t/ha) and T7 (94.8 %) (9.48 t/ha).

Table 7 : Evaluation of different IPM module options against french bean diseases and insect-pests

Treatment	Root rot (%)	Rust (%)	ALS (%)	BB (%)	Sucking Bug (%)	Yield (t/ha)
T1 - ST Th(11+28)+SA Lantana+FS Cow dung extract+FS BSKE	12.63^{bc} (20.80)	19.83^{bc} (26.20)	12.28^{bcd} (20.13)	6.60^{bc} (14.85)	2.75a (9.50)	5.5^{ab}
T2 -ST Th11+28)+SA Lantana+FS Azoxystrobin+FS Cartap hydro	12.97^{b} (21.08)	3.66^{a} (10.78)	2.92^{abc} (9.67)	2.40^{a} (8.72)	1.75a (7.52)	9.55^{a}
T3 - ST Th 11+28 + SA Lantana + FS Panchgavya + FS BSKE	13.10^{b} (21.16)	20.95^{bc} (26.65)	11.58^{abcd} (19.58)	4.83^{ab} (12.64)	2.73a (9.42)	6.60^{b}
T4 - ST with Th 11+28 + SA Parthenium + FS Azoxystrobin @ 0.1 + FS Cartap hydrochloride	9.53^{bc} (17.97)	3.53^{a} (10.66)	2.20^{a} (8.34)	1.33^{a} (6.60)	1.68a (7.35)	9.66^{a}
T5 - ST with Th 11+28 + SA Parthenium + FS Cow dung extract @ 50 % + FS BSKE @ 10 %	10.87^{bc} (19.25)	17.89^{b} (24.89)	12.42^{cd} (20.43)	4.27^{ab} (11.83)	2.80a (9.61)	6.53^{b}
T6 - ST with Th 11+28 + SA Parthenium + FS Panchgavya @ 3 % + FS BSKE @ 10 %	13.20^{b} (21.30)	18.92^{bc} (25.44)	10.50^{abcd} (18.69)	6.77^{bc} (14.82)	2.68a (9.37)	6.4^{b}
T7 - ST Carbendazim + Azoxystrobin @ 0.1 FS + cartap hydrochloride FS	8.63^{a} (17.08)	3.50^{a} (10.71)	2.50^{ab} (8.97)	2.17^{a} (8.41)	1.50a (6.84)	9.48^{a}
T8 - control	29.00^{c} (32.57)	35.58^{c} (36.58)	19.00^{d} (25.64)	12.13^{c} (20.29)	17.50^{b} (24.43)	3.65^{c}
Turkey HSD (P = 0.05)	2.32	7.08	6.88	2.98	4.38	1.57

(Means of three replications)

Figures in parentheses represent arc sine transformed values

Means in the same column followed by different letters are significantly different ($P < 0.05$).

ALS: Angular leas spot, BB: Bacterial blight

The integrated disease and pest management involves a total system approach for the suppression of pathogens and insect populations to a level where higher yields can be obtained and enables the farmers to achieve maximum economic return. In the present study, more emphasis has given on use of bioagents, biopesticides, botanicals/plant extracts, and judicious use of pesticides in compatible or complimentary manner. Similarly, Pande *et al.* (2009) emphasized about the use of Integrated Disease management (IDM) modules for important foliar and viral diseases of legumes including bean. Stevens *et al.* (2003) reported that long-term effectiveness of IPM plus soil solarization reduced soil borne diseases of vegetables for more than two years after solarization. The IPM technology has been found economically viable as the yield on IPM farms has been found higher by about 46 per cent, cost of cultivation has been less by about 21 per cent and the net returns have been higher by 119 per cent (Gajanana *et al.,* 2006). Hence there is lot of scope for co-operative awareness among the farmers in enhancing the use of IPM practices especially in vegetable cultivation.

CONCLUSION

The present study concludes that bioagent fortified composts, organic amendments, botanicals, fungicides and insecticides alone or in combination have potential to enhance the germination, controlling soil and foliar diseases and insect populations of french bean. Acute toxicity assays ($LC-_{50}$) revealed deltamethirn as the highly toxic insecticide for sucking bug, *Chauliops choprai* followed by diafenthiuron and indoxacarb. But in field conditions, deltamethrin was not statistically superior to cartap hydrochloride in efficacy against sucking bug with the percent pest reduction of 82.7 and 79.2 per cent. In IPM trial, *Melia azedarach* (batain) extract 5 per cent and

cartap hydrochloride were found to be effective against the sucking bug registering a per cent pest reduction of 78.8 ad 89.6 per cent, respectively and thus can be recommended.

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Original Research Paper

Pollen germination studies in Giant Himalayan Lily (*Cardiocrinum giganteum* Wall.) a high value of ornamental plant in Western Himalayan region

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ABSTRACT

Giant Himalayan lily (*Cardiocrinum giganteum* Wall.) is a perennial bulbiferous herb of Liliaceae and an endemic species in the eastern Himalayan region, which has become one of the new flower crops because of its high ornamental value. The present investigationwas conducted to study pollen morphologyi.e., fresh pollen grains size was measured in different media, *viz.*, water, acetocarmine, glycerol and without any medium (dry). The pollen grains of Giant Himalayan lily exhibited the average size (length x width) of (22.64 x 19.72µ) in water suspension. The result revealed that in glycerine and dry condition shape of pollen grains was perprolate. However, in water and acetocarmine it looked was round in shape. The result also shown that highest pollen germination (54.70%, 63.69%) and pollen tube growth (89.24µ, 175.85µ) in 10% sucrose solution for 12 hours and 24 hours respectively. In control light treatments, red light was found to be best in pollen germination and pollen tube growth.

Keywords : Pollen morphology, pollen tube, growth regulators, pollen germination.

INTRODUCTION

The Giant Himalayan lily (*Cardiocrinum giganteum* Wall.) is the largest species of the lily plants, belong to the family Liliaceae. The genus *Cardiocrinum* consists of three species distributed geographically in the Himalayas (*C giganteum),* China (*C. cathayanum)* and Japan (*C. cardatum)* among that *C. giganteum* (Wallich.) Makino [syn. Lilium giganteum (Patterson and Givnish 2002)], commonly known as giant Himalayan lily. It has two varieties: *C. giganteum* var. *Giganteum* and *C. giganteum* var. *yunnanense*. *C. Giganteum* var. giganteum is native to the Himalayan region of tropical Asia (Bhutan, India, Nepal and Mayanmar) and *C. Giganteum* var. *Yunnanense* to temperate Asia (Southeast China) (USDA- ARS 2009). In India, *C. giganteum* var. *giganteum* grows in dense deciduous moist temperate forest woodlands at altitudes of about 1,500-3,000 m in the Himalayas (Genders 1994 and Guar 1999).The plants produce bulblets at the base of the taxon (Fox 1985 and Huxley 1992). It is monocarpic in nature and produce flowers in monsoon (July- August) and seed setting occur in the autumn (October- November). *Cardiocarinum giganteum* is grown as an ornamental plant in temperate region of the Northern Hemisphere including the United States (Phartyal *et al*., 2012). Oldfield (1991) confirmed its continuation in international trade in horticultural bulbs of wild origin from India. In addition to being an introduced ornamental, the species is hermaphroditic and has been reported to be an invasive species in its introduced habitat. Phartyal *et al.,*(2012) stated that Giant Himalayan Lily has are suitable for screening purpose, grown in shaded and Bog Garden, border making and bedding purpose. The flowers can be used as a cut flower formatting bouquet, Flower arrangement and decorations. Beside ornamental value; plants known to contain bioactive compounds, such as isopimarane type diterpenoids (Liu,1984) locally used for medicinal purpose. The starchy bulbs of *C. giganteum* are the staple food of local people in Guangxi and Yunnan (Li, 1997). The great economic value of *Cardiocrinum* species has brought about over exploitation and habitat fragmentation/ isolation of their natural populations (Li *et al.,* 2012) which might decrease not only population size but also genetic diversity. Present studies on by keeping the above points in considered to the study of pollen germination and pollen tube growth for further biological studies.

MATERIALS AND METHODS

A field experiment was conducted at College of Horticulture, VCSG, UUHF, Bharsar, Pauri Garhwal, Uttarakhand, India during June 2020. The experimental site located at an altitude of 1900 m asl at a Longitude of 78.99^0 E and Latitude of 30.056^0 N. The climate of Bharsar is typically temperate type with mild summer, higher precipitation during rainy season and severe cold prolonged winter with occasional snowfall (Bisht and Sharma 2014).

The size of pollen grains was measured with the help of ocular micro indexed against stage micrometre (Aneja, 2003). The pollen morphology size measurement was done in different media, *viz.,* water, acetocarmine, glycerol and no medium (dry). The experiment used replicated five times in complete randomized block design (Gomez and Gomez 1984). In second experiment effect of different chemicals *viz.,* sucrose, IAA, IBA and GA_3 (1, 5, and 10 ppm each) on pollen germination was done. The treatment was replicated three times in complete randomized block design. In third experiment effect of different light colours *viz.,* red, blue, green violet and pink in pollen were also tested and replicated five times in complete randomized block design. Pollen was collected from mature anthers. Bulk of the pollen was distributed into germination media in cavity slides and placed at room temperature (15±22 ºC) for 12 and 24 hours. Germination was quantified as the percentage of germinated pollen grains per 100 evaluated. Pollen grain was considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel, 2000).

RESULTS AND DISCUSSION

Pollen morphology (Size and shape)

The pollen grains of Giant Himalayan lily (Table 1 and Fig. 1) exhibited the maximum size (length x width) of 22.64 x19.72µ in T_1 (water suspension).The minimum size of pollen grains in length acetocarmine staining 19.02µ and width dry conditions 11.37µ. The shape of pollen grains in glycerine and dry condition were found perprolate whereas, in acetocarmine and water it looked round. The result of present investigation was accordance with the finding of Selvarasu *et al.,* (2019) found the average fresh pollen size was 65.24 µm in *G. rothschildiana*. In dry conditions and glycerol shape of pollen was perprolate which was actual shape of pollen grains, while in other media *i.e.,* acetocarmine and under water conditions it looked like round. The perprolate shape in glycerine and dry conditions might be due to inner gradient matter which in not equally proportion to the exine material of pollen grains. Prativa *et al.* (2012) the differences in the pollen diameter among the varieties might be due to their genetic makeup in rose. Hemanta *et al.,* 2017.The comparison of pollen diameter between the small bud and large bud showed that the large bud had bigger pollen diameter than the small bud. The reason for bigger pollen diameter of large bud might be due to the bigger size of bud and maturity.

Pollen germination and pollen tube length

The data of pollen germination and pollen tube length in Giant Himalayan Lily were taken at 12 hours intervals. Presented in Table 2 and Fig. 1. The data revealed that the maximum pollen germination

Table 1 : Variation in pollen shape and size in different media

Treatment	Size of pollen grain (µ) ± SE (m)	
	Length	Width
T_1 - Water (control)	22.64 ± 0.56	19.72 ± 0.40
T_2 - Dry conditions	21.80 ± 0.18	11.97* ± 0.18
T_3 – Acetocarmine	19.02*± 0.89	17.81* ± 0.66
T_4 – Glycerol	20.42*± 0.39	13.05 *± 0.24
SE(d)	0.810	0.590
$CD_{(0.05)}$	1.732	1.260
C.V	6.107	5.960

µ = micron (unit of pollen length and width)
*Significant at 5 % level of significance as compared with (control)

Table 2 : Pollen germination percentages and pollen tube elongation at different incubation time

Treatment	Pollen germination (%) ± SE (m) (12 hours)	Pollen germination (%) ± SE (m) (24 hours)	Pollen tube length(µ) ± SE (m) (12 hours)	Pollen tube length(µ) ± SE (m) (24 hours)
T_1 Water	20.37±0.74	32.36±1.05	16.63±0.96	32.25±3.86
T_2 1 % Sucrose	40.73*±1.29	55.86*±5.73	38.65±12.30	55.57*±9.15
T_3 5 % Sucrose	49.46*±4.62	56.74*±1.63	61.18*±4.74	119.06*±11.16
T_4 10 % Sucrose	54.70*±2.32	63.69*±3.56	89.24*±39.73	175.85*±8.50
T_5 20 % Sucrose	36.26*±3.15	40.45*±2.09	21.86±1.46	44.41±3.14
T_6 1 ppm GA_3	37.73*±0.82	48.50*±1.88	22.24±1.38	36.60±1.01
T_7 5 ppm GA_3	32.43*±0.36	38.02±1.12	23.41±2.80	32.50±1.81
T_8 10 ppm GA_3	27.68*±0.34	35.81±1.23	24.83±2.69	32.48±1.33
T_9 1 ppm IBA	25.12±0.97	38.08±0.78	21.04±0.87	73.46*±2.09
T_{10} 5 ppm IBA	29.19*±4.92	35.28±0.84	47.09±6.14	73.21*±9.97
T_{11} 10 ppm IBA	25.87±2.99	34.08±1.03	22.93±2.42	39.35±3.26
T_{12} 1 ppm IAA	26.50±0.57	39.34*±0.54	20.96±1.70	40.78±2.75
T_{13} 5 ppm IAA	25.20±2.03	37.21±0.54	29.45±1.84	51.91*±12.94
T_{14} 10 ppm IAA	23.46±0.56	35.53±1.26	28.41±5.55	40.65±1.58
SE (d)	3.355	3.032	16.276	9.277
$CD_{(0.05)}$	6.907	6.243	33.511	19.100
C.V	12.649	8.798	59.635	18.754

µ= micron (unit of the pollen tube length and width)
*Significant at 5% level of significance as compared with control

percentage was recorded with T_4 (54.70%) 10% sucrose solution. The minimum pollen germination percentage was recorded with T_1 (20.37%) water. All the treatments were found to be significant as compared to control except T_9 (IBA 1ppm), T_{11} (IBA 10ppm), T_{12} (IAA 1ppm), T_{13} (IAA 5ppm) and T_{14} (IAA 10ppm). The maximum pollen tube length was recorded in T_4 (10% sucrose) 89.24 µ and found statically at par with T_3 (5% sucrose) 61.18µ. Minimum pollen tube length (16.63 µ) was recorded in T_1 water (control).

The pollen germination and pollen tube length in Giant Himalayan lily at 24 hours interval has been depicted in Table 2 and Fig.3. It was recorded that the maximum pollen germination percentage was recorded in T_4 (63.69%) 10% sucrose solution. The minimum pollen germination percentage (32.36%) was in T_1water (control) and found statically at par with treatmentsT_7 ($GA_3$5ppm), T_8 (GA_3 10ppm),T_9 (IBA 1ppm), T_{10} (IBA 5ppm), T_{11}(IBA 10ppm),T_{13}(IAA 5ppm) andT_{14}(IAA 10ppm). The maximum pollen tube length was recorded in T_4 (175.85 µ). 10% sucrose solution. The minimum pollen tube length (32.25 µ) was recorded in T_1 water (control) and found statically at par with treatments T_5(Sucrose 20%), T_6(GA3 1ppm), T_7(GA3 5ppm), T_8(GA3 10ppm), T_{11}(IBA 10ppm), T_{12}(IAA 1ppm) and T_{14}(IAA 10ppm). Hemanta *et al.* (2017) who reported highest pollen germination with 15 % sucrose and 15% sucrose + 60 ppm boric acid in Tuberose. Chaudhary (1991) and Jisha (1999) who reported the best pollen germination of gladiolus under 15 % sucrose + 75 ppm boric acid in germination medium. Yuxin *et al.* (2005) also found that sucrose and boron have great effects on the germination of lily pollen. Assessment of pollen viability has direct relevance in hybridization as pollen of male parent takes part in the fertilization process. Therefore, pollen germination study is an important activity in order to determine the potentiality of male parent for fertilization and seed setting after crossing

(Shivanna *et al.,* 1991). Mascarenhas and Mermeistein (1981) also emphasized the need for newly synthesized protein for tube growth. An array of plant growth regulators and other chemicals have been empirically added to the culture medium to promote pollen germination and tube growth and the positive effects of some of these substances have led to speculation about their biochemical functions. Further, pollen germination and tube elongation are independent processes governed by separate sets of conditions (Malik, 1985).

Effect of light color on pollen germination and tube elongation

The pollen germination and pollen tube length in Giant Himalayan lily plants at 12- and 24-hours interval is depicted in Table 3 and Fig. 2. It was observed that pink light (no germination) and violet light (20.85%) inhibited pollen germination, and also pollen tube growth. The maximum pollen germination and pollen tube elongation were observed in red light recording 35.99% and 46.23µ respectively. Finding is accordance with report by Nautiyal *et al,* (2009) in *Aconitum balfourii* (Benth) Muk. and *Aconitum heterophyllum* Wall. found highest pollen germination and pollen tube growth in red and green light respectively. Maximum pollen germination and tube elongation in red color suggest the involvement of phytochromes, as red synthesizes phytochrome protein and its biological manifestation (Sharma and Malik, 1978; Korner 1999).

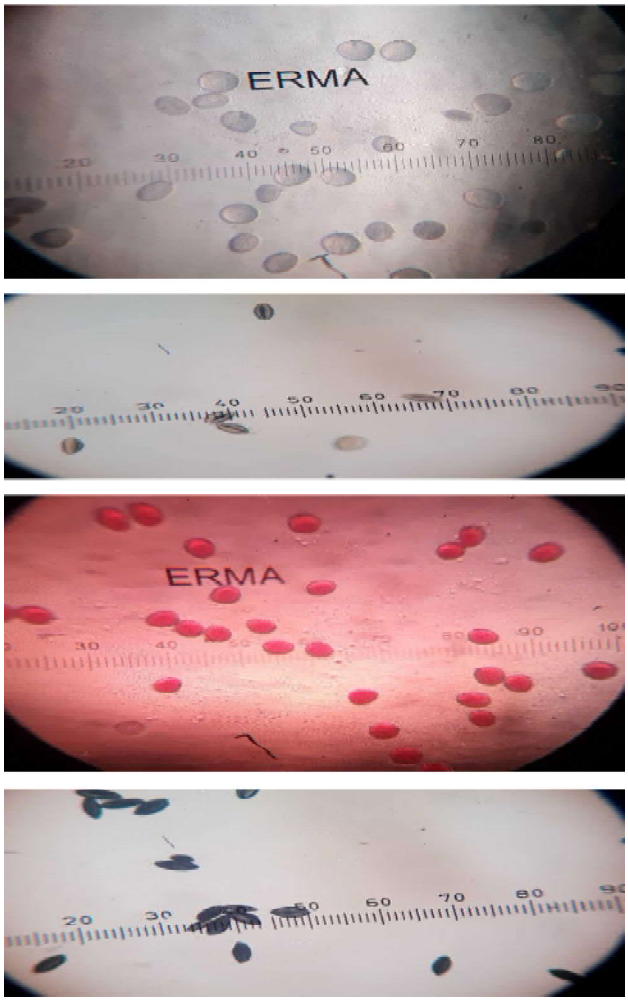

Fig. 1 : Pollen morphology Giant Himalayan Lilly a. water, b. acetocarmine, c. glycerine and d. dry (fresh condition)

Table 3 : Effect of light on pollen germination and pollen tube elongation at different incubation time

Light colour	Pollen germination (%) ± SE(m) 12 hours	Pollen germination (%) ± SE(m) 24 hours	Pollen tube length (µ) ± SE(m) 12 hours	Pollen tube length (µ) ± SE(m) 24 hours
T_1 Red	29.62±2.12	35.99±3.07	29.33±1.63	46.23±2.86
T_2 Blue	17.29*±0.98	27.38*±1.77	22.88*±1.36	31.94*±2.36
T_3 Green	23.50*±1.81	29.05*±2.21	27.49±3.80	36.10*±2.39
T_4 Violet	16.50*±1.33	20.85*±1.78	17.66*±0.80	30.87*±1.12
T_5 Pink	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SE(d) 2.054	2.037	2.805	2.885	
$CD_{(0.05)}$ 4.315	6.052	5.891	6.060	
C.V. 18.683	20.105	22.770	15.711	

µ= micron (unit of pollen tube length and width)
*Significant at 5% level of significance as compared with T_1

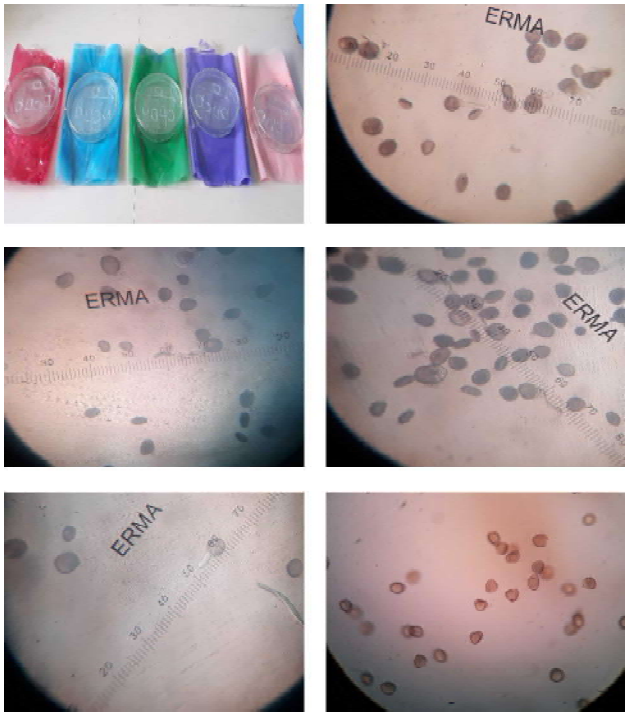

Fig. 2 : Effect of light on pollen germination and pollen tube elongation: a. different colours of light, b. red, c. blue, d. green, e. violet and f. pink.

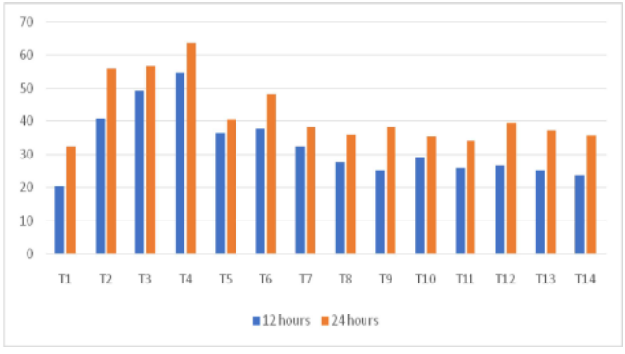

Fig. 3 : Percentage of pollen germination in Giant Himalayan Lilly

CONCLUSION

The pollen germination studies of *Cardiocrinum giganteum* Wall. Showed that maximum pollen size was recorded in T_1(water). The perprolate shape of pollen grain were obtained in glycerine and dry conditions whenever in water and acetocarmine it was found round shape. The maximum pollen germination and pollen tube growth were record in treatment 10% sucrose at 12- and 24-hours intervals. The result also showed among different light used red light is effective in improving pollen germination and pollen tube elongation.

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Original Research Paper

Ex-situ conservation of an endangered medicinal plant *Andrographis paniculata* by plant tissue culture

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ABSTRACT

An effective and rapid *in vitro* regeneration protocol of Kalmegh (*Andrographis paniculata*) was established by investigating the factors like combinations of plant growth regulators and explant types (stem, leaf and midrib). To find out the effective medium for callus induction and shoot regeneration, different explants of *A. paniculata* were cultured on MS media enriched with several concentrations of 6-benzylaminopurine (BA), á-naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxy acetic acid (2,4-D). Stem explant was noticed more responsive than leaf and midrib explant both in callus initiation and shoot regeneration. The ranges of callus initiation from stem, leaf and midrib explants were 26.67 - 100%, 20 - 93.33% and 13.33 - 73.33%, respectively. The calli obtained from midrib explants were not used in shoot initiation because of its poor size. The stem explant exhibited the maximum 73.33% shoot regeneration frequency in a comparison with leaf explants (60%). The maximum callus induction (100%) and shoot regeneration (73.33%) from stem explants were noticed in MS medium strengthened with 0.5 mg/L NAA and 2.0 mg/L BA and half strength MS media complemented with 0.1 mg/L NAA and 3.0 mg/L BA respectively. The highest shoot regeneration from the stem explant may be due to presence of more active parenchymatous cells than that of leaf explant. Half MS medium fortified with 2.0 mg/L IBA considered as best root initiation medium as it resulted in maximum rooting (93.33%). After acclimatization, the plants were transferred to field and found identical to the mother plant.

Keywords: Callus, Kalmegh, medicinal plant, organogenesis, stem explants.

INTRODUCTION

Kalmegh (*Andrographis paniculata*) is a medicinal plant of immense worth, which has immeasurable uses in Ayurveda and Unani medicines for treating many ailments like as common cold, fever, asthma, cough, urinary tract infections,tuberculosis, bronchitis, acute diarrhea, dysentery, acidity, skin diseases, snake bites *etc*. (Wang *et al.,*2014, War *et al.,* 2018). It is placed at 17th position among the 32 prioritized medicinal plants which is ranked by the Indian National Medicinal Plants Board (INMPB) (Verma *et al.,* 2019) and it is the major ingredients of at least 26 Ayurvedic compositions stated by the Indian Pharmacopoeia (Mishra *et al.,* 2007; Verma *et al.,* 2019). It is also included in Pharmacopoeia of People's Republic of China as a "cold property herb" utilized to get recovery from fever and eliminate toxins (Abhilasha and Arpita, 2017). Thailand's Ministry of Public Health has also recorded this plant in "National List of Essential Drugs A.D. 1999" (Pholphana *et al.,* 2004; Abhilasha and Arpita, 2017). The plant carries a wide range of curative properties like anti-oxidant (Trivedi and Rawal, 2001), anti-viral (Wiart *et al.,* 2005), anti-fungal (Sule *et al.,* 2012), immune enhancement and anti-HIV activities (Calabrese *et al.,* 2000), cardio-protective (Ojha *et al.,* 2012), anti-cancer and immune-non stimulatory (Kumar *et al.,* 2004), hepatoprotective (Nagalekshmi *et al.,* 2011), anti-hypertensive and anti-microbial (Jindal *et al.,* 2015).

Approximately half of the death throughout the world caused by communicable diseases and it is reported that more than 50% of hospital deaths are occurred due to communicable diseases (Tandon *et al.,* 2015). And these numbers are increasing rapidly due to the occurrence of bacterial resistance to some specific

antibiotics and introduction of new infectious diseases like COVID-19. In the meanwhile, in this pandemic situation almost 3.7 million excess deaths occurred across all the countries and the situation is getting worsen constantly (Karlinsky and Kobak, 2021). So, there is a dire need for research to investigate the alternative source of existing drugs to combat different diseases. *A. paniculata* can be an alternative source of existing drugs due to its high medicinal value.

In recent years, a noticeable amount of medicinal plant species is rapidly dissipating in the world and are under serious risk of extinction as a result of anthropogenic habitat destruction (Ayuso *et al.*, 2019). The declining rate of natural habitats coupled with the over-exploitation as a source of pharmaceutical drugs make this species endangered that cause genetic diversity shrinkage and demolition of biological communities, that are very essential for ecosystem functioning as well as human well-being (Silveira *et al*., 2016). International Union for Conservation of Nature (IUCN) included this species in the list of threatened plants. This species was also classified as vulnerable by CAMP (Conservation Assessment and Management Prioritization) exercise in India in 2003 (Gowthami *et al.,* 2021). Again, Bangladesh National Herbarium (BNH) has already identified about 97 of such over-used and threatened plants in Bangladesh and *A. paniculata* is one of them (Khan *et al.,* 2001). Nevertheless, the traditional vegetative propagation of *A. paniculata* can't meet the ever-increasing requisite for pharmaceutical industries because of its slower propagation rate. Hence, divergent ordinated programs are direly necessary to protect and conserve the existence of these precious plant resources. Recently in India, INMPB emphasized 32 medicinal plants for conservation and *A. paniculata* have also been on that list (Gowthami *et al.,* 2021).

The intense pressure on this species will rise continuously unless proper initiative for conservation and sustainable use are taken. To protect threatened plants, appropriate management of wild populations as well as natural habitats protection are urgent through several *in situ* conservation strategies (Ayuso *et al.,* 2019). But in case of *A. paniculata*, very low seed production and delay in root initiation from seedlings reduce its production from seeds (Martin, 2004) and make a great obstacle for the *in-situ* conservation of this species. Conversely, *in situ* conservation is getting troublesome day by day because the natural habitats are utilized to meet the ever-increasing requirements of human beings as well as its natural habitats cannot be expanded. However, in critical situations, *ex situ* strategies play a vital role in the preservation of endangered plants and offer some scopes of saving wild resources against the loss of their natural habitats (Nam off *et al.,* 2010; Ayuso *et al.,* 2019). Plant i*n vitro* culture has arisen as a promising biotechnological technique for expeditious production of enormous number of individuals from limited number of plant material and conserving the species within a short period and confined space, which hardly disturbs wild populations. The popularity of *in vitro* culture has developed in recent years for the protection of endangered, endemic and rare species (Bunn *et al.*, 2007). Moreover, *in vitro* regeneration provides easy access to the re-introduction of plants to their natural habitats and offers some distinct advantages over alternative strategies. Furthermore, to do some improvements on the drawback side of conventional plant breeding, like reduced growing rate, very lower seed production, delayed rooting of seedlings and lower production of secondary metabolites, *in vitro* culture of plant becomes a powerful biotechnological technique to study the plant secondary metabolism and appears as an effective system to produce bioactive compound (Rusedski *et al.,* 2017; Isiah *et al.,* 2018; Hu *et al.,* 2019).

Considering these points, plant tissue culture can provide an additional strategy for mass scale production of this species which can overthrow the constrains of extracting valuable metabolites and can act as a 1st step for the *ex-situ* conservation of this plant's germplasm. Although several reports were found worldwide, few studies (Al-Mamun *et al.,* 2015; Roy, 2014) with limited success were found on the regeneration of *A. paniculata* in Bangladesh. In Bangladesh, its medicinal properties are not well known, hence no activities are in place to conserve this valuable plant. However, *ex-situ* strategies also help for the conservation of this species as well as give additional conservation options. Therefore, the present study is undertaken to develop as an economical and reliable regeneration protocol for its large-scale production to meet the increasing demand of phyto-pharmaceutical industries and to protect this highly valued medicinal plant from extinction.

MATERIALS AND METHODS

Plant materials

Healthy cuttings of *A. paniculata* were collected from Moulvibazar and Sunamganj district of Bangladesh. Collected cuttings were planted in the research field and rooftop of the Department of Genetics and Plant Breeding for explant preparation.

Explant and media preparation, and culture method

For explant (stem, leaf and midrib) preparation, at first a shoot was excised from healthy plant of *A. paniculata* and swabbed with 70% alcohol-soaked cotton and the shoot was cut into small pieces. After that the small pieces were washed in running tap water for 20 min followed by washing with household detergent for 5 to 7 min. They were brought to laminar air flow after washing with distilled water. Then the shoot pieces were treated with 70% ethyl alcohol (MERCK, Germany) for 2 min for surface sterilization and rinsed in sterile double distilled water 3 to 4 times. Again, the shoot bits were surface sterilized with 10% Chlorox (Sodium hypochlorite, The Clorox Company, Oakland, USA) for 8 minutes, and were rinsed with sterile double distilled water 3 to 4 times to remove the effect of surface sterilizing agents. The explants were then split into little pieces and used as explants after being surface sterilized. Finally, the segment of stem, leaf and midrib (5-7 mm in length) from excised shoot were used as explant for the callus induction.

Stem, leaf and midrib explants (5-7 mm in length) were inoculated in MS (Murashige and Skoog, 1962) media complemented with several concentrations of BA (99%, Duchefa Biochemie, the Netherlands) (0.5, 1.0, and 2.0 mg/L), NAA (98%, Duchefa Biochemie, the Netherlands) (0.1, 0.5 and 1.0 mg/L) and 2,4-D (96%, Duchefa Biochemie, the Netherlands) (0.1, 0.5 and 1.0 mg/L) to find the best medium for callus initiation. The process was carried out entirely in a laminar airflow cabinet. In each culture jar containing 50 ml callus induction media, five explants were put. On the surface of the medium, the stem, leaf, and midrib segments were inoculated horizontally (Fig. 1a, b & c) and implanted in culture room with high light intensity (16/8 hours light/dark) produced by 144 W white fluorescent lamp, a temperature of 25±2°C and a relative humidity of 70%.

After attaining a convenient size, the calli were re-cultured on freshly prepared shoot induction media (Fig. 1 d & e) containing MS salts and vitamins, 3% sucrose, 1% agar and different strengths of 2,4-D (0.5 and 1.0 mg/L), NAA (0.1, 0.5 and 1.0 mg/L) and BA (1.0, 2.0 and 3.0 mg/L). When in *vitro* raised shoots grew about 2-3 cm in height was aseptically transferred into full strength and half strength MS media separately supplemented with different strengths of NAA (0.5, 1.0 and 2.0 mg/L) and IBA (0.5, 1.0 and 2.0 mg/L). A permanent marker was used to mark the cultivated vessels after being sealed with parafilm to denote each treatment. They were then placed in a culture room.

After development of adequate root system, 4-6 cm plantlets were removed cautiously from the cultivated vessels without damaging roots and rinsed smoothly in running tap water to eliminate agar medium and sucrose trace elements to avoid fungal infection. Then the complete rooted plantlets were transferred into plastic pots containing wet soil and provide a glassware cover (beaker) to prevent dehydration (Fig. 1k). When the plantlets were successfully adapted to the natural environment then they were transferred to field condition (Fig. 1l).

Statistical analysis

The experiment was designed following completely randomized design (CRD) with three replications. The recorded data on the percentage of callus initiation, shoot regeneration and root regeneration and statistically analyzed to ascertain the significance of the experimental results. MS Excel 2010 was used for calculating the mean and standard deviation for all treatments. Dunkan's Multiple Range Test (DMRT) was used for evaluating the significance and difference between means by utilizing R software (version Rx64 3.4.3).

RESULTS AND DISCUSSION

The optimal medium for callus induction

Callus initiation was started within a week from the stem, leaf and midrib explants of *A. paniculata* after incubation of explants on MS medium containing several combinations of BA (0.5, 1.0 and 2.0 mg/L), 2, 4-D (0.1, 0.5 and 1.0 mg/L) and NAA (0.1, 0.5 and 1.0 mg/L). Among the combinations tested, MS medium supplemented with 0.5 mg/L NAA and 2.0 mg/L BA showed the highest callus initiation frequency for all three types of explants with significant heterogeneity among the explants (Fig. 2 and Fig. 3). The highest callus formation was 100%,

93.33% and 73.33% for stem, leaf and midrib explants respectively in 0.5 mg/L NAA and 2.0 mg/L BA combination, while the lowest recorded callus formation for stem (26.67%), leaf (20%) and midribex plant (13.33%) found in MS medium supplemented with 0.1 mg/L 2,4-D and 0.5 mg/L BA combination (Fig. 2). However, the callus initiation frequency of stem, leaf and midrib explants on rise with the increase of NAA and 2,4-D up to 0.5 mg/L individually in a combination with BA up to 2mg/L. Afterword, with the increase of NAA and 2,4-D the callus initiation frequency has decreased. The phytohormone 2,4-D in a combination with BA produced 80%, 66.67% and 53.33% callus for stem, leaf and midrib explants respectively, whereas, NAA along with BA induced 100%, 93.33% and 73.33% for stem, leaf and midrib explants respectively. So, it is clear that callus initiation frequency is mostly regulated by NAA and 2, 4-D and NAA was more responsive compared to 2, 4-D (Fig. 2). On the other hand, higher concentration of NAA induced excessive rooting in the callus (Fig. 6a) and eventually reduced the shoot forming capacity of the callus. However, in hormone free MS media, none of the explants produced callus.

In this study, stem and leaf explants showed better response for callus induction than midrib explants are in conformity of previous finding of War *et al.,* 2018. Jindal *et al.,* 2016 reported that best callus induction was obtained as MS medium containing 1 mg/L 2, 4-D and 1 mg/L NAA by using leaf explant. War *et al.,* 2018 suggested that optimal callus induction medium for leaf explant was MS medium containing 2, 4-D (1.0 mg/L) and Kinetin (0.5 mg/L) followed by 2,4-D (1.5 mg/L) and Kinetin (1.0 mg/L) and optimal callus induction medium for stem explant is MS medium supplemented with 2,4-D + Kinetin (1.5 + 1.0 mg/L). Sharma and Jha (2012) reported that, highest callus initiation was achieved on MS media consist of 1 mg/L 2,4-D and 1 mg/L NAA as well as combinations of 1 mg/L 2,4-D and 0.5 mg/L Kin and 1 mg/L BAP and 1 mg/L NAA.

The physical appearance of a callus is influenced by the type of explants and the composition of the culture media (Sharma and Nautiyal, 2009). In this study, several types of distinguishable callus in terms of consistency and colour were generated from different types of explants (Fig. 3) in different auxin (NAA and 2, 4-D) and cytokinin (BA) combinations (Fig. 4). When the concentration of auxin (NAA and 2, 4-D) and cytokinin (BA) were increased (above 1 mg/L) very compact, brownish, brownish white or white calli were found. Whereas, at low NAA, 2,4-D and BA (below 0.5 mg/L) concentrations fragile, brownish or whitish colour calli were produced (Fig. 4). Again, in the presence of NAA and BA, leaf explants generate compact, green-colored callus. On the other hand, 2,4-D and BA combinations generated friable white callus from leaf explant.Therefore, compact, fragile, greenish, whitish and brownish calli derived from various explants in various combinations and concentrations of growth regulators. However, these types of callus morphology were also found in some other plants like *Ipomoea obscura* L. (Mungole *et al.,* 2009), *Gynura procumbens* (Nurokhman *et al.,* 2019). A green compact callus was found in *Orthosiphonsta mineus* from a balanced concentration of NAA and BA (Elangomathavan *et al.,* 2017). In contrast, higher concentration of NAA and BA provided yellowish white compact callus in *Dianthus caryophyllus* (Arif *et al.,* 2014).

However, it is revealed that, varied concentration and combination of plant growth regulators also influenced the callus morphological responses. Differences in plant growth regulator concentrations and combinations have a significant impact on morphogenic responses of callus and the morphogenic response is affiliated with the mineral nutrition that is applied (Avilés *et al.,* 2009). Additionally, color of callus can change due to changes in chlorophyll as a result of reaction among different plant growth regulators; explant types and environmental culture factors like light exposure and temperature (Elias *et al.,* 2015).

The optimal medium for shoot regeneration

In this experiment, stem and leaf explants had a stronger callus induction response and produced robust, vigorous calli than the midrib explants. Hence, calli derived from stem and leaf explants were employed in shoot regeneration.Two types of auxins (2,4-D and NAA) and cytokinin (BA) were used to find out the best medium for shoot regeneration. 21 days old healthy calli were cultured on half strength MS medium containing a variety of BA, 2,4-D and NAA combinations and concentrations (Fig. 5). After three weeks of callus culture, shoot bud formation started from the calli. Both the calli and calli with the shoot buds was sub cultured on shoot regeneration

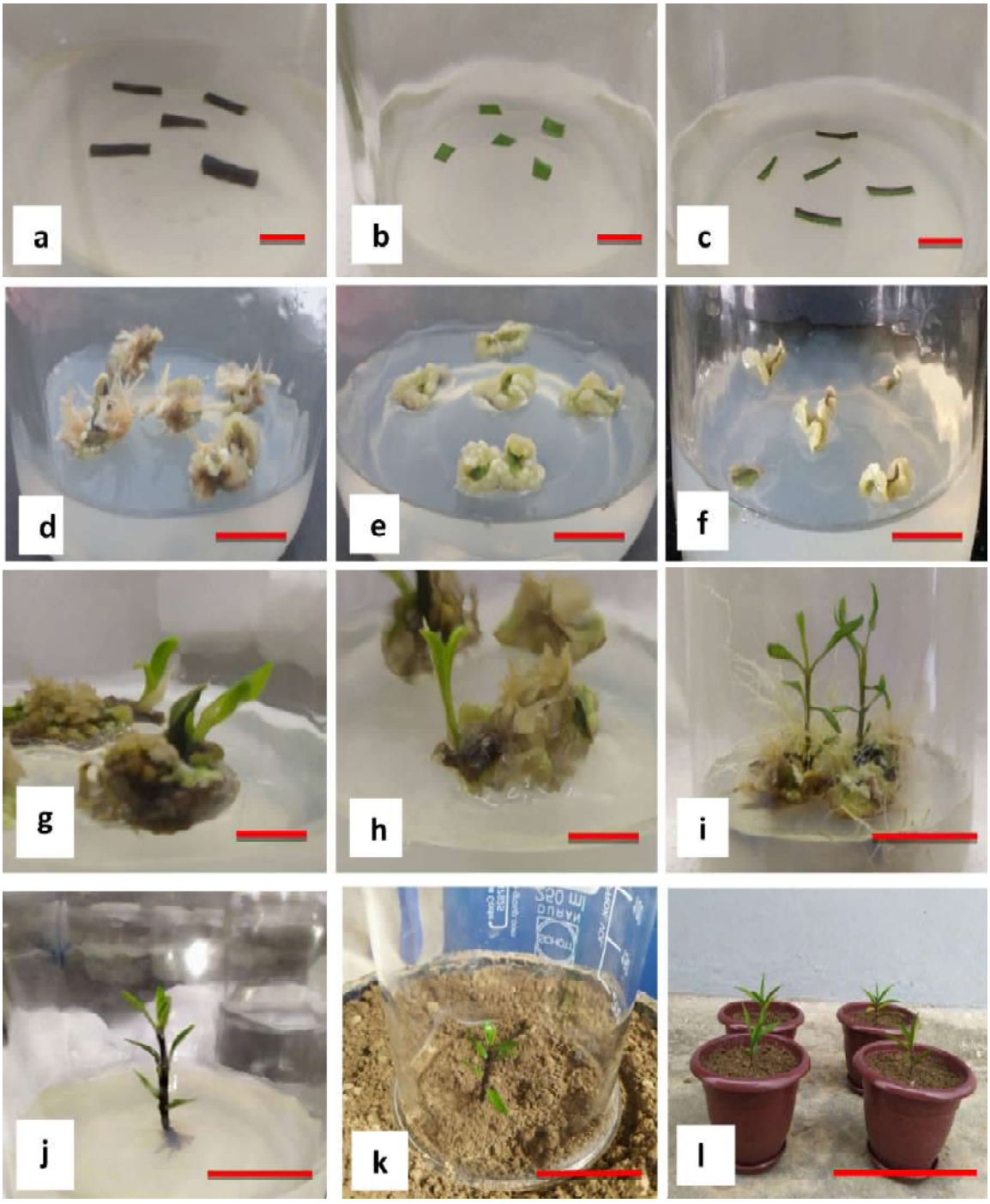

Fig. 1 : *In vitro* regeneration of Kalmegh (a) Stem explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1st day of culture, (b) Leaf explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1stday of culture, (c) Midrib explants on callus induction medium (MS + 0.5NAA + 2.0BA) at 1st day of culture, (d) 15 days old stem callus on callus induction media, (e) 15 days old leaf callus on callus induction media, (f) 15 days old midrib callus on callus induction media, g) shoot bud initiation from stem callus in shoot induction medium (½MS + 0.1NAA + 3.0BA) on 27 days of culture, (h) shoot bud initiation from leaf callus in shoot induction medium (½MS + 0.1NAA + 3.0BA) on 27 days of culture, (i) multiplication of shoot in shoot induction medium (MS + 0.1NAA + 3.0BA) on 6 weeks of culture, (j) initiation of root in MS + 2 IBA medium, (k) acclimatized plant in soil, (l) plants that survived in natural condition.*Scale bars* represent 0.5 cm (a, b & c), 1 cm (d, e, f, g & h), 2.5 cm (i, j & k), 1.0 cm (l).

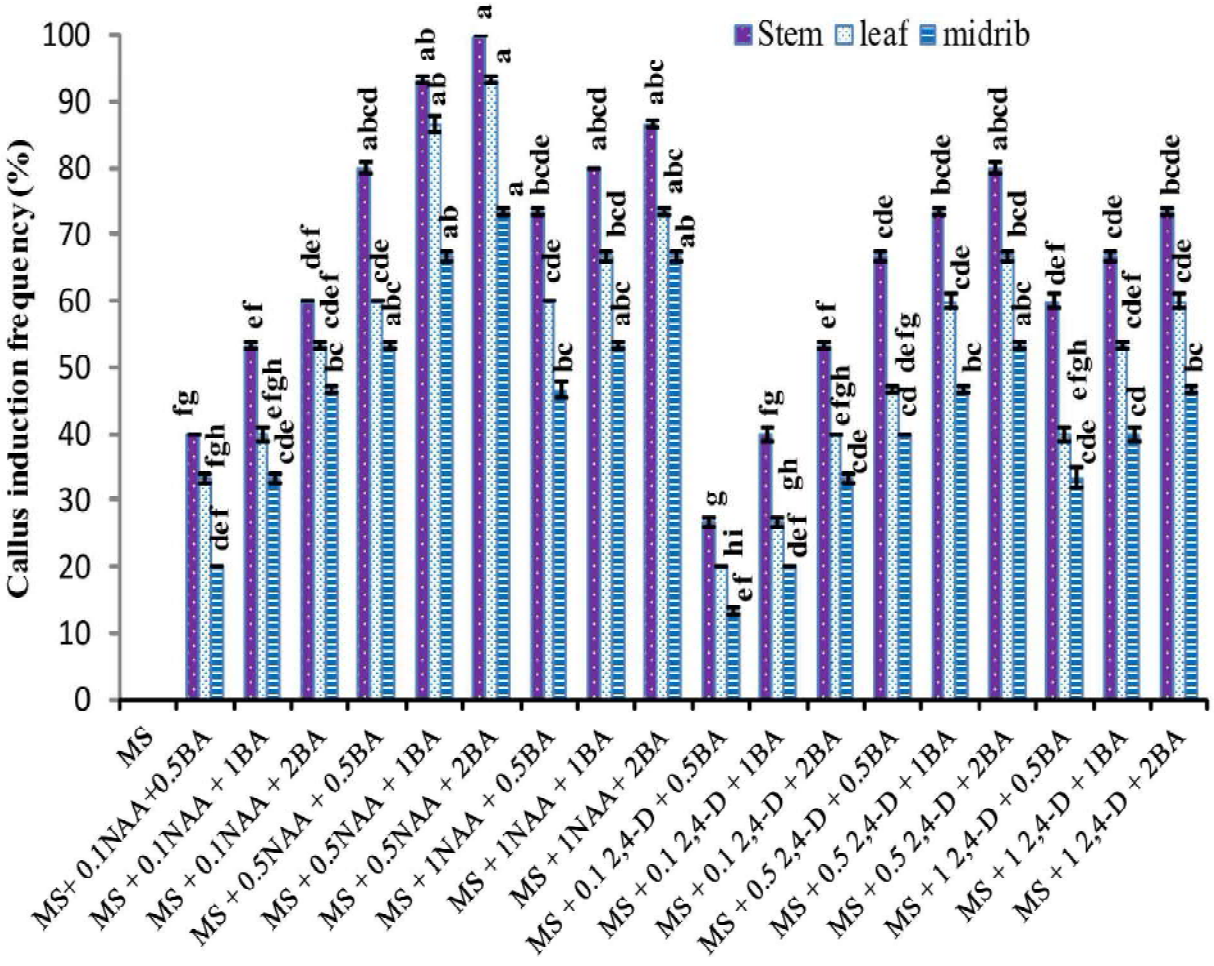

Fig. 2 : Callus initiation frequency from *In vivo* stem, leaf and midrib explants of *A. paniculata* on MS media fortified with various concentrations of BA, NAA and 2,4-D. Data consist of three replications and 5 explants were utilized in each replication. Bars reflect the standard deviation of means. Values with different letters are significantly different at *P* value = .05 (DMRT).

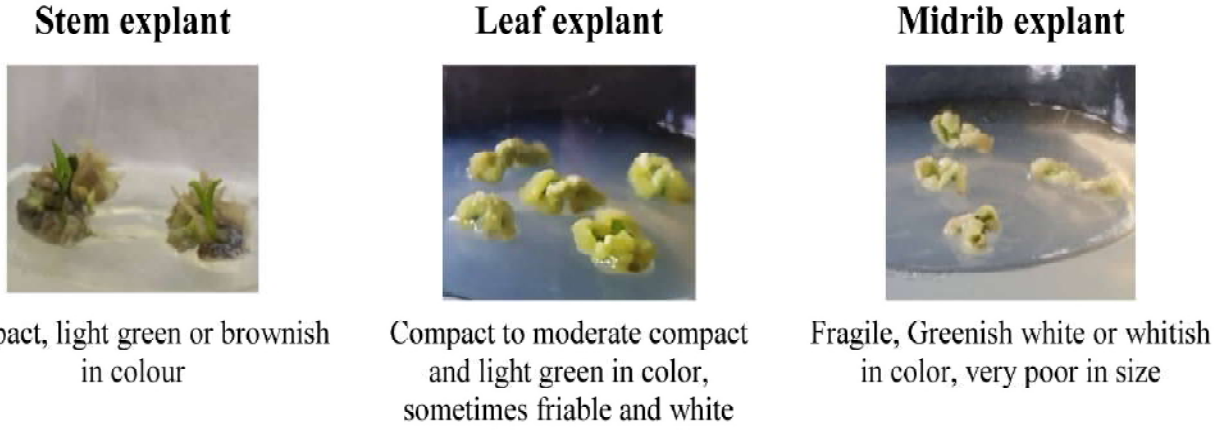

Fig. 3 : Morphology of callus at 3rd week of culture periodbased on explant type

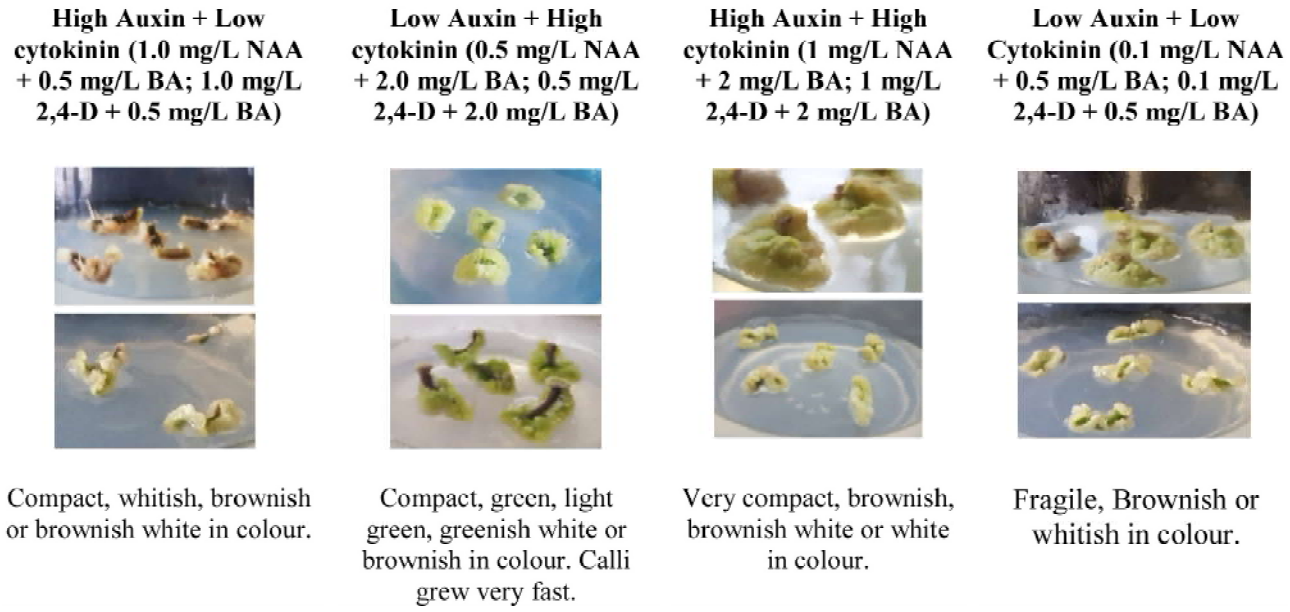

Fig. 4 : Morphology of callus at 3rd week of culture period based on PGR concentration and combination

media to obtain complete shoot buds. The percentage of shoot regeneration was varied with the difference of concentrations and combinations of growth hormones. Out of different combinations, ½ MS + 0.1 mg/L NAA + 3.0 mg/L BA was showed the highest 73.33% and 60% shoot regeneration frequency for leaf as well as stem explants respectively (Fig. 5). This result has proven that, ½ MS + 0.1 mg/L NAA + 3.0 mg/L BA is the best media combination for shoot initiation. Other findings of *in vitro* shoot regeneration of *A. paniculatas* (Bansi and Rout, 2013) also suggest that ½ MS in a combination with 3.0 mg/L BA produce higher number of shoots. However, the better performance of stem explant in shoot regeneration is also supported by the findings of Roy (2014).

The strength of MS medium also influenced the shoot regeneration from stem and leaf callus. Full MS medium showed browning of the callus (Fig. 6c) and reduced shoot elongation process (Fig. 6b). These observations might be influenced by the high mineral concentrations, present in full strength MS media, excessive in amount for shoot morphogenesis (Purkayastha *et al.,* 2008; Kataky and Handique, 2011).

From the above data, it was found that the frequency of shoot regeneration from stem explant was higher than that of leaf explant (Fig. 5). To investigate the reason behind more shoot regeneration frequency of stem explants compared with leaf explant, anatomical study of stem and leaf segment of *A. paniculata* was carried out. Transverse section (T.S.) of stem possesses epidermis, collenchyma, chlorenchyma, sclerenchyma, phloem, xylem, pith cells (Fig. 7) whereas T.S. of leaf contains layer of epidermis, palisade parenchyma, spongy parenchyma (Fig. 7). Among them chlorenchyma, pith, palisade parenchyma, spongy parenchyma is parenchymatous cell. In microscopic observation, number of active parenchymatous cells was found more in stem than that of leaf (Fig. 7). And collenchyma cell was found in the stem which is absent in the leaf (Fig. 7).

Possibility of the highest shoot regeneration from the stem explant was due to presence of more active parenchymatous cells than leaf explant (Fig. 7). On the other hand, collenchyma cells serve the growing part of the plant like leaf and shoot which is absent in the leaf (Fig. 7). So, the highest shoot regeneration in the stem explant may be due to the presence of more active parenchymatous cells and collenchyma cells in the stem compared to the leaf explants (Abhilasha and Arpita, 2017).

Initiation of roots

For root initiation, well grown shoots were rescued and sub-cultured into MS media supplemented with different concentration of NAA and IBA (0, 0.5, 1.0 and 2.0 mg/L). Among the fourteen tested media, the

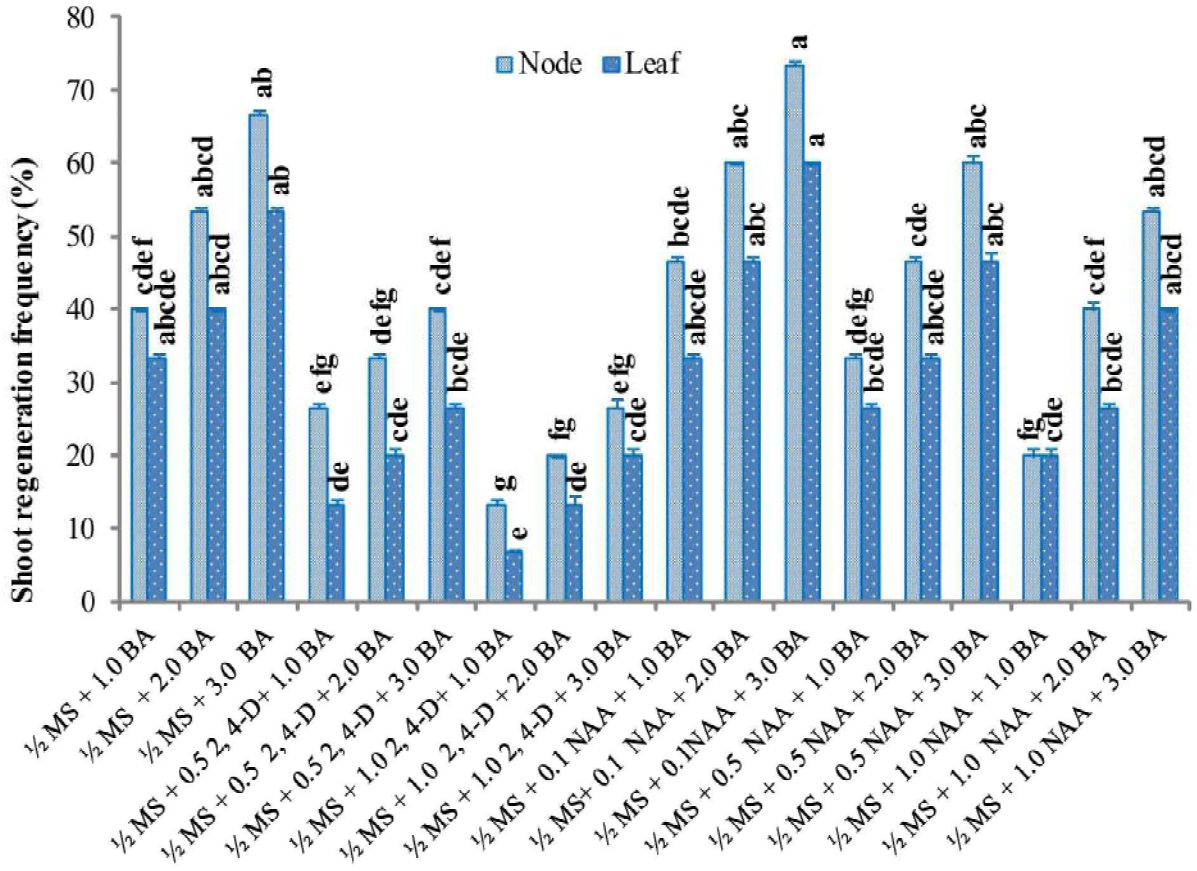

Fig. 5 : Shoot regeneration frequency at 6 weeks of culture of stem and leaf explant of *A. paniculata* on half strength MS media strengthen with various concentrations of BA, NAA and 2,4-D. Data consist of three replications and 5 explants were utilizedin each replication. Bars reflect the standard deviation of means. Values with different letters are significantly different at *P* value = .05 (DMRT).

best result was obtained in half strength MS media containing 2.0 mg/L IBA. The maximum percentage of rooting (93.33%) was noticed in half MS medium fortified with 2.0 mg/L IBA whereas the minimum root formation frequency (33.33%) was found in full strength MS medium reinforced with 0.5 mg/L NAA (Table 1).Root development was noted within 7 days of transplanting, and plantlets established a well-developed root system by 15 days (Fig. 1j). After that the plants were transferred to pot soil of acclimatization room for acclimatization (Fig. 1k). The acclimatized plantlets were grown successfully in field condition.

Fig. 6 : (a) Excessive rooting in the callus-on-callus induction medium contained higher concentration of NAA and 2,4-D (more than 1 mg/L), (b) no elongation of shoot in full strength MS medium, (c) browning of callus on full strength MS medium.*Scale bars* represent 1 cm (a, b, c).

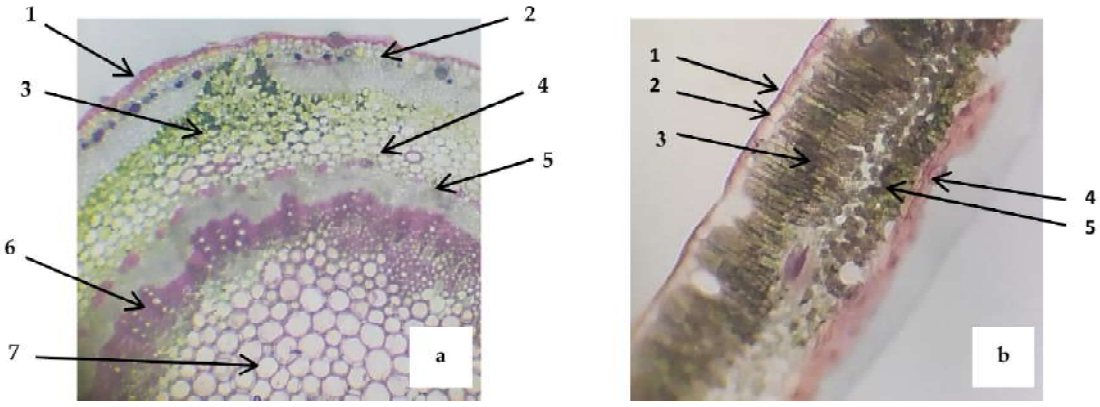

Fig. 7. (a) T.S. of Stem of *A. paniculata* Nees (× 40) where 1. Epidermis, 2. Collenchyma, 3.Chlorenchyma, 4. Sclerenchyma, 5. Phloem, 6. Xylem and 7. Pith cells; (b), T.S. of Leaf of *A. paniculata*(× 40) where 1. Cuticle, 2. Lower epidermis, 3. Palisade parenchyma, 4. Upper epidermis and 5. Spongy parenchyma.

Table 1 : Root initiation frequency of A. *paniculata* on different strength of MS media reinforced with various concentrations of NAA& IBA.

Medium	Concentrations of growth hormone (mg/L)		Days of root initiation	Root formation frequency(%)
	NAA	IBA		
Full Strength MS Media	0	0	-	0^{f}
	0.5	0	12-15	33.33 ± 0.58^{e}
	1.0	0	10-12	40 ± 1^{e}
	2.0	0	8-10	53.33 ± 0.58^{cde}
	0	0.5	10-12	40 ± 1^{e}
	0	1.0	8-12	66.67 ± 0.58^{bcd}
	0	2.0	8-10	73.33 ± 0.58^{abc}
Half Strength MS Media	0	0	-	0^{f}
	0.5	0	10-15	46.67 ± 0.58^{de}
	1.0	0	8-12	53.33 ± 0.58^{cde}
	2.0	0	8-10	73.33 ± 0.58^{abc}
	0	0.5	10-12	73.33 ± 0.58^{abc}
	0	1.0	8-12	86.67 ± 0.58^{ab}
	0	2.0	7-10	93.33 ± 0.58^{a}

Data consist of three replications and 5 explants were used for each replication. The mean values were compared by DMRT. Mean ± SD followed by same letters are not significantly different at $P = 0.05$.

Growth hormone, NAA or IBA alone or in a combination provide positive effects on rooting *in vitro* for *A. paniculatas* (Bansi and Rout, 2013; Roy, 2014; Kataky and Handique, 2011). In this study, two auxins (NAA & IBA) were used for root initiation. Six weeks old shoots,obtained from the subsequent culture in callus initiation and shoot formation media, were used for root formation. However, the regenerated shoot did not produce any roots in control (full and half strength MS medium without hormones) even after six weeks of culture. This type of *in vitro* rooting behavior of *A. paniculatas* also reported by Kataky and Handique

(2011); Bansi and Rout (2013); Jindal *et al.* (2015) and Chandran *et al,* (2017). Root initiation was observed in full and half strength MS media supplemented with NAA or IBA after 7 days of inoculation. The highest (93.33%) rooting was found in half strength MS medium supplemented with 2.0 mg/L IBA whereas the lowest (33.33%) rooting was occurred in MS media with 0.5 mg/L NAA (Table 1). Similarly, Jindal *et al.* (2015); Chandran *et al.* (2017); Deshmukh *et al*. (2017); found maximum root formation in 2.0 mg/L IBA.

In this study, half strength MS media responded better to root initiation than full strength MS media which is in accordance with Chandran *et al*. (2017); Deshmukh *et al,* (2017); Roy (2014); Bansi and Rout (2013); Kataky and Handique (2011). This could be owing to half-strength MS media having lower osmotic strength and nutritional content than full-strength MS media. Half-strength MS media provided low osmotic potential for ease acclimation of plants and also created partial stress, causing plants to develop more roots and stimulate rhizogenesis.

Any medium with lower concentrations of auxin showed a poor response in root formation as compared to medium with higher concentrations of auxin and IBA was proved more responsive than NAA which is in accordance with Roy (2014) and Purkayastha *et al.* (2008) findings.

CONCLUSION

In conclusion, the present study demonstrates an easy, cost effective, rapid and highly efficient *in vitro* regeneration protocol of *A. paniculata*. Stem explants showed better response both for callus initiation and shoot regeneration. MS medium complemented with 0.5 mg/L NAA and 2.0 mg/L BA was found as proper medium forhigh frequency callus initiation.The medium ½MS medium enriched with 0.1 mg/L NAA and 3.0 mg/L BA resulted in maximum shoot regeneration. The best medium for root initiation of *A. paniculata* was MS medium enhanced with 0.2 mg/ L NAA. Commercial utilization of this protocol will allow the pharmaceutical industries to multiply vast quantities of this key medicinal plant species for developing novel drugs to fight against infectious disease with more safety.

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Original Research Paper

Comparison of leaf volatile aroma constituents and phenolic acid profiles of the seedling originated polyembryonic mango (*Mangifera indica* L.) genotypes

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ABSTRACT

In mango, leaf and fruit volatile aroma profiles are variety specific which can be used as fingerprint of a variety. Such biochemical markers can also discriminate the nucellar and zygotic seedlings in polyembryonic mango varieties. In order to validate the applicability of volatile as well as phenolic acid profiles as biomarkers, the open pollinated seedlings of three polyembryonic varieties of mango were compared with their mother trees. Leaf volatile and phenol acid profiling were done using Gas Chromatography/Mass Spectrometry (GCMS) and Liquid Chromatography/Mass Spectrometry (LCMS) methods respectively. The sesquiterpene hydrocarbons were the most abundant in all the genotypes studied. Monoterpenoids were the major compounds in cultivars Vellaikolumban and Olour, while the sesquiterpenoids were the major compounds in *cv*. Turpentine. While terpinolene was the major monoterpenoid compound in Vellaikolumban and limonene in *cv*. Olour, the sesquiterpene á-gurjunene was the major compound in *cv*. Turpentine. Volatile profiling showed clear differences between the varieties but was similar within a variety. Among the 15 phenolic acids quantified in the leaves, P-coumaric acid, gallic acid, and ferulic acids were predominant whereas, vanillic acid, syringic acid, gentisic acid, benzoic acid, and sinapic acids were low in quantity. Phenolic acid profile did not show significant diversity among the varieties and therefore cannot be used for identification of varieties. The volatile profiling can be used for the identification and differentiation of polyembryonic mango genotypes.

Keywords: GCMS, LCMS, mango, nucellar seedling, polyembryony

INTRODUCTION

India has a large diversity of mangoes, with more than 1000 varieties (Salvi and Gunjate, 1988) that are grouped based on the number of embryos in the seed into monoembryonic and polyembryonic types (Mukherjee 1997). Most of the commercially grown varieties in India are monoembryonic while the polyembryonic varieties are used as rootstock since their apomictic seedlings arising from nucellus are known to be true to type. Each cultivar is distinguished by a unique combination of characters such as plant architecture, fruit size, color, taste, and flavor. Correct identification of varieties as well as discrimination of zygotic and nucellar seedlings is very important for crop improvement as well as for clonal rootstocks, even though morphological and molecular assessments have greatly aided in cultivar identification (Naik and Gangolly 1950, Ravishankar *et al*., 2000, Karihaloo *et al.,* 2003, Pandit *et al*., 2007). To complement this work, more reliable variety specific biochemical markers are a desirable attribute. There is a reliable variability in the volatile profile in mango cultivars (Andrade *et al.,* 2000). More than 270 aroma volatile compounds have been reported in various mango cultivars, including monoterpenes, sesquiterpenes, esters, aldehydes, ketones, alcohols, acids, aliphatic hydrocarbons (Shibamoto and Tang, 1990). Each of these volatile substances has its own distinct odour, and the combinations, quantities, and ratios of these molecules impart unique fragrance traits (Araguez and Valpuesta 2013). Mango leaves are a rich source of phenolic compounds such as xanthone-C-glycosides, gallotannins, benzophenones, flavonol glycosides, 5-alkyl- and 5-alkenylresorcinols and many other miscellaneous phenols (Barreto *et al.,* 2008) such as

kaempferol, quercetin, catechin, rhamnetin, gallic acid, benzoic acid, ellagic acid, tannins, flavonols, benzophenone, and their derivatives (Mwaurah *et al.,* 2020, Dorta *et al.,* 2014). In this study an attempt has been made to study the variability in leaf volatile and phenolic acid profiles of polyembryonic mango genotypes to identify their suitability as biochemical marker to identify the polyembryonic seedlings.

MATERIALS AND METHODS

Plant material

Three weeks old fresh mango leaves (top three) were taken from the OP seedlings of polyembryonic genotypes (Vellaikolamban, Olour, and Turpentine) conserved in the field gene bank of ICAR- IIHR, Bengaluru for HS-SPME and phenol profiling analysis. The volatile flavor constituents were analyzed by headspace-solid phase micro-extraction (HS-SPME) technique using GC–MS/MS and the phenol profiling were done by LC-MS/MS technique.

Volatile profiling

Solid phase micro extraction (SPME) of volatiles

The adsorption of analytes from the coated phase of fused silica fibre and partitioning of analytes between the stationary phase of the fibre and the extraction medium as gas constitute solid phase micro extraction. It consists of a 1-2 cm long fused silica fiber, coated with a stationary phase such as poly dimethyl siloxane (PDMS), divinyl benzene (DVB) and carboxen (CAR) or the mixture of all the three and bonded to a stainless-steel plunger and holder. These fibres are to be first conditioned at 250°C for 2-3 hours in the injector port of GC with the continued flow of Helium gas. In our study, ten grams of the fresh leaf was powdered using liquid nitrogen and taken in 100 ml conical flask along with a magnetic stirrer and then previously conditioned SPME fibre (Facundo *et al.,* 2013) was inserted to absorb the head-space volatiles for 2 hours. Fibre was subsequently injected into the GC-MS for the separation and identification of compounds.

GC-MS analysis

GC-MS analysis was performed on Varian-3800 gas chromatograph coupled with Varian 4000 GC-MS/MS ion trap mass selective detector. The MS column was a fused-silica capillary column of 30 cm x 0.25 mm id, 0.25mm film thickness for the analysis. The injector temperature was set at 250ºC and all injections were split-less mode for 0.2 min, detector temperature was 270°C, and the temperature programs for the column was as follows: 40°C for 2 min at an increment of 3°C/min to 190°C, held for 1 min, then 5°C/min to 220°C and maintaining the constant temperature for 5 min. The mass spectrometer was set in the external electron ionization mode (EI) with the carrier gas helium at 1.5 mL/min; injector temperature at 250°C; trap temperature at 180°C, ion source-heating at 190°C, transfer line temperature at 260°C, EI-mode at 70 eV, with full scan-range 50-350 amu (Atomic mass unit). The total volatile production was calculated by the individual peak areas in the chromatogram, individual compounds identified by comparison of the spectra against the retention index determined using homologous series of n-alkanes (C5 to C32) as standard using two spectral libraries available as Wiley and NIST-2007, and expressed as relative percent area.

Profiling phenols by LCMS

The phenolic acids for LC-MS/MS analysis was extracted using 80% methanol as previously described by Weidner *et al*. (2000) and Chen *et al*. (2001) with slight modification. 10 g sample was homogenized in methanol (80%), centrifuged and made up to 50 mL. 20 mL extract was taken and evaporated near to dryness under vacuum at 45°C and then diluted to 5 mL with water later extracted thrice with petroleum ether then in 40 mL of ethyl acetate using separating funnel. The aqueous layer was discarded and extract was ethyl acetate evaporated to dryness under vacuum at room temperature. To the dry residue, 4 mL of 2N NaOH was added and allowed to hydrolyze for overnight. Once acidifying to pH 2 using 5 mL 2N HCl, again re-extracted with 50 mL ethyl acetate. Ethyl acetate layer was again re-extracted twice with 25 mL of 0.1N NaHCO3. The ethyl acetate layer which carried the flavonoids was evaporated to complete dryness under vacuum, the residue was dissolved in 2 mL MS grade methanol filtered through 0.2µm nylon filter prior to injection in LCMS MS for flavonoids estimation. The aqueous layer was further acidified to pH 2 with 5 mL 2N HCl and extracted thrice with 25 mL ethyl acetate, the ethyl acetate layer was dried completely in rotary evaporator and the residue was dissolved in 2 mL MS grade methanol filtered through 0.2µm nylon filter prior to injection in LCMS MS for phenolic acid estimation.

LC and MS-MS conditions

The phenolic acids were resolved on the analytical column BEH-C18 (2.1 x 50 mm, 1.7 µm) from Waters India ltd., protected by a Vanguard BEH C-18 (Waters, USA) with the gradient flow of organic and aqueous phase with the flow rate of 0.3mL/min. The column temperature was maintained at 25°C during analysis and the sample injection volume was 2µL. The eluted phenolic acids and flavonoids from the UPLC column effluent pumped directly without any split into the TQD-MS/MS (Waters, USA) system optimized for the analysis of the phenolic acid.

Statistical analysis (Pearson Correlation) was performed by the web-based portal OPSTAT (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

Volatile profiling

In the three polyembryonic seedling originated plants of three varieties, the leaf volatile profile was generated, using GCMS/MS. The volatiles varied significantly among the genotypes. The most abundant hydrocarbons were monoterpenes and sesquiterpenes in all the three genotypes. In Vellaikolumban and Olour genotypes (Table 1 and 2), the monoterpenoids were maximum while the sesquiterpenoids were minimum but in *cv*. Turpentine (Table 4) sesquiterpenes were maximum. Among the monoterpenoids, the terpinolene was the major volatile compound followed by α-Pinene in the 3 seedling originated plants of cv. Vellaikolumban while sesquiterpenoids β-elemene, γ-cadinene and δ-Cadinene were found to be the minor

Table 1 : Relative peak area (%) of leaf volatile compounds of genotype Vellaikolumban using SPME based GC-MS analysis and their correlation among plants

Volatile compound	VP_1	VP_2	VP_3
α-Pinene	10.577	7.218	7.195
Camphene	1.077	0.729	0.700
β-Pinene	3.618	2.817	3.055
Sabinene	1.906	2.140	1.628
3-Carene	5.541	6.494	5.830
α-Terpinene	2.072	2.269	1.034
Limonene	2.416	2.359	1.974
cis-Ocimene	1.314	1.315	1.115
trans-Ocimene	1.870	2.156	1.184
Terpinolene	49.423	57.821	50.252
α-Copaene	0.585	0.367	0.865
(-)-β-Elemene	0.288	0.126	0.449
β-Caryophyllene	3.921	3.883	6.805
α-Humulene	2.072	1.917	4.313
Germacrene D	3.452	0.908	3.499
γ-Cadinene	0.369	0.368	0.712
δ-Cadinene	0.801	0.612	1.890
Pearson correlation matrix			
	VP_1	VP_2	VP_3
VP_1	1		
VP_2	0.995**	1	
VP_3	0.993**	0.995**	1

Table 2 : Relative peak area (%) of leaf volatile compounds of genotype Olour using SPME based GC-MS analysis and their correlation among plants

Volatile compound	OP_1	OP_2	OP_3
trans-2-Hexenal	0.208	0.756	0.649
cis-3-Hexen-1-ol	0.166	0.389	0.261
α-Thujene	0.128	0.182	0.128
α-Pinene	19.567	11.412	17.241
Camphene	0.329	0.181	0.235
Sabinene	0.813	0.399	0.331
β-Pinene	2.276	1.554	1.713
trans-Ocimene	4.101	4.111	4.447
α-Phellandrene	5.417	5.631	5.687
Limonene	56.958	62.001	57.140
α-Terpinene	0.801	0.754	0.663
Terpinolene	0.368	0.378	0.357
Nerol	0.029	0.203	0.095
2-methyl-2-bornene	0.277	0.959	0.759
Allo-Ocimene	0.019	0.034	0.026
4-Terpineol	0.016	0.177	0.114
Methyl salicylate	0.495	1.229	0.570
)-Elemene	0.199	0.261	0.368
Germacrene B	2.733	3.478	5.044
(-)-α-Cubebene	0.201	0.211	0.372
Pearson correlation matrix			
	OP_1	OP_2	OP_3
OP_1	1		
OP_2	0.988**	1	
OP_3	0.998**	0.993**	1

volatile compounds. The correlation analysis between the volatile compounds (Table 1) of three plants of Vellaikolumban were found to be significantly and positively correlated to each other (r = 0.993- 0.995). In Olour (Table 2), limonene was the major monoterpenoid followed by α-pinene and allo-ocimene. The correlation matrix (Table 3) indicated that volatiles of all the three plants of cv. Olour were highly correlated to each other (r = 0.988-0.993). In Turpentine (Table 3), sesquiterpenoids were the major group with α-gurjunene being the highest followed by β-sellinene in all the three seedling originated plants. Volatiles of all the 3 plants were highly correlated with each other (Table 4) (r = 0.991-0.998). Genotypes can be identified based on the volatile profile. Monoterpene and sesquiterpene hydrocarbons are the most abundant volatile components in all mango cultivars, accounting for 70–90% of total volatiles. Wetungu *et al*. (2015) studied the chemical profile of six mango varieties and reported that the mango leaves were rich in monoterpenes and sesquiterpenes. The α-pinene, phellandrene, limonene and ocimene were important monoterpene compounds which clearly distinguished the variability among 34 appemidi genotypes and sesquiterpenes composition was observed in genotype Gaddemara (90.39%) followed by Kalwaguda (78.73%). Among sesquiterpenes, α-humulene and caryophyllene were the major

Table 3 : Relative peak area (%) of leaf volatile compounds of genotype Turpentine using SPME based GC-MS analysis and their correlation among plants

Volatile compound	TP_1	TP_2	TP_3
α-Pinene	2.61	3.09	2.41
Sabinene	0.42	0.25	0.36
α-Phellandrene	5.62	3.09	2.36
β-Elemene	0.48	0.57	0.52
α-Gurjunene	40.12	37.76	38.01
β-Caryophyllene	14.57	16.25	15.13
α-Humulene	5.94	7.31	6.94
Allo-aromadendrene	0.33	0.48	0.41
(+)-9-Aristolene	3.12	3.56	4.10
β-Sellinene	22.56	23.53	25.69
γ-Gurjunene	2.69	2.94	2.58
γ-Cadinene	1.21	1.02	1.44
Pearson correlation matrix			
	TP_1	TP_2	TP_3
TP_1	1		
TP_2	0.995**	1	
TP_3	0.991**	0.998**	1

compounds in all the genotypes (Veena, 2018). Ma *et al.* (2018) detected α-pinene and terpinolene in mango varieties and these compounds are considered to be important volatiles. Cultivars Pingguo and Guixiang contained the highest level of α-pinene and limonene respectively. Moreover, limonene was a predominant component in five mango cultivars, including Cuba Delicioso, Super Hadden, Ordoez, Filipino and La Paz (Pino *et al.,* 2005). 3-carene was the dominant volatile in *cv*. Boluoxiang, but limonene was not found. Sesquiterpene hydrocarbons form the second largest group of aroma volatiles in mango (Pandit *et al*., 2009). Significant differences in the composition of total sesquiterpenoids were recorded among genotypes by Donald (2019) and the highest per cent of sesquiterpenoids composition was observed in genotype Rumani (91.48%) followed by H-151 (90.17%), while, the least content was noticed in genotype Dashehari (26.22%). In the case of sesquiterpenoids, caryophyllene, α-gurjunene and α-humulene contributed the maximum to the leaf volatiles in the genotypes studied indicating that the leaf volatile profile can be used as a fingerprint for varietal identification and could be important for clearly distinguishing the variability among mango genotypes (Donald, 2019, Veena, 2018, Gebara *et al.,* 2011, Dzbreveamic *et al.,* 2010, Liu *et al.,* 2013). Dzbreveamic *et al.* (2010) reported that the leaves of *M. indica* was rich in sesquiterpenes (70.3%) and δ-3-carene, α-gurjunene, β-selinene and β-caryophyllene were dominant compounds in mango leaf oil. In conclusion, mango cultivars differ in terms of total volatile concentration, both qualitatively and quantitatively. The volatile profiling of polyembryonic genotype was found to be different between the genotypes, but was strongly correlated with the seedling originated plants within a genotype. The three seedling originated plants of Vellaikolumban, Olour and Turpentine genotypes were also found to be morphologically similar within the group. Hence it is proved that the volatile profiling can be successfully used to identify the seedling originated plants of polyembryonic genotype.

Phenolic acid profiling

The phenolic acid profile of mango leaves was determined using liquid chromatography-Mass spectrometry (LC-MS/MS). Fifteen phenolic acids

Table 4 : Phenolic acid (mg/gm) profiling of genotypes *viz* Vellaikolumban, Olour and Turpentine and their correlation among genotypes

Phenolic acid	VP_1	VP_2	VP_3	OP_1	OP_2	OP_3	TP_1	TP_2	TP_3
Vanillic acid	0.05	0.96	4.67	0.09	2.97	4.74	7.66	7.46	9.37
Syringic acid	0.18	0.11	0.07	0.00	0.00	0.01	0.02	0.04	0.05
Ferulic acid	541.31	635.65	522.05	306.61	223.91	355.38	272.26	378.66	344.17
Caffeic acid	17.90	29.01	5.95	9.24	4.13	6.97	9.02	6.66	15.37
Gallic acid	564.95	705.11	383.15	144.47	145.30	272.86	437.98	514.02	742.97
p-Coumaric acid	1096.94	1266.06	927.67	872.10	606.84	1657.16	967.13	1088.20	1411.17
o-Coumaric acid	72.08	86.21	54.47	83.80	60.77	133.59	67.77	136.14	148.89
2,4-Dihydroxy benzoic acid	24.44	18.81	0.68	5.28	3.21	6.60	91.88	85.89	101.45
Gentisic acid	57.51	5.90	1.76	7.60	0.00	0.62	40.80	43.60	204.64
Protocatechuic acid	27.95	43.01	0.60	0.93	0.00	7.48	178.99	157.59	1.20
p-Hydroxy benzoic acid	36.30	28.96	19.79	24.03	26.99	35.94	31.80	29.34	32.63
Salycylic acid	59.60	17.16	15.43	22.05	10.01	10.07	34.45	47.56	94.12
Benzoic acid	4.74	1.40	9.43	3.93	3.50	1.37	3.01	0.67	0.42
3-Hydroxy benzoic acid	49.45	35.74	24.26	30.14	34.16	48.07	40.86	40.13	39.64
Sinapic acid	2.51	2.01	0.52	1.80	5.26	3.65	1.92	1.92	3.81
Pearson correlation matrix									
	VP_1	VP_2	VP_3	OP_1	OP_2	OP_3	TP_1	TP_2	TP_3
VP_1	1								
VP_2	0.998**	1							
VP_3	0.992**	0.990**	1						
OP_1	0.946**	0.934**	0.960**	1					
OP_2	0.965**	0.956**	0.974**	0.997**	1				
OP_3	0.927**	0.915**	0.932**	0.991**	0.988**	1			
TP_1	0.966**	0.964**	0.947**	0.943**	0.955**	0.950**	1		
TP_2	0.981**	0.980**	0.966**	0.951**	0.966**	0.950**	0.995**	1	
TP_3	0.967**	0.961**	0.938**	0.926**	0.942**	0.936**	0.974**	0.978**	1

(Table 4) were identified in the leaves of all the 3 genotypes. Among them, P-coumaric acid, gallic acid and ferulic acids were found to be the major phenolic acids. On the other hand, vanillic acid, syringic acid, gentisic acid, benzoic acid and sinapic acids were minor contributors in phenol profiling. P-Coumaric acid was the predominant phenolic acid in all the genotypes followed by gallic acid, ferulic acid in Vellaikolumban and Turpentine but in Olour it was ferulic acid followed by gallic acid. The correlations between the seedlings originated from the same kernel indicated a highly significant correlation (r = 0.915-0.998) (Table 4). Correlations between the genotypes also showed significantly higher values indicating that this parameter is not variety specific. Earlier reports indicate that the proportion and profile of polyphenols in mango vary depending on the variety and also plant part (Ma *et al*., 2011). Ocampo *et al*. (2020) reported variations in the phenolic profiles among mango types. Gallic, vanillic, syringic, and ferulic acids were all

found in the peels of all mango genotypes, while coumaric and chlorogenic acids were not detected. Gallic acid has also been identified as a common phenolic acid present in the mango types Keitt, Sensation, and Gomera 3 (Dorta *et al*., 2014). Our results showed that based on phenolic acid profiling, it is not possible to distinguish the genotypes. On the contrary to these findings, Ocampo *et al*. (2020) reported that the phenolic acid profile could be utilised as a marker/fingerprint in the future to correctly identify types such as the Carabao mango, which is well-known in the Philippines for its flavour.

CONCLUSION

Volatile aroma and phenolic acid profiling from the mango leaf using GCMS and LCMS/MS techniques indicated that leaf volatile profile is variety specific and can also be used successfully to identify the nucellar seedlings of polyembyonic varieties which are similar to the mother plant. Leaf volatiles are stable which gives unique aroma to a particular genotype. However, the phenolic acid profiling could not differentiate the varieties.

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Original Research Paper

Possibility of early detection of graft incompatibility in some commercial plum cultivars by phenolic compounds analysis

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ABSTRACT

The incidence of incompatibility signs in the grafting point can be delayed, and the analysis of phenols is used as an applicable early sign for the detection of graft incompatibility. Accordingly, this study mainly aimed to investigate compatibility/incompatibility in 10 commercial plum cultivars grafted on myrobalan and apricot rootstocks, followed by determining the role of phenols in graft incompatibility. The evaluated cultivars included Santarosa, Ghatreh tala, Shams, Dargazi, No. 16, No. 17, Laroda, Simka, Bokhara, and Stanley. The results showed significant differences in the stem diameter. The union graft location in Shams, Laroda, Simka, Stanley, and Ghatreh tala cultivars on apricot rootstock was thicker than the scions and stocks. Phenolic compounds in the union graft decreased in all plum cultivars on myrobalan rootstock in comparison with other sites. Finally, the most phenolic accumulation belonged to the union graft on Santarosa, Ghatreh tala, and Shams on apricot rootstocks. Therefore, it seems that phenolic compounds in plums can be used as a biochemical marker in graft incompatibility.

Keywords: Apricot rootstock, incompatibility, myrobalan rootstock, phenolic content, plum

INTRODUCTION

Plum (*Prunus* spp.) is one of the most commercially important fruit species in Iran. Plums are temperate zone fruits, but they are widely grown throughout the world, from the cold climate of Siberia to the sub-tropical conditions of the Mediterranean region (Son, 2010). Prunus species such as *P. cerasifera*, *P. domestica*, *P. institia* and *P. Salicina* are widely grown throughout the world. The European plum (*P. domestica*) and the Japanese plum (*P. salicina*) are more important in terms of commercial production (Ozbek, 1978).

Grafting is largely used in the production of vegetable and fruit-bearing crops in order to increase uniformity, vigor, and adaptation to biotic and abiotic stresses. The compatibility of rootstock and scion plays a crucial role in establishing highly efficient root systems through grafting (Goldschmidt, 2014; Warschefsky *et al*., 2016). However, this trait varies significantly even between closely related species, which necessitates the evaluation of compatibility before grafting a specific scion genotype into the rootstock. For the stone fruit industry that heavily relies on vegetatively propagated cultivars (*i.e*., individual genotypes) via grafting, the long-term vitality of the union between the rootstock and scion is crucial (Lee *et al*., 2011; Guan *et al*., 2012).

Graft incompatibility generally occurs at the early stage of graft development when the vascular connection is forming. However, symptoms may manifest at large growth stages such as low plant development related to physiological differences in the stem diameter, which impairs the normal flow of photoassimilates and the lignification of grafted tissues (Souza *et al*., 2018), thus decreasing the hydraulic conductivity of the graft union (Tworkoski and Fazio, 2015). These symptoms appear during the plant fruiting period when the plant is subjected to a high demand for water transport (Martinez-Ballesta *et al*., 2010). Incompatibility does not permanently become

apparent immediately after grafting. It may take several years to manifest failure with establishing graft-union leading to major economic losses to growers and nurseries. In addition, the significant delay in the appearance of incompatibility symptoms renders the evaluation and transfer of new fruit tree genotypes to industry time-consuming, expensive, and laborious (Gainza *et al*., 2015; Pina *et al*., 2017).

Fruit trees are typically formed by a combination of the scion and rootstock. A good union between a scion and rootstock is necessary for a successful combination (Errea *et al*., 2001). Graft incompatibility symptoms in woody species include bark thickening in the connection region, chlorotic leaves, premature leaf fall, budding delay, vigor differences between the rootstock and scion, excessive stem thickening below, above, or at the point of the graft union. Other symptoms are graft union disruption, reduced vegetative growth, low productivity, and premature plant death (Zarrouk *et al*., 2010; Hartmann *et al*., 2011).

The grafted partners frequently belong to the same species or genus although the use of genetically divergent genotypes is also common. Incompatibility repeatedly occurs in the plum when it is grafted on other Prunus species such as the apricot graft. Different reasons may influence graft success, including the inherent system of cellular incompatibility, the formation of plasmodesmata, vascular tissue connections, and the presence of growth regulators and peroxidases (Usenik *et al*., 2006). Macromolecules (phloem proteins, RNA, and hormones) that are present in the sap phloem might also be important during vascular differentiation in the compatibility process (Pina and Erea, 2005). Different methods for an early detection of graft incompatibility have already been used, including in vitro techniques (Errea *et al*., 2001), isozyme analysis (Fernandez-Garcia *et al*., 2004; Gulen *et al*., 2002), and phenol analysis (Musacchi *et al*., 2000). Such compounds are important to the early growth stages of connections between scion-rootstock combinations since the cell walls of xylem tissues are dynamic structures composed of polysaccharides, phenolic compounds, minerals, and proteins (Herrero *et al*., 2014). Moreover, the presence of phenolic compounds has been identified as an important marker for the evaluation of graft compatibility between scions and rootstocks (Prabpreea *et al*., 2018).

The analysis and recognition of structural phenol diversity are of particular interest because of their physiological roles during the first steps of graft establishment (Usenik *et al*., 2006). The presence of phenols was generally associated with small cells in incompatible combinations, which did not lead to successful unions (Errea *et al*., 2001). Higher concentrations of catechin and epicatechin were found in quince-incompatibility cultivars before the appearance of visible incompatibility symptoms (Musacchi *et al*., 2000). In less compatible apricot combination higher level of flavanols, catechin, and epicatechin, was characteristics (Errea *et al*., 2000).

In several apricot combinations grafted on prunus rootstocks, graft incompatibility resulted in breakdown of the trees at the union years after planting, therefore an early selection process could help in detecting a comparatively compatible combination. Analysis of the phenol content at the graft union can be used as a technique for the estimation of graft incompatibility (Dogra *et al*., 2018).

Several studies have shown that phenolic compounds in incompatible combinations move from vacuole to cytoplasm and cause inhibition of lignification which is required during early stages of establishment of scion–stock connections. The cell wall of xylem vessels are dynamic in nature composed of phenolic compounds (for example, lignins), minerals, polysaccharides and proteins (Liu, 2012; Herrero *et al*., 2014). Plant hormones, especially auxins determine the compatibility of a rootstock-scion combination by interacting with phenolic compounds. Incompatibility has been associated with increased levels of phenolic compounds above the graft union which adversely affect the auxin transport (Errea, 1998). Low auxin concentration in incompatible combinations in turn affect the differentiation of vascular tissues and lignification (Aloni, 2010; Koepke and Dhingra, 2013). All these changes will lead to the formation of weak unions which may cause huge economic losses to the growers. More information about the compounds responsible for inducing graft incompatibility is needed (Gainza *et al*., 2015).

Given the above-mentioned explanations, the current study mainly sought to evaluate the relationship between graft incompatibility and the total phenolic content in some commercial plum cultivars, as well as to determine whether such analysis can be a useful tool for the early detection of graft incompatibility.

MATERIALS AND METHODS

Plant material

This research was conducted at at Golmakan Horticultural Research Station (59° 17' N; 36° 32' E), north east of Iran/Mashhad, with an average altitude of about 1176 m. The mean temperature for growing season was 13.4°C and total seasonal precipitation was 239.7 mm. The nursery soil was sandy loam with low organic matter. Drip irrigation was applied in the nursery. The trees were planted at a spacing of 100 × 10 cm (100.000 trees ha-1) and budded (T-budding technique) 10 cm above the ground level. All rootstocks (apricot and myrobalan) were seedlings, and the samples were taken from 1-year-old plum trees. The content of total phenols above, below, and at the union graft in 10 plum cultivars (i.e., Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, No. 16, No. 17, Bokhara, Shams, and Simka) grafted on myrobalan and apricot seedling rootstocks was analyzed as well.

Field study

Trees were used one year after grafting for the study. The stem diameters of scions, stocks, and the graft union were measured using a pair of caliper. The units of measurment was to mm.

Phenol extraction

Three trees from each grafting combination were analyzed, and the samples were collected in June. The small sections of the bark above, below, and at the union graft (1 cm above and below the graft union, 1.5 cm in length) were removed with a knife and immediately frozen in liquid nitrogen. Phloem with cambium was used for analysis.

The samples were extracted with a 1.5 ml methanol-acetone-water solution (7:7:1 v/v/v). In a mortar, 50 mg of the plant material was homogenized with a 1.5 ml extraction solution. Next, the samples were centrifuged at 6000Xg for 20 min using a bench centrifuge. Then, the solvents were evaporated in rotary at 40 ^{Ú}C, and the residue was dissolved in 5 ml of deionized water. The extracts were stored at -80 ^{Ú}C until the analysis of the total phenolic content (Mngomba *et al*., 2008). The applied chemical reagents were obtained from Merck Company.

Total phenolic content analysis

The amounts of the total phenol content in pulm cultivar extracts were determined with the Folin-Ciocalteau reagent using the method of Spanos and Wrolastad (1990), as modified by lister and Wilson (2001). To this end, 0.5 ml of Folin-Ciocalteau reagent and 2 ml of Na_2CO_3 (7.55/a, w/v) were added to 100 µl of each sample (three replicates) and then incubated at 45 ^{Ú}C for 15 min. The absorbance of all samples was measured at 620 nm using a SPECTRA max-PLU5384 UV-Vis spectrophotometer. The results were expressed as milligrams of catechol acid equivalent per gram of dry weight (Ganji Moghadam *et al*., 2007).

Statistical analysis

The trial was laid out in a factorial experiment based on completely randomized design with three replications where each replication contained 10 trees. Factors a contains cultivars in 10 levels (Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, No. 16, No. 17, Bokhara, Shams, and Simka), factor b contains rootstock in 2 levels (myrobalan and apricot seedling) and Factor c contains 3 levels (above, below, and at the union graft). Three replicates of each sample were used for statistical analysis using MSTAT-C, version 1.42. Data were subjected to the analysis of variance, and means were compared by the least significant difference. Differences at $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Significant differences in stem diameters were observed above, below, and at the union graft. The stem diameters below the graft unions were visibly greater than those of above and the union graft on myrobalan rootstock and Santarosa, Dargazi, Bokhara, No. 16, and No.17 cultivars on the apricot rootstock. The unions were thicker than scions and stocks in Ghatreh tala, Shams, Laroda, Stanley, and Simka on the apricot rootstock (Table 1).

In the study of the independent effect of the total phenol content in the union graft, the highest total phenolic content was detected in the below graft union while the lowest content was found in the above graft union. Above and union graft differences were not significant (Figure 1a).

Based on the evaluation of the effect of the union graft in apricot and myrobalan rootstocks, the highest and

Table 1 : Thickness (mm) above, below, and at union graft of different plum cultivars grafted on apricot and myrobalan rootstocks

Graft Combination	Above the Union	At the Union	Below the Union
Apricot rootstock			
Santarosa	6.82*	11.95a	12.37a
Ghatreh tala	8.2c	16.08a	11.57b
Shams	7.24a	9.09a	7.93a
Laroda	5.8b	11.30a	10.95a
Dargazi	7.15c	12.98b	16.18a
Simka	5.16b	11.79a	9.21a
Bokhara	5.9c	10.27b	11.86a
Stanely	6.86a	13.72a	11.11a
No. 16	6.03b	12.13a	14.41a
No. 17	5.03b	11.62ab	18.21a
Myrobalan rootstock			
Santarosa	6.93b	12.71b	20.55a
Ghatreh tala	8.41c	11.57b	16.84a
Shams	6.76b	10.23b	17.74a
Laroda	7.94c	12.16b	16.38a
Dargazi	9.53c	13.49b	18.48a
Simka	8.22c	13.61b	19.02a
Bokhara	7.00c	9.28b	11.31a
Stanely	8.41b	13.67a	16.99a
No. 16	7.18b	10.88b	19.44a
No. 17	6.83b	11.23b	15.11a

Note : *Means with the same letters within a row are not significantly different at P<0.05.

the lowest total phenolic contents were observed in the below graft union on myrobalan and apricot rootstocks, respectively (Figure 1b). The highest total phenolic content was detected in the below graft union of Laroda, Shams, Stanley, Santarosa, and Dargazi cultivars grafted on the myrobalan rootstock whereas the lowest content was found in the below graft union

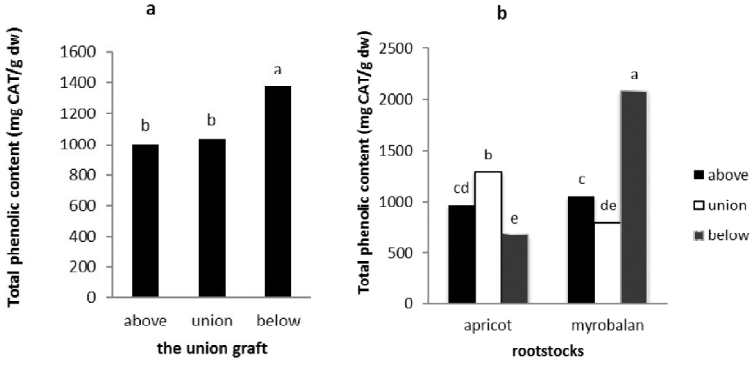

Fig. 1 : Effects of Independent Union Graft (a) and Interaction Rootstocks and Graft Union (b) on the Total Phenolic Content (mg Catechol Acid Equivalent per g of Dry Weight)

of Shams, Laroda, Ghatreh tala, and Santarosa cultivars grafted on the apricot rootstock. The compared differences in the total phenol content in the union and below graft demonstrated the significant accumulation of phenol in the union graft in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on the apricot rootstock while it decreased on myrobalan rootstock (Table 2).

The stem diameters below the graft unions were visibly greater compared to the above and at union graft on myrobalan rootstock and Santarosa, Dargazi, Bokhara, No. 16, and No.17 cultivars on the apricot rootstock. The unions were thicker than the scions and stocks in Ghatreh tala, Shams, Laroda, Stanley, and Simka on the apricot rootstock. The highest total phenolic contents were detected in myrobalan rootstocks while the lowest contents were found in apricot rootstocks. The composition of phenols depends on the genetic constitution of the plant species, and hence, some plants accumulate more than others. These results are in agreement with those of Pina ane Errea (2005) indicating that some apricot cultivars grafted onto a plum rootstock demonstrated only some callus differentiation occurred on cambium and vascular tissues while a large portion of the callus never demonstrated a differentiation. This interrupts vascular connections because of the lack of differentiation that brings discontinuities in the cambium and the formation of a band of

Table 2 : The Amount of the total phenol content (mg gallic acid equivalent per g of dry weight) in above, below, and at the union graft of different plum cultivars grafted on apricot and myrobalan rootstocks

Graft Combination	Above the Union	At the Union	Below the Union
Apricot rootstocks			
Santarosa	1033.79a	1274.88a	441.01b
Ghatreh tala	1051.14b	1905.93a	430.14b
Shams	1010.95a	1424.65a	317.81b
Laroda	810.05a	902.28a	401.82a
Dargazi	1114.15a	1302.28a	747.94a
Simka	1166.21a	1513.24a	839.27a
Bokhara	677.17a	951.59a	555.25b
Stanely	762.56a	1250.23a	698.63a
No. 16	925.15b	1135.16a	1091.33ab
No. 17	807.31b	1347.91a	1204.56a
Myrobalan rootstocks			
Santarosa	611.78b	363.47b	2230.13a
Ghatreh tala	1421.91a	663.93b	1476.86a
Shams	805.47b	1112.32b	2413.69a
Laroda	1378.99b	536.07b	3215.52a
Dargazi	830.14b	449.31c	1221.46a
Simka	1053.88b	763.47b	1789.95a
Bokhara	838.36b	989.04b	1813.69a
Stanely	1120.59b	1114.15b	2291.33a
No. 16	629.23c	1309.59b	1882.19a
No. 17	1828.31a	619.18b	2122.51a

Note : *Means with the same letters within a row are not significantly different at P<0.05.

parenchymatous cells. Based on the findings regarding the evaluation of the independent effect of the total phenol content in the union graft, the highest and lowest total phenolic contents were detected in the below and above graft unions, respectively. The results related to the effect of the union graft on apricot and myrobalan rootstocks, the highest and lowest total phenolic contents belonged to below graft union on myrobalan rootstock and apricot rootstock, respectively. Mngomba *et al*. (2008) reported that the accumulation of phenol deposits at the place of the graft union might inhibit graft compatibility. Usenik *et al*. (2006) also demonstrated that differences in phenol accumulation below and above the graft union might serve as an indicator of incompatibility.

The highest total phenolic content was detected below the graft union of Laroda, Shams, Stanley, Santarosa, and Dargazi cultivars grafted on myrobalan rootstock whereas the lowest content was found in the below graft union of Shams, Laroda, Ghatreh tala, and Santarosa cultivars grafted on apricot rootstock. The comparison of differences in the total phenol content in union and below graft showed the significant accumulation of phenol in the union graft in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on apricot rootstock while it decreased on myrobalan rootstock. In apricot/plum combinations, a high concentration of phenolic compounds was observed in undifferentiated callus at the scion-rootstock interface of plants previously categorized as incompatible (Pina *et al*., 2012), and thus they are involved in the processes of differentiation of vascular tissues (Usenik *et al*., 2006), which is in line with our results. The statistically significant accumulation of phenol in the graft union was ascertained in Santarosa, Ghatreh tala, Laroda, Stanley, Dargazi, Bokhara, Shams, and Simka cultivars grafted on apricot rootstock when compared with the content below the graft union while phenol above the graft union decreased in plum cultivars on myrobalan rootstock. The highest accumulation of phenol in the union graft that can be used as a biochemical marker of graft incompatibility are observed in Ghatreh tala, Shams, and Santarosa on apricot rootstock, which corroborates with the findings of Prabpreea *et al*. (2018), implying that the presence of phenolic compounds has been identified as an important marker for the evaluation of graft incompatibility between scion and rootstocks in the union graft.

CONCLUSION

The early phase of graft incompatibility is complex and needs further evaluation. Phenol analysis is an applicable early sign for the prediction of graft incompatibility, especially when there are new cultivar/ rootstock combinations. The results showed that Ghatreh tala, Shams, and Santarosa on apricot rootstock have the highest graft incompatibility, respectively.

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Original Research Paper

Identification of circular RNAs in resistant tomato genotype in response to *ToLCBaV* infection

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ABSTRACT

Circular RNAs (CircRNAs) are covalently closed non-coding RNAs that play an important role in a variety of biological processes. CircRNA profiling helps to understand biological process associated with various abiotic and biotic stresses. In tomato genotype IIHR- 2611 (resistant to ToLCBaV), a total of 193 CircRNAs were discovered, of which 72 and 121 were found in control (RC) and ToLCBaV inoculated (RI) plants respectively. Among them, 103 (53 %) were exonic CircRNA regulating the expressions of their parent genes. Relative expression of CircRNAs 2:45295638|45295796, 2:51520741|51530067 and 7:67566489|67566691 and their respective parent genes *Solyc02g080530.3* (peroxidase), *Solyc02g088950.2* (superoxide dismutase) and *Solyc07g065840.2.1* (heat shock protein 90) response to ToLCBaV infection were analysed at different time intervals. A significantly positive correlation was observed for the expression profiles of all three circRNAs and their parent genes. Furthermore, the differential expression across samples as well as time interval indicates that CircRNA mediated gene expression is involved in viral resistance. The results of the expression assays of both superoxide dismutase and peroxidase were consistent with enzyme analysis. Overall findings demonstrated the importance of CircRNAs in ToLCBaVD resistance and suggested that CircRNAs could be key regulators of gene expression during disease resistance in tomato.

Keywords: CircRNAs, RNA sequencing, ToLCBaVD resistance, tomato

INTRODUCTION

Tomato is a globally important vegetable crop and its cultivation is severly hampered by various pests and diseases including *Tomato leaf curl virus* (ToLCV). The losses due to infestation of ToLCV often exceeds 90 per cent (Varma and Malathi, 2003; Singh *et al*., 2015). Majority of genomic studies relied on molecular markers and functional analysis of genes. In order to understand the structural and functional concept of genomic regions, it is important to employ high-throughput next generation sequencing technologies (Barone *et al*., 2008; Wang *et al*., 2018). RNA sequencing also known as transcriptomics is one such technology that allows researchers to examine both known and unknown transcripts. Non-coding RNAs (ncRNAs) are transcripts that are not part of protein-coding genes (Wang *et al*., 2018). Among them Circular RNAs (CircRNA) are diverse and unique family of endogenous non-coding RNAs found in plant cells (Wang *et al*., 2018).

CircRNAs are abundant in the eukaryotic transcriptome. Their discovery and functional involvement in biological processes has opened up a new perspective so as to know how genomic regions interact in a variety of ways. However, their specific role is yet to be understood (Zhang *et al*., 2020; Litholdo *et al*., 2018). The majority of CircRNAs are conserved across species, although their expression varies according to tissue or developmental stage, as well as during biotic and abiotic stresses. CircRNAs interact with the transcriptional complex and influence the transcriptional and post-transcriptional regulation of gene expression (Zhang *et al*., 2020; Shao *et al*., 2021). They regulate parent gene expression by acting

as miRNA sponges and RNA binding protein (RBP) sponges (Hansen *et al*., 2013; Ashwal-Fluss *et al*., 2014; Shao *et al*., 2021). Their biogenesis competes with linear mRNA splicing to target alternative splicing mechanism of gene regulation (Shao *et al*., 2021). CircRNAs also regulate the translation of parental genes through interaction with trans-acting elements (Shao *et al*., 2021).

CircRNA were found to be differentially expressed during pathogen interaction in Arabidopsis (Sun *et al*., 2016; Zhang *et al*., 2020), pathogen invasion in kiwi fruit (Wang *et al*., 2017) and interaction with leaf curl virus in tomato (Wang *et al*., 2018); they also have regulatory roles in response to cotton verticillium wilt and maize Iranian mosaic virus (Xiang *et al*., 2018; Ghorbani *et al*., 2018). However, there is no information on the involvement of CircRNA in ToLCV tolerance in tomatoes. Keeping this in view, the present study investigated the potential role of CircRNA in regulating ToLCV resistance in tomato. Using high-throughput sequencing technology and appropriate bioinformatic tools, we analysed transcriptome data and identified CircRNAs. Abundance of CircRNAs, chromosome distribution and their corresponding genes were analysed and further the differential expression of few selected CircRNAs and their corresponding parent genes at different interval after ToLCBaV infection were analysed through gene expression studies.

MATERIALS AND METHODS

Plant infection and RNA sequencing

Tomato genotype (Acc No. IIHR 2611) resistant to ToLCBaV was grown under control green house conditions at ICAR-IIHR, Bengaluru. Ten day old seedlings were inoculated with white fly (*Bemisia tabaci*) carrying ToLCBaV. At 0, 3, 5, 9, 15 and 21 days post inoculation (DPI) leaf samples were collected. Total RNA from all the periods with three biological replications in each sample was isolated using RNA iso-Plus (TAKARA, BIO INC. Japan). The quality of total RNA was measured using NABI UV/vis Nano Spectrometer. Total RNA of control plants of all the intervals (0, 3, 5, 9, 15 and 21 DPI) were pooled as sample RC and total RNA of infected plants of all the intervals (3, 5, 9, 15 and 21 DPI) were pooled as sample RI and sent for RNA-sequencing at M/S Eurofins Genomics facility, Bengaluru. The libraries were made from the pooled RNA samples and sequenced on an Illumina Hiseq1500 sequencing platform with 150-bp paired-end reads following manufacturer's instructions. The raw reads were filtered to obtain the clean reads by removing reads containing adaptors and uncertain nucleotides N>10%, and also reads with low quality nucleotides (base quality <5 and Q score <20%). The RNA sequence data of both control and infected tomato (IIHR 2611) was submitted to NCBI (SRR 13493714).

Bioinformatic analysis to detect CircRNAs

CircPlant is composed of four modules. Based on total/polyA- RNA sequencing reads, CirePlant using BWA-MEM software detects plant CircRNAs and the modified CIRI2 (Gao *et al*, 2015; Gao *et al*, 2019; Zhang *et al*., 2020). CIRIexplore2 tool was used to identify CircRNAs with the following criteria: both ends of splice sites should be GU/AG; mismatch d" 2; Back-spliced junctions reads e" 1; The distance between two splice sites d" 100 kb (Zhang *et al*., 2016). The functional role of the parent genes of identified CircRNAs involved in viral resistance was taken from Sol Genomics Network (https://solgenomics.net) and also from other publications.

Validation of CircRNAs and their parent genes using qRT-PCR assay

Following the manufacturer's instructions, cDNA for RNA samples of IIHR-2611 at both control and infected conditions (0, 3, 9 and 15 DPI) with three biological replications were synthesized using Hi-cDNA synthesis kit (Mol Bio HIMEDIA: MBT076-100R). qRT-PCR was performed in Quantstudio 7 Flex thermal cycler (Applied biosystems) using the intercalation dye TB Green Premix Ex Taq II (TaKaRa Cat# RR820A). PCR mixture composition and data analysis were carried out as previously described (Sorrequieta *et al*. 2010). PCR conditions were 30 sec at 95 °C and 40 cycles of 5 sec at 95 °C, 40 sec at 59 °C and 30 sec at 72 °C. A melting curve for every target analysed was included using the following conditions: 95 °C for 15 sec, 60 °C for 1 min, and 95 °C for 15 sec. Primer sequence for CircRNA and their parent genes is listed in Table 1. The relative expression level was calculated using the $2^{"\Delta\Delta Ct}$ method (Livak and Schmittgen, 2000). Each sample contains three biological replications and three technical replications. The housekeeping gene EF-1α (elongation factor-1α) was used to normalize the transcript levels in the RNA samples (Lacerda *et al*.,

Table 1 : List of primers used in the study

CircRNA_ID	CircRNA primer sequence 5 '-3'	Corresponding gene primer sequence 5'-3'
2:45295638|45295796 (Peroxide, POX)	F: TGCTTGGTCTCACACATTCA R: GCAAGATGTATAATGCGATGGAT	F: GGAATCAACACCCCTGGAGTT R: ACTTCTGGATATAAACGGTGTACCAA
2:51520741|51530067 (Superoxide dismutase)	F: GCTTGTTCCCAAATCCTGCA R: TTACCCGAGTTCCATCCACC	F: GCGACACTTGAACTTCCTTCCT R: AGCACTTCCCCACAGAATAATTTG
7:67566489|67566691 (Heat shock protein 90)	F: ACATGGAGAGAATTATGAAGGCC R: TCACCTTACACAGTCCCTCA	F: TATGAAGGCACAGGCACTTAGG R: ATGATGGAGTTCTCTGGGTTGATC

2015). Correlation was performed to mean values of $\log_2$ (FC) in excel to analyse relationship between CircRNA and their parent gene expression.

Antioxidant enzyme analysis

In order to complement CircRNAs data, we examined SOD (superoxide dismutase) and peroxidase (POX) activity, spectrophotometrically in control and infected plants following Du and Bramlage (1994) and Chander, S. (1990) respectively. Absorption was measured at 560 nm for SOD and the increase in absorbance was measured at 450 nm up to 5 min at 1 min interval for POX. Enzyme activity was expressed in Unit/mg FW. The enzymes activity between control and ToLCBaV infected tomato samples with three replications were compared statistically by two factor analysis of variance (ANOVA) using online statistical software package for Agricultural Research-OPSTAT (Sheoran *et al*., 1998). In all analyses, $P < 0.05$ was taken to indicate statistical significance and Tukey's HSD Test was performed for multiple comparisons.

RESULTS AND DISCUSSION

Identification of CircRNAs in ToLCBaV infected resistant tomato genotype

A total of 193 CircRNAs were identified in IIHR-2611 genotype using CircPlant (CircRNA Identifier-CIRI2 software (Gao *et al.*, 2015; Gao *et al.*, 2019), of which 58 were specifically expressed in uninfected plant samples and 107 CircRNAs in ToLCBaV infected samples (Fig. 1A). While 14 CircRNAs are common to both RC and RI conditions (Fig.1A). The analysis of CircRNAs across chromosomes (Chr.) showed that all chromosomes harbour CircRNA. However, Chr. 2 has maximum CircRNAs both in control and infected conditions, accounting for 23.83 per cent of total CircRNAs identified (Fig. 1B). The distribution of identified CircRNAs differs with chromosomes where, Chr. 1, 4 and 6 had more CircRNAs in infected sample compared to control (Fig. 1B).

The results showed that CircRNAs were formed from various genomic regions. Out of 193 total CircRNAs identified from both RC and RI, 103 (53 %) CircRNAs were generated from exonic region, four (2 %) CircRNAs were from intergenic region and 86 (45 %) CircRNAs are from other regions of the genome (Fig. 1C). The CircRNAs length analysis showed that most of the exonic CircRNAs were up to 10kb and intergenic CircRNAs were <500bp (Fig. 1D). A few parent genes of identified CircRNAs involved in virus resistance includes SOD (Solyc02g088950.2) and POX (Solyc02g080530.3) in Chr. 2, Zinc finger transcription factor 33 (Solyc04g057990) and Ariadne-like ubiquitin ligase (Solyc04g079780) on Chr. 4 and Chaperonin (Solyc01g028810) and Chaperonin Cpn60 (Solyc01g028810) on Chr. 1 (Table 2).

Validation of tomato CircRNAs in response to ToLCBaV infection

A total of 193 novel CircRNAs were discovered from control and ToLCBaV infected seedlings of the resistant genotype (IIHR-2611). 108 of them were specific to infected samples induced due to viral infection. Among them, we listed parent genes of CircRNAs based on their functional role in defence against biotic stress (Table 2) and experimentally tested the predictions of their expressions using qRT-PCR analysis. Relative expression pattern of CircRNA and their parent genes was found to be significantly positively correlating across different interval of viral infection in all three genes with correlation coefficient of 0.89, 0.61 and 0.97 for 7:67566489|67566691 (HSP 90: Solyc07g065840.2.1), 2:45295638|45295796 (POX: *Solyc02g080530.3*) and 2:51520741|51530067 (SOD: *Solyc02g088950.2* respectively (Fig 2). The expression of SOD gene (up to 6.0 $\log_2$FC)

Table 2 : List of a few identified CircRNAs with their parental genes

CircRNA_ID	Gene_ID	Corresponding gene	Reference
1:41290623|41290915	Solyc01g028810	Chaperonin	Solgenomics
1:41290675|41291268	Solyc01g028810	Chaperonin Cpn60	Solgenomics
2:44939803|44947800	Solyc02g080050.2	Cysteine-rich receptor-like protein kinase 25	Van *et al*., (2017)
2:45295638|45295796	Solyc02g080530.3	Peroxide, POX	Xue *et al*., (2020)
2:51520741|51530067	Solyc02g088950.2	Superoxide dismutase	Li *et al*., (2020)
4:55042616|55042849	Solyc04g057990	Zinc finger transcription factor 33	Solgenomics
4:64205208|64205372	Solyc04g079780	Ariadne-like ubiquitin ligase	Solgenomics
6:39320133|39320546	Solyc06g061200	Glycine-rich protein	Padmanabhan *et al*., 2019
6:39320160|39320804	Solyc06g061200.1	Glycine-rich protein TomR2	Padmanabhan *et al*., 2019
7:67566489|67566691	Solyc07g065840.2.1	Heat shock protein 90	TGRD
9:66931468|66931629	Solyc09g074680.2.1	Cullin 1B (ubiquitin-protein ligase activity)	Solgenomics
9:69558474|69566030	Solyc09g084465.1	Wound-induced proteinase inhibitor 1	Fan *et al*., (2019)
11:40246227|40246508	Solyc11g040050.2	TBP-associated factor 15	TomAP

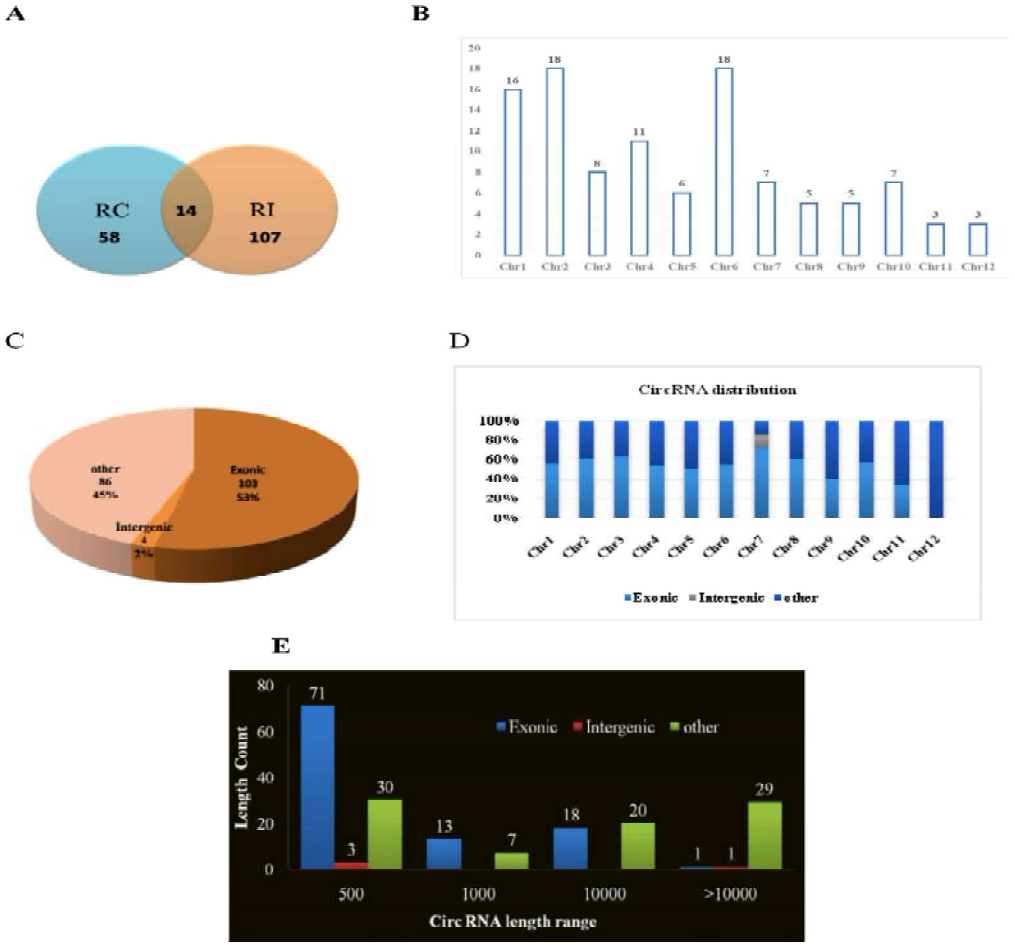

Fig. 1. Identification and characterization of CircRNAs in response to ToLCBaV in a resistant tomato genotype. (A) Number of CircRNAs identified in RC and RI. (B) CircRNAs distribution on each chromosome. (C) The number and percentage of CircRNAs originated from exon, intergenic and other genomic regions. (D). Percent distribution of exonic, intergenic and other CircRNAs across chromosomes. (E) Classification of CircRNAs based on length range.

and its CircRNA (up to 5.63 log_2FC) was significantly higher during early stages of infection whereas, gene. HSP 90 (up to 14.92 log_2FC) and its corresponding CircRNA (up to 7.17 log_2FC) expression was higher during later stages of viral infection (Nine and 21 DPI) (Fig. 2). While, the gene POX relative expression was up to 5.34 log_2FC and that of its CircRNA was up to 8.38 log_2FC (Fig. 2).

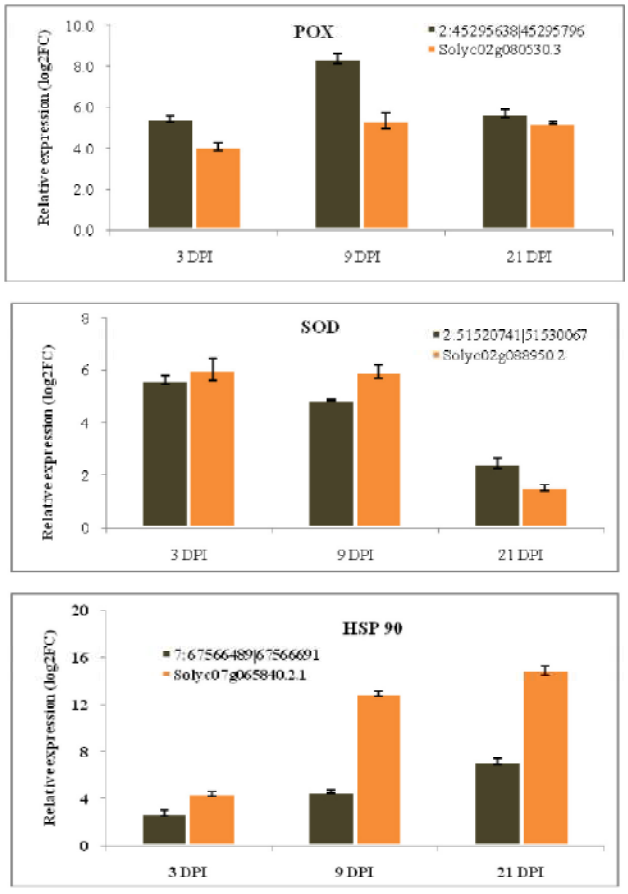

Fig. 2: Expression profiling of ToLCBaV resistant genotype for CircRNAs and their corresponding parent genes at different days post infection. The data were normalized to Elongation factor-1, presented as the means (Standard error, n = 3, three biological replicates, correlation coefficient r=0.89, 0.61 and 0.97 for HSP 0, POX, and SOD respectively).

The enzyme analysis done to support the CircRNAs data showed that ToLCBaV infection had significantly altered the enzymatic activity of POX and SOD in resistant tomato (Fig. 3). The uninfected tomato plants had lower POX and SOD activity compared to those infected plants in all five intervals and this difference was probably due to the presence of the virus. In infected samples, the POX activity was highest at 9 and 21 DPI followed by both five and 15 DPI, while the SOD activity was significantly higher at three and five DPI followed by nine and decreased at 21 DPI. The difference in SOD and POX activity between the intervals to be more pronounced was likely due to the presence of virus. These relative expression results were in accordance with the enzyme analysis of both SOD and POX at respective day intervals and provide some insights into the important role of CircRNAs association with antioxidant enzymes in disease response against ToLCBaV in resistant plant. These findings point out to a possible functional role for CircRNAs in plant defence against viral infection.

CircRNAs are covalently closed non-coding RNA molecules. They predominantly comprise of exonic sequences and are spliced at canonical splice sites and were first discovered in humans and mouse, although they are found in all eukaryotes (Salzman, 2016). Use of high-throughput sequencing technologies and *in silico* analyses, have reported the CircRNAs-mediated gene regulation in plant immune system (Litholdo *et al*., 2018). In tomato plants, CircRNAs identification have been performed on tomato fruit ripening (Yin *et al*. 2018), tomato fruit coloration (Hong *et al*., 2020), fruit pigment accumulation (Yang *et al*., 2020), responsive to *Phytophthora infestans (*Zhou *et al*., 2020), TYLCV infection and tomato leaves responding to multiple stresses of drought and heat (Tan *et al*., 2017) and also low temperature treatments (Yang *et al*. 2020). In this study, identification of CircRNAs in tomato genotype resistant to ToLCBaV was examined and their functions in response to virus infection process are discussed.

In our study, a total 121 CircRNAs were generated from diverse genomic regions across all chromosomes in response to viral infection. Our result showed that Chr 1, 2, 4 and 6, had a greater number of induced CircRNAs (Fig. 1B). Few of them were involved in defence response (Table 2). Similar results were found where chromosome 01 had the most CircRNAs from susceptible tomato in response to TYLCV (Wang *et al*. 2018) and in response to multiple stresses of drought and heat (Zhou *et al*., 2020). Chr. 4 and Chr. 6 harbour TYLCV resistance loci Ty-5 (Hutton *et al*., 2012) and Ty-1/3 respectively (Dong *et al*., 2016) and

induced CircRNAs on Chr. 4 and 6 might be having the regulatory roles on these resistance genomic regions. CircRNAs are mainly located at exons of genes, but scarcely distributed at introns or intergenic regions (Zuo *et al*. 2016; Yang *et al*. 2020). Similar pattern was observed in our experiment that CircRNAs were generated from various genomic regions and out of 193 total CircRNAs identified from both RC and RI, 103 (53 %) were from exonic region (Fig 1C). Similar trend was observed when the CircRNAs (62 % from exonic region) were analysed in susceptible tomato (Wang *et al*. 2018).

The expressions of exonic CircRNAs were significantly correlated with the expressions of parent genes (Ye *et al*., 2015; Wang *et al*., 2018). In our study, we observed a significant positive correlation between relative expression of selected CircRNAs and their parent genes (correlation coefficient r=0.89, 0.61 and 0.97 for HSP 0, POX, and SOD respectively) (Fig. 2). HSP90 function through 26S proteasome mediated proteolytic machinery in eukaryotic cells (Sadanandom *et al*., 2012). Due to decrease in the degradation of the TYLCV protein V2 by the 26S proteasome, silencing of HSP90 led to enhanced accumulation of TYLCV CP and DNA levels as infection develops (Moshe *et al*., 2016). There is a significant positive correlation between 7:67566489|67566691 CircRNAs and its parent gene Solyc07g065840.2.1 (HSP90) (Fig. 2). TYLCV infection enhances defence mechanism through the activity of the antioxidant's enzymes, *i.e.,* SOD, CAT, PPO and POX in tomato (Dieng *et al*., 2011; Sofy *et al*., 2017). The corresponding biochemical activity of POX and SOD was in similar trend with the CircRNA expression across different intervals after ToLCBaV infection (Fig. 2 and 3).

CircRNAs in plants are differentially expressed both spatially and temporally in plants, acting as important functional modulators involved in biological processes (Pan *et al*., 2018; Wang *et al*., 2016; Zhou *et al*., 2017). CircRNAs (Slcirc017 parent gene) regulated TYLCV infection in susceptible plant and the silencing of its parent gene (Solyc01g080200.2) resulted in decreased TYLCV virus accumulation (Wang *et al*., 2018). In this study also differential expression of CircRNA parent genes (*Solyc02g088950.2* and *Solyc02g080530.3*) was observed between control and

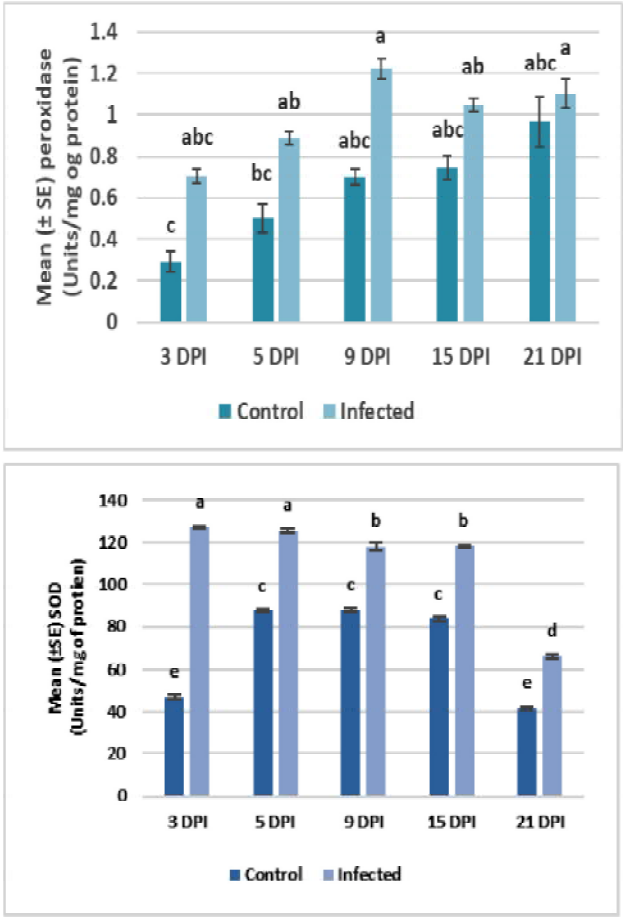

Fig. 3: Response of SOD and POX activity in IIHR-2611 to ToLCBaV infection. Bar charts of different colour and with different letters are significantly different ($P < 0.05$, $F = 1.702$, $df = 18$, $P = 0.021$ for POX and $F = 3.75$, $df = 18$, $P = 0.021$) based on Tukey's HSD test. Data presented as means ± SE (n = 3)

infected conditions at different intervals (Fig. 2). The results indicate that the CircRNAs- 2:51520741|51530067 and 2:45295638|45295796 positively influence the plant response to disease through genes involved in ROS scavenging enzymes (SOD and POX) providing some insights into the role of CircRNAs in association with antioxidant enzymes against ToLCBaV. The increasing activity of peroxidase after virus infection (Fig. 2 and 3) might be due to structural defence of peroxidase which was known to perform polymerization, suberization, cell wall elongation, controlling virus multiplication and wounding (Bahar *et al*., 2020). TYLCV infection enhances the activity of SOD and POX and further these enzymes activate the plant defence mechanisms (Dieng *et al*., 2011; Sofy *et al*., 2017).

CircRNAs analysed in this study might be acting as miRNA sponges and functioning through miRNA involved regulatory pathways or any of the other transcriptional, translational and posttranslational regulation mechanisms mentioned above. Furthermore, the precise mechanism by which CircRNAs regulate parent gene expression need to be investigated by identification of miRNA targets for the parent genes, in order to determine whether CircRNAs acting via miRNA mediated pathway or CircRNAs directly acting as parent gene regulator at transcription and translational level. The association between CircRNAs and interacting miRNAs was induced using the rice transgenic plants developed using agroinfection of rice calli with CircRNAs expression cassette (Sharma *et al*., 2021). There are various methods like artificial miRNA-mediated CircRNA knockdown, gain-of-function study, full-length CircRNA identification followed by CircRNA-protein interaction (Feng and Yu, 2021) to study and characterize the biological function of identified CircRNAs.

CONCLUSION

CircRNAs are emerging as a key player in RNA mediated gene regulation, having roles in several biological processes at both transcriptional and posttranscriptional stages. Many new studies on CircRNA profiling to diverse stresses found that the exonic CircRNAs positively regulates the expressions of their parent genes. This is the first report on CircRNAs for ToLCBaV resistance and we found a positive correlation between few CircRNAs and their parent genes. We hypothesised that these circRNAs must be acting as miRNA sponges and as regulators of miRNA mediated pathways in positively regulating their parental genes or acting as regulatory check points at transcription and translational level of parent gene expression. CircRNAs mediated regulation of some of their parent genes were found to be involved in host defence against viral disease. Further, research on understanding precise mechanism of these CircRNAs during viral infection and resistance against virus would help to identify target specific CircRNAs in plant viral disease resistance.

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Original Research Paper

Taxonomic revision of the cultivated species of *Mimusops* (Sapotaceae) in Egypt, with new records

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ABSTRACT

During the process of updating horticultural records of this genus in Egypt, five problems were identified: lack of publications, lack of clarity between species, numerous errors of identifications, loss of earlier documented records of identity, as well as, the introduction and cultivation of new plants during the 19th Century added to the complexity of the problem. In this study, the taxonomic aspects of genus *Mimusops*, were thoroughly studied to identify the most reliable characters for taxon delimitation. Our assessment was based on morphological characters representing habit, leaves, petioles, flowering pedicels, buds, floral parts, fruit and seed. Fieldwork have revealed the presence of four species, of which *Mimusops kummel* and *M. zeyheri* are new records. The latter species is represented in Egypt by *M. zeyheri* var. *laurifolia*. This variety has been neglected by many authors. Additionally, *Mimusops elengi* L. was believed to be cultivated in Egypt, but no materials have been encountered that could confirm it. The specimens earlier identified as *M. elengi* actually belong either to *M. kummel* or to *M. laurifolia.* A detailed description of the genus and species with photographs, an identification key, and synonymy for each taxon are provided.

Keywords : Cultivated - species, new records, *Mimusops*, Sapotoideae, Sapoteae, Taxonomy

INTRODUCTION

Sapotaceae Juss. (1789) is a well-marked family characterized by its well-developed system of latex; two-armed, unicellular trichomes; often coppery leaves beneath; axillary, ramiflorous or cauliflorous inflorescence; simple or complex flower structure; oppositipetalous stamens; and sometimes with staminodes. It is a woody tropical and subtropical family, and is represented by 59 genera with 1250 species, of worldwide distribution. [Pennington, 1991; Govaerts *et al*., 2001; Swenson *et al.*, 2007; Gautier *et al.* 2013]. *Sapotaceae* is a member of *Ericales* (APG III, 2009), sister to Ebenaceae and Primulaceae (Rose *et al.* 2018).

Recent molecular analyses (Swenson & Anderberg, 2005; Smedmark *et al*., 2006), place *Mimusops* L. in the tribe *Sapoteae* (=Mimusopeae sensu Pennington, 1991) of subfamily *Sapotoideae*; while *Sapoteae* s.str. only includes subtribe *Mimusopinae* and *Manilkarinae* (with the exception of *Northia*) proposed earlier by Pennington (1991). Moreover, *Sapoteae* has been circumscribed as monophyletic, with the monophyletic *Mimusops* (Smedmark et al., 2006, Gautier *et al.*, 2013). *Mimusops* is a palaeotropical genus (with exception of *M. elengi* in Australia), with 47 species (Govaerts *et al.*, 2001). Members of *Mimusops* are unarmed trees and shrubs, and diagnosed by minute caducous stipules; complex flower structure with biseriate calyx of 4 sepals each; corolla-lobes 8, always 3-segmented (one erect median clasping the opposing stamen and two laterals attached dorsally); stamens 8, in one whorl; staminodes 8 , well developed, alternating with the stamens, hairy, inflexed and often forming a sheath round the gynoecium; ovary 8-loculed; fruit 1-6-seeded; seeds generally with shiny brown testa, and with a small, basal to basi-ventral seed-scar. In ancient Egypt, *Mimusops laurifolia* was considered as a sacred tree. The twigs were used at funerals and have been found in the Egyptian tombs with mummies. In the tomb of Djoser (3rd Dynasty, Saqqara), the fruits of Persea were deposited as offerings; while in the tomb of Tutankhamun (18th Dynasty, Luxor), the leaves and twigs were used in making funerary garlands and floral

bouquets (Täckholm, 1951; Darby *et al.*, 1977; Friis 1981).

For Egypt, a relatively few short accounts of *Mimusops* have been published (Bircher, 1960; Diwan et al., 2004; Hamdy *et al.*, 2007; Youssef *et al.*, 2012; Youssef & Hamdy, 2013; Gamal, 2018). Table (1) summarizes the available information about the genus in Egypt. It is clear that different authors have treated differently the cultivated species. This has led to a considerable confusion in species identifications. The introduction and cultivation of new plants in Egypt during 19th Century has added to the complexity of this problem.

Schweinfurth (1883) recorded one species: *Mimusops schimperi* Hoscht. ex A. Rich. [currently treated as *M. laurifolia* (Forssk.)Friis] from Egyptian tombs. He identified the evergreen folded leaves frequently found with Egyptian mummies. Later, *M. laurifolia* has appeared regularly in the Egyptian texts. Delchevalerie (1899) and Sickenberger (1901) reported the occurrence of *M. elengi* L. in the gardens of El Rhodah and Cairo respectively. This has been followed by later investigators and has appeared regularly in the Egyptian literature (Bircher, 1960; Diwan *et al.* 2004; Hamdy *et al.* 2007; Hamdy, 2010; Youssef & Hamdy, 2013). In the study of the Gardens of the Hesperides, Bircher (1960) cultivated eleven species of *Mimusops* s.l. in Al-Saff Garden. This includes species with 3-merous flowers (now included in Manilkarinae); while only three are conspecific to *Mimusops*. These are: *M. elengi*, *M. caffra* (a new addition), and *M. laurifolia* (=*M. schimperi*). She stated that all the species were cultivated in her garden. Today, Al-Saff Garden has been vanished due to urbanization and land degradation. Recently in Diwan *et al*. (2004), the number of species had been reverted to only two (*M. caffra* and *M. elengi*) with short notes in arabic. Hamdy *et al*. (2007) listed the plant distribution of three species in three historic gardens in Egypt (Zohriya, Orman, and Zoo). They reported *Mimusops elengi*, *M. laurifolia*, and *M. caffra* in Orman; *M. elengi* and *M. laurifolia* in Zohriya; and *M. laurifolia* in the Zoological Garden. Hamdy (2010) reported three species in Aswan, of which *Mimusops kummel* Bruce ex A.DC. had been added. However, *M. kummel* sensu Hamdy was actually *M. laurifolia.* Both of Youssef *et al.* (2012) and Youssef & Hamdy (2013) listed three species which were previously recorded; while Gamal (2018) only recorded *M. laurifolia*.

The aim of the present study is to update the list of the cultivated species of the genus *Mimusops* in Egypt; to provide an identification key for these species; to study thoroughly the taxonomic parameters for taxon delimitation; and to give a detailed description with photographs, and synonyms for each taxon. These data are presented for the first time.

Table 1 : Historical review of *Mimusops* species cultivated in Egypt. (+=present, -=absent, ×=present but cited under a synonym, *= a new record).

Taxa	Schweinfurth (1883)	Delcheveralerie (1899)	Sickenberger (1901)	Täckholm (1951)	Bircher (1960)	Diwan *et al.* (2004)	Hamdy *et al.* (2007)	Hamdy (2010)	Youssef et al. (2012)	Youssef & Hamdy (2013)	Gamal (2018)	Present study (2020)
M. elengi L.	-	+	+	-	+	+	+	+	+	+	-	-
M. caffra E. Mey. ex A.DC.	-	-	-	-	+	+	+	+	+	+	-	+
M. kummel Bruce ex A.DC.	-	-	-	-	-	-	-	-	-	-	-	*
M. laurifolia (Forssk.)Friis	×	-	-	×	×	-	+	×	+	+	+	+
M. zeyheri Sond. var. *laurifolia* Engl.	-	-	-	-	-	-	-	-	-	-	-	*
	1	1	1	1	3	2	3	3	3	3	1	4

MATERIALS AND METHODS

In this study, morphological data were scored from examination of Egyptian herbarium specimens, digital photographs of the authentic material kept in BM, BR, C, FT, K and LG; examination of fresh material collected during conducted investigations; and the contribution sources of Heine (1963), Meeuse (1963), Hemsley (1968), Friis (1981, 2003, 2006), Kupicha (1983), Pennington (1991) and Govaerts *et al*. (2001).

The herbarium study was based on the examination of specimens kept in the major Egyptian herbaria (CAI, CAIM, MAZHAR, Orman, and Aswan) [acronyms follow Holmgren *et al*., 1990].

Living material included those collected during investigations in different botanical garden of Cairo [Egyptian Museum, El-Nahr and El-Zohriya]; Giza [National Gene Bank (NGB), Mazhar, Orman, and the Zoological gardens]; and Aswan [Plant Island]. These investigations were performed to obtain fresh material for the in vivo study of the vegetative, floral, and fruit characters; for preparing exsiccate; and to make field observation in several localities. Voucher specimens have been deposited in the Cairo University Herbarium (CAI). The examined representative specimens were geographically arranged according to the phytogeographical territories of Egypt proposed by El Hadidi (2000: 14-22). Localities and collectors are given in Appendix 1.

For each species, nomenclature, authentic type specimens and synonyms are given with photographs of fresh material, as well as, phenology using the information from herbarium labels and investigations. Type and authentic material seen by the authors are followed by (!). Citation of the authors follows Brummitt and Powel (1992).

RESULTS AND DISCUSSION

I. The genus Mimusops L. in Egypt

The present work resulted in a total number of four cultivated species, of which *Mimusops kummel* Bruce ex A.DC. and *M. zeyheri* Sond. are new additions. Today, both are only cultivated in El-Zohriya Garden. This is attributed to the introduction and cultivation of new plants in our Egyptian gardens. *Mimusops zeyheri* is a polymorphic taxon, in which two varieties are currently accepted: var. *zeyheri* and var. *laurifolia* Engl. (Engler, 1904). The relationship of staminode length to its stamens and corolla lobes was successfully employed for the authors to recognize *M. zeyheri* var. *laurifolia* Engl. in Egypt. All herbarium specimens, identified by earlier investigators as *M. elengi* have been re- identified either as *M. laurifolia* or as *M. kummel*. Therefore, the occurrence of *M. elengi* in Egypt has not been supported by this research.

II. Taxonomy

Mimusops L., Sp. Pl., ed., 1: 349 (1753) - Gen. Pl., ed.5, 165 (1754).

Synonyms:

Elengi Adans., Fam. Pl.2: 166 (1763).

Binectaria Forssk., Fl. Aegypt.-Arab. 82 (1775). Type: *B. laurifolia* Forssk.

Phlebolithis Gaertn., Fruct. Sem. Pl. 1:201 (1788). Type*: P. indica* Gaertn.

Imbricaria Comm. ex Juss., Gen. Pl. 152 (1789). Type: *I. barbonica* J.F.Gmel.

Radia Noronha, Verh. Batav. Genootsch. Kunsten 5(4):3 (1790). "nom. nud.

Mimusops sect. Quaternaria A.DC.in DC., Prodr. 8: 202 (1844).

Mimusops sect. Imbricaria (Comm. ex Juss.) Hartog, J. Bot.17: 358 (1879).

Semicipium Pierre, Not. Bot. 10 (1890). Type: S. *boivinii* Hartog ex Pierre.

Kaukenia Kuntze, Revis. Gen. Pl. 2:406 (1891).

Mimusops subgenus *Imbricaria* (Comm. ex Juss.) Engl. in Engl. & Prantl, Nat. Pflanzenfam. 4(1):142 (1891).

Description : Unarmed trees or shrubs, with abundant to scarce milky latex. **Young vegetative parts** commonly adpressed-pubescent with ferruginous, brownish, cinereous or yellowish indumentum, later glabrous or glabrescent. **Leaves** thinly to firmly coriaceous, simple, alternate or spirally arranged, clustered at the end of branches or not, with brochidodromous venation, and with ± thickened paler margin; petiolate or subsessile; stipules minute and caducous. **Flowers** bisexual actinomorphic, 1-4(-8), borne in the axils of persistent or fallen leaves, sometimes on brachyblasts (short, stout spur shoots), pedicelled; pedicel shorter to longer than the petiole, not or slightly accrescent in fruit, adpressed-pubescent. **Calyx** of 8 sepals; sepals in two dissimilar whorls of

4, free or slightly connate at base, persistent, not or slightly accrescent in fruit, hairy on both surfaces but less internally; outer sepals valvate; inner narrower, and paler in colour. **Corolla** gamopetalous, white, cream, yellowish, brownish or pink, frequently as long as calyx or slightly longer, rarely shorter; corolla-tube frequently much shorter than the lobes; corolla-lobes 8, 3-segmented; median segment entire, erect and clasping the stamen, sometimes incurved against the gynoecium; the two lateral segments shorter to longer than the median segment, entire (undivided) or further divided. **Stamens** 8, opposite to the corolla lobes, in one whorl, inserted at the top of the corolla-tube and adnate to it; anthers relatively large, extrorse, apiculate, frequently hairy and longer than the filaments; filaments free or partly fused with the staminodes; **staminodes** 8, well developed, alternate with the stamens, simple, with entire or dentate apex, commonly inflexed and connivent to form a conical sheath concealing the gynoecium, densely pilose dorsally and along margins. **Ovary** ovoid to globose or cylindrical, hairy, 8-loculed; locules uniovulate; style slender, exserted from the floral parts or not, glabrous or with few scattered hairs. **Fruit** indehiscent, baccate, fleshy to rather leathery, ovoid to ellipsoid or globose, with persistent calyx at the base and remnants of the style at the apex, 1-6-seeded; **seeds** laterally compressed, with hard and shining testa; attachment scar small, basal or basi-ventral, circular or ellipsoid; embryo with copious endosperm, and thin foliaceous cotyledons.

A paleotropical genus, with the exception of *M. elengi* in Australia. It comprises 47 species, with 25 species in Africa; 15 in Madagascar and the Comoros; 3 in the Mascarenes; one in Seychelles; and 3 in the Indo-Pacific (one in Asia through Malesia to the Pacific, one in Andamans Islands, and one in Sri-Lanka) [Govaerts *et al.*, 2001].

Key to *Mimusops* species cultivated in Egypt:

1a. Leaves firmly coriaceous, obcordate, cuneiform or obovate, 2-7 cm long, lateral veins 5-8 pairs, margin revolute; petiole 0.5 - 1cm long; bole twisted, seldom straight……**1. *M. caffra***

b. Leaves coriaceous to thinly coriaceous, may be obovate, never obcordate or cuneiform, (4 -) 7-14.5 cm long, lateral veins 10–25 pairs, margin unrevolute; petiole 1 –5 cm long; bole straight 2

2a. Flowering pedicels (1.5–) 2–4 cm long, longer than petiole; flower buds 7–10 mm long, 2.0–2.5 times as long as broad, acute; calyx 10 –12 mm long; corolla 9 –12 mm long; stamens 5 –6 mm long; gynoecium 12–15 mm long; style 10–12 mm long, distinctly exserted; leaves at least 5 times as long as the petiole, lateral veins 15 –25 pairs..... **2. *M. Kummel***

b. Flowering pedicels 1.0–2.0 cm long, shorter than petiole; flower buds 3–7 mm long, 1–2 times as long as broad, blunt to rounded or acute; calyx 5–10 mm long; corolla 6–9 mm long; stamens 2–5 mm long; gynoecium 4–11 mm long; style 2–9 mm long, included or slightly exserted; leaves up to 5 times as long as the petiole, lateral veins 10–15 pairs.....3

3a. Leaves clustered at the end of branches; flower buds blunt or rounded, 3-5×3-4 mm, 1.00–1.25 times as long as broad; corolla-tube 4 mm long, longer than corolla-segments; lateral segments irregularly incised; stamens c.2 mm long; gynoecium 4-5 mm long; style 2-3 mm long, included, hairy below; fruiting calyx reflexed; fruit green when ripe, 1-4 seeded; seed scar basi ventral, circular; bole buttressed..... **3. *M. laurifolia***

b. Leaves not clustered; flower buds acute, 5–7 × 3–5 mm, 1.5–2 times as long as broad; corolla-tube 1–2 mm long, shorter than corolla-segments; lateral segments entire; stamens 4 –5 mm long; gynoecium 7–11 mm long; style 5–9 mm long, included or slightly exserted, glabrous; fruiting calyx clasped to the fruit or spreading; fruit brittle yellow or orange when ripe, 1–seeded; seed scar basal, oblate; bole not buttressed...4. ***M. zeyheri***

1. *Mimusops caffra* E. Mey. ex A. DC. in DC., Prodr.8: 203 (1844) "Coast red milkwood"

Type: Southern Africa (Eastern region): Pondoland or Natal. Between Umtentu and Um Zimkulu Rivers, 1837, *Drège J.F. s.n.*, K000435321, isotype, (K-image!).

Homotypic synonym:

≡ *Kaukenia caffra* (E. Mey. ex A.DC.) Kuntze, Revis. Gen. Pl. 2:406 (1891).

Heterotypic synonym:

=*Mimusops revoluta* Hochst. apud Krauss, Flora 27: 825 (1844). Type: Southern Africa: Port NATAL. In woods on the dunes near Durban, 1840, *Krauss F*. 76, K000435320, isotype, (K-image!).

Description: Much branched, shrub or small- to medium-sized tree, up to 15 m high, with milky latex, and rounded crown; bark dark grey, rough, shallowly and longitudinally fissured, wrinkled; bole seldom straight, often twisted, and not buttressed. **Young vegetative parts** densely adpressed-pubescent, with yellowish-brown to ferruginous indumentum, soon glabrescent. **Leaves** firmly coriaceous, not clustered, petiolated; blade obovate, obcordate or cuneiform, 2.0–7.0 cm long, 1.0–4.0 cm wide, 2–2.5 times as long as broad; apex retuse, emarginated or rounded; base acute; margin yellowish, thickened, and revolute; upper surface glaucous, glossy, with yellowish-brown to ferruginous hairs when young, soon glabrous; lower surface pale green, mat, adpressed-pubescent, with persistent fulvous indumentum along the midrib; midrib prominent below and flush above on both surfaces; lateral veins 5– 8 pairs; petiole canaliculate towards the leaf base on upper surface, dilated at the base, 0.5–1.0 cm long, shorter than the leaf blade (leaf blade = 5– 15 x petiole), adpressed-pubescent, with ferruginous indumentum. **Inflorescence** (1–) 2–3(–4)–flowered; flower 10–20 mm in diam.; flower buds 8–10×3–5 mm, 2–3 times as long as broad, acute, pedicelled; pedicel erect to ascending or commonly deflexed, dilated at apex, 20–30 mm long, not accrescent in fruit, sulcate when dry, longer than the petiole (pedicel= 3–4 × petiole), densely adpressed-pubescent, with fulvous indumentum. **Calyx** 8–10 mm long; calyx-tube c. 1.0 mm long; outer calyx lobes lanceolate, 8–9×2 –3 mm, with a dorsal midvein ceases below the acute apex, and with 1–2 lateral veins along the midvein on each side, densely adpressed-pubescent with fulvous indumentum outside and inside (less inside); inner calyx-lobes shorter, paler, and narrower, deltoid lanceolate, 7–8×1 mm, with a prominent, dark brown, median dorsal groove, and with fulvous hairs. **Corolla** white or cream, as long as the calyx; corolla-tube 1–2 mm long; median segment lanceolate, 8–9 × 0.5–1.0 mm, pluri-nerved, with acute apex and involute margins; lateral segments 6–7 mm long, slightly shorter than the median segments, each shallowly to deeply divided into two narrowly deltoid laciniae. **Stamens** 5–6 mm long; filaments subulate, 2.0–2.5 mm long, hairy at base; anthers lanceolate, 3–4×1 mm, apiculate (apicula c. 0.5 mm long), hairy on and along the connective tissue; staminodes deltoid-lanceolate, 3–4 mm, shorter than the stamens and corolla-segments, acute, densely pilose outside (especially along the margins). **Gynoecium** 9–11 mm long; ovary ovoid to globose, with black brown colour at apex and base, 1.5–2.0 x 1.0–1.5 mm, pilose; style black or reddish-brown, slender, 7–9 mm long, as long as corolla or slightly exserted, sparsely hairy below; stigma c. 0.2 mm wide, hairy. Fruiting calyx clasped to the fruit; **fruit** orange-red or red when ripe, globose or subglobose to ovoid, 2.0–2.5 x 1.5–2 cm, 1.0–1.5 times as long as broad, with rotundate or shortly rostrate apex (beaked/pointed tip), usually crowned by the persistent style at least when young, 1-seeded, ovoid to ellipsoid, 10-15 x 7-9 x 5-7 mm, glabrous; testa brown, shiny, with basal ellipsoid seed scar. (Fig.1)

Phenology: Flowering season May-July.

Global distribution: Native to the coastal scrub of Southern Africa (Cape Province to Natal) northwards to Mozambique in South Tropical Africa.

Taxonomic note: Unfortunately, our specimens are without fruits. Therefore, characters of the fruit and seed are derived from authentic specimens and from literature of Meeuse (1963), Baehni (1965), and Kupicha (1983).

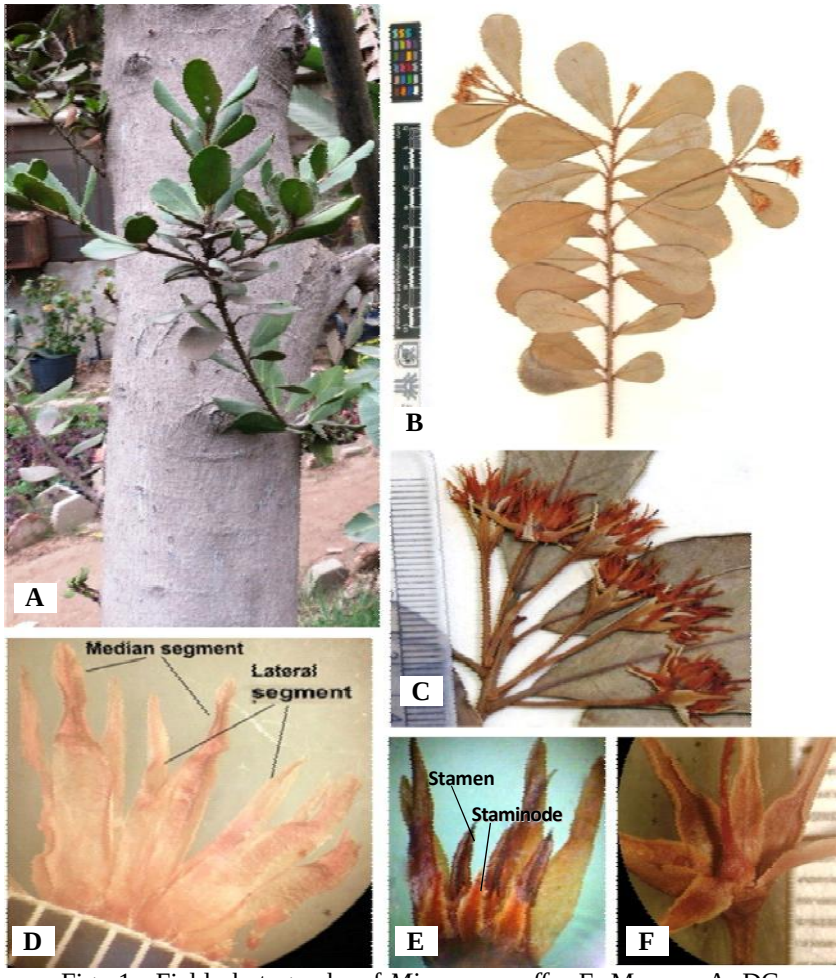
Uses: As ornamental tree.

2. *Mimusops kummel* Bruce ex A.DC. in DC., Prodr. 8: 203 (1844) "*Red milkwood*"

Type: Northeast Tropical Africa: Ethiopia. Tigre. In faucibus montium et ad declivia septentrionalia Montis Scholoda, 20June -31December 1837, *Schimper G.H.W.* 280, K000435270, lectotype by Hemsley, 1968 (K, image!).

Homotypic synonym:

≡ *Kaukenia kummel* (Bruce ex A.DC.) Kuntze, Revis. Gen. Pl. 2: 40 (1891).



Fig,. 1 : Field photographs of *Mimusops caffra* E. Mey. ex A. DC.

A: Trunk; B: Twig with leaves and inflorescences; C: Inflorescence; D: Corolla longitudinally sectioned and opened, ventral view; E: Corolla opened with 3 corolla-lobes, showing median and 2 lateral segments; F: Corolla and one sepal removed, showing gynoecium [Aswan Botanic Garden (Plant Island); *Abdelmohsen* s.n. (CAI)].

Heterotypic synonyms:

= *Imbricaria fragrans* Baker in Oliv., Fl. Trop. Afr. 3: 509 (1877). Type: West Tropical Africa: South Nigeria. Yoruba, s.d., *Barter* C. 1217: K000435260, holotype (K, image!).

=*Mimusops fragrans* (Baker) Engl. in Engl. & Prantl, Nat. Pflanzenfam. 4(1): 152, fig. 82 N-S (1891).

=*Binectaria fragrans* (Baker) Kuntze, Revis. Gen. Pl. 2: 406 (1891).

=*Mimusops djurensis* Engl., Monogr. Afr. Pflanzenfam. 8: 75, tab. 30/B (1904). Type: Northeast Tropical Africa: Sudan. Seriba Ghattas, im lande der Djur Reise nach central Africa & Sudan, 11 April 1869, *Schweinfurth G.* 1379, K000435271 & *Schweinfurth G.* 2428, K000435273, isosyntypes, (K, images!).

=*Mimusops langenburgiana* Engl., Monogr. Afr. Pflanzenfam. 8: 70, tab. 28/D (1904). Type: East Tropical Africa: Tanzania. Rungwe District, near Tukuya [Langenburg], s.d., *Goetze S.G.*864, BR0000006282295, isotype, (BR, image!).

= *Mimusops stenosepala* Chiov., Atti Reale Accad. Italia, Mem. Cl. Sci. Fis. 11: 47 (1940). Type: Northeast Tropical Africa: Ethiopia. Neghelli, poscoli, 16 March 1937, *Senni L.* 1015, FT 002542, holotype (FT, image!).

Description: Small-to large-sized evergreen tree, up to 25 m high, with milky latex; crown much branched; bark dark grey, longitudinally fissured and wrinkled;

bole straight, not buttressed. **Young vegetative parts** densely adpressed-pubescent, with ferruginous hairs, soon glabrescent. **Leaves** coriaceous, not clustered, broadest part at the middle or above and petiolated; blade elliptic to oblong-elliptic, ovate-oblong or obovate (5.0-)7.0-14.5 cm long, 2.0-5.0 cm wide, 2-4 times as long as broad; apex frequently acuminate to cuspidate or acute, rarely emarginated or obtuse; base acute or cuneate; margin entire; upper surface dark green, glossy, densely adpressed-pubescent, with ferruginous indumentum, later glabrous; lower surface paler, mat, with persistent ferruginous hairs along the midrib; midrib prominent on both surfaces; lateral veins 15-25 pairs; vein reticulation obscure above, obscure or slightly raised beneath; petiole canaliculate, sulcate when dry, 0.5-2.0(-2.5) cm long, much shorter than the leaf blade [blade = 5-9 × petiole], densely adpressed-pubscent, with ferruginous indumentum. **Inflorescence** (1 -)2-4–flowered; flowers 10 -13 mm in diam.; flower buds 7-10 × 4-5 mm, 2-2.5 times as long as broad, acute, pedicelled; pedicel erect to ascending, or commonly recurved, slender, (15 -) 20-40 (-50, not in ours) mm long, longer than the petiole [pedicel= (1.5-) 2-3×petiole), densely adpressed-pubescent, with ferruginous hairs. **Calyx** 10-12 mm long; calyx-tube c. 1 mm long; outer calyx-lobes lanceolate, 10-11 × 2-3 mm, with acute apex, paler margin, and densely pubescent with ferruginous hairs on both surfaces (less hairy inside especially at the darker base); inner calyx-lobes slightly shorter and narrower, 9 –10 × 1–2 mm, acute, with brown dorsal groove, with paler hairs. **Corolla** white or cream, 9-12 mm long, as long as calyx or slightly shorter; corolla-tube 1–2 mm long; median segment oblong-elliptic or lanceolate, 9-10×2.0 mm, with 2-3 pairs of prominent lateral veins along the prominent midvein, apex acute to obtuse; lateral segments 8–10 mm long, as long as the median segments or slightly shorter, entire or often divided into 2 -3 lanceolate-linear laciniae. **Stamens** 5-6 mm long; filaments reddish-brown, 2.0-3.5 mm long; anthers lanceolate, 2.5-4.0 × 1.0-1.5 mm, hardly longer than filaments, with an apiculate apex (apicula 0.3–0.5 mm long), hairy on and along the connective tissue; staminodes deltoid lanceolate or lanceolate-linear, 4-6(-7) mm long, gradually or abruptly narrowed above into an acute apex with an extended, paler and glabrous tip (1-2 mm long), frequently as long as the stamens or shorter, rarely slightly longer, shorter than the corolla-segments, externally densely pilose throughout except the extended apex. **Gynoecium** 12–15 mm long; ovary ovoid, 2–3 × 1–2 mm, densely covered with ferruginous hairs; style reddish-brown, slender, 10-12 mm long, distinctly exserted, glabrous; stigma 0.2–0.3 mm wide, hairy. Fruiting calyx clasped to the fruit; **fruit** yellowish-orange or orange- red, ellipsoid to ovoid, 2.0 –2.5 × 1.0–1.5 cm, 2.0–2.5 times as long as broad, with acute to obtuse or commonly with shortly rosrate/beaked apex, usually crowned by the persistent style at least when young, glabrous, 1-seeded; **seed** ellipsoid, laterally compressed, 18–20 × 9 ×7 mm; testa brown, shiny, with basi –ventral scar; scar ellipsoid, c. 3.5 × 2 mm, (Fig. 2).

Phenology: Flowering season May-July, October; Fruiting season: January, June, October.

Global distribution: It is widely distributed in Tropical Africa. It occurs in West and West-Central Africa (Ghana, Guinea, Ivory Coast, Nigeria, Togo, Central Africa, and Cameroon); Northeast Africa (Ethiopia, Somalia, and Sudan), East Africa (Kenya, Tanzania, and Uganda), and Malawi in South Tropical Africa.

Taxonomic notes: It is unique by its entire or divided lateral segments with lanceolate-linear laciniae, as well as the anthers hardly longer than filaments. In all our cultivated specimens, the staminodes are 4 –6(–7) mm long, frequently as long as stamens or shorter, rarely slightly longer.

Hamdy *et al*. (2010) reported the occurrence of *Mimusops kummel* from Aswan, but it was actually belong to *M. laurifolia* (Forssk.) Friis.

In Zohriya garden, the tree was initially mistaken for *M. elengi* L. Both species are within the same range of leaves (shape, size, apex, lateral veins, and petiole). However, indumentum colour, floral and fruit characters are diagnostic to distinguish both. In *Mimusops kummel*, the indumentum is with ferruginous hairs [vs. rufous hairs] along the midrib beneath, narrower (10-13 mm wide) flowers [vs. 15-20 mm wide]; longer (10-12 mm long) calyx [vs. 7-9 mm long]; shorter (1-2 mm long) corolla-tube [vs. 2-4 mm long]; entire or divided lateral segments [vs. entire]; longer (10-12 mm long) style [vs. 3-5 mm long]; narrower (1.0-1.5 mm wide) fruit [vs. 1.5-2.5 mm wide], and with an ellipsoid seed scar [vs. circular].

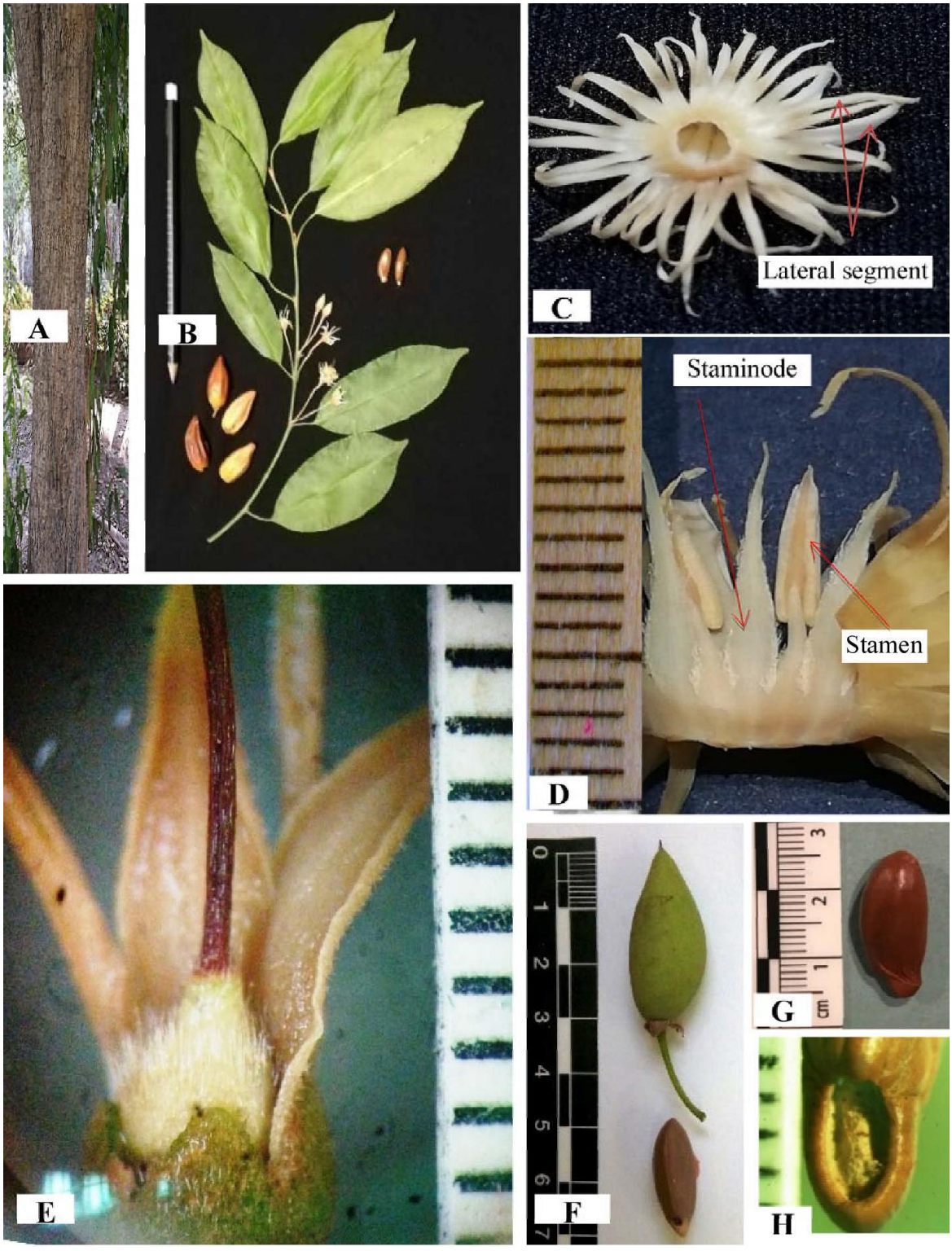


Fig. 2 : Field photographs of *Mimusops kummel* Bruce ex A. DC.

A: Trunk; B: Twig with leaves, inflorescences, fruits, and seeds; C: Lateral segments with its entire or divided laciniae, viewed from below; D: Corolla opened with stamens and staminodes, dorsal view; E: Corolla and most sepals removed showing gynoecium; F: Fruit (above) and seed (below); G: Seed, lateral view; H: Seed with seed-scar, ventral view [Zohriya Garden; *Abdelmohsen* s.n. (CAI)].

Uses: As ornamental tree

3. *Mimusops laurifolia* (Forssk.) Friis, Kew Bull. 35(4): 787, figs. 1-3 (1981) "*Persea*"

Type: Arabian Peninsula: Yemen. Bayt al Faqih "Beit el Fakih", 1763, *Forsskål P.* 359, C10001840 and *Forsskål P.* 360, C10001841, syntypes (C, images!).

Homotypic basionym:

≡ *Binectaria laurifolia* Forssk., Fl. Aegypt.-Arab.: CX No. 252,82, Cent III No. 54 (1775).

Heterotypic synonyms:

=*M. Kauki* Vahl, Symb. Bot. 1:27 (1790) [non L., 1753].

=*M. schimperi* Hochst. ex A. Rich., Tent. Fl. Abyss. 2:22 (1851). Type: Northeast Tropical Africa: Ethiopia. Tacazze; infra Dscheladscheranne, 09 December 1839, *Schimper W.G.* 697, LG0000090026638 (LG, image!), Isosyntype; Tigre; "Inter Dscheladscheranne et Selassaquilla", in convalle fluvii Tacazze, 01 May 1840, *Schimper W.G.* 873, K000435265, syntype (K, image!).

= *Mimusops kummel* sensu Hamdy, Gard. Hist. 38 (2): 276 (2010) [non Bruce ex A. DC., 1844].

Description: Small-to medium-sized, evergreen tree, up to 15 m high, with milky latex; crown rounded; bark brown, longitudinally fissured and wrinkled; bole straight and buttressed. **Young vegetative parts** densely adpressed-pubescent, with ferruginous indumentum, later glabrescent. **Leaves** subcoriaceous, densely clustered at the end of branches or on spurs (c. 2.0–3.0 cm long) if present (not in ours), broadest part at the middle or below and petiolated; blade elliptic to oblong-elliptic or lanceolate, 4.0–12.0 cm long, 2.0–5.0 cm wide, leaf blade 2–3 times as long as broad; apex acute or bluntly apiculate, rarely emarginate; base cuneate or obtuse; margin yellow, entire; upper surface dark green, glossy, densely adpressed-pubescent with ferruginous hairs, later glabrous; lower surface paler, mat, with persistent ferruginous hairs on and along the midrib; midrib prominent below and flush above on both surfaces; lateral veins 10–15 pairs; petiole pale in colour, canaliculated towards the leaf base on upper surface, sulcate when dry, 2.0–5.0 cm long, shorter than the leaf blade [blade = 2–4×petiole], adpressed-pubescent, with ferruginous indumentum. **Inflorescence** (1–)2–4–flowered; flowers 10–12 mm in diam.; flower buds 3.0–5.0 × 3.0–4.0 mm, 1.0–1.25 times as long as broad, with blunt or rounded apex, pedicelled; pedicel erect to patent, 10–20 mm long, accrescent in fruit (up to 2.5 cm long), shorter than the petiole (petiole = 2–3× pedicel), adpressed-pubescent with ferruginous hairs. **Calyx** 5.0–7.0 mm long; calyx tube c. 1.0 mm long; outer calyx-lobes green, lanceolate, 5.0–6.0 × 2.0–3.0 mm, with obtuse apex, and with white or paler stripe along the margin, adpressed-pubescent, with ferruginous hairs on both surfaces (less hairy inside); inner calyx-lobes paler, narrower, oblong-lanceolate, 4.0–5.0 × 2.0 mm, as long as or slightly shorter than the outer ones, dorsally with a distinct green median groove ceases below the ± mucronulate apex, hairy on both surfaces (less hairy inside).**Corolla** yellowish-white, 6.0–7.0 mm long, as long as the calyx or slightly longer; corolla-tube c.4 mm long, slightly longer than the corolla lobes; median segment narrowly spathulate or spathulate-oblong, 2.0–3.0 × 0.5–1.0 mm, with descending lateral veins along the midvein which ceases below the ± acute apex, and with uneven margin above; lateral segments c.3.0 mm long, as long as the median segments or slightly longer, each irregularly divided (incised) into narrowly oblong-lanceolate to linear laciniae. **Stamens** c. 2.0 mm long; filaments subulate, c. 0.5 mm long; anthers lanceolate, c. 2.0 × 1.0 mm, apiculate (apicula c. 0.2 mm long), hairy; staminode lanceolate-linear, c. 2.5 mm long, slightly longer than stamens, slightly shorter than the corolla-lobes or subequal, densely hairy throughout. **Gynoecium** 4.0–5.0 mm long; ovary globose or subglobose, ribbed, c.2.0 mm wide, hairy (more along ribs); style reddish, ± slender, included, 2.0–3.0 mm long, hairy below and glabrous above; stigma reddish, c. 0.3 mm wide, hairy. Fruiting calyx reflexed; **fruit** green, ovoid to ellipsoid, 3.0–4.0 × 2.0–2.5 cm, with acute or commonly with shortly rostrate (beaked) apex, glabrous, 1–4–seeded, 1.5–2 times as long as broad; **seeds** ellipsoid, laterally compressed, 17–20 × 10–12 × 10 mm; testa pale (toffee) or dark brown, shiny; scar small, basi-ventral, circular, 2.0–3.0 mm in diam, (Fig. 3).

Phenology: Flowering season: March-June, October; Fruiting season: June-August, October.

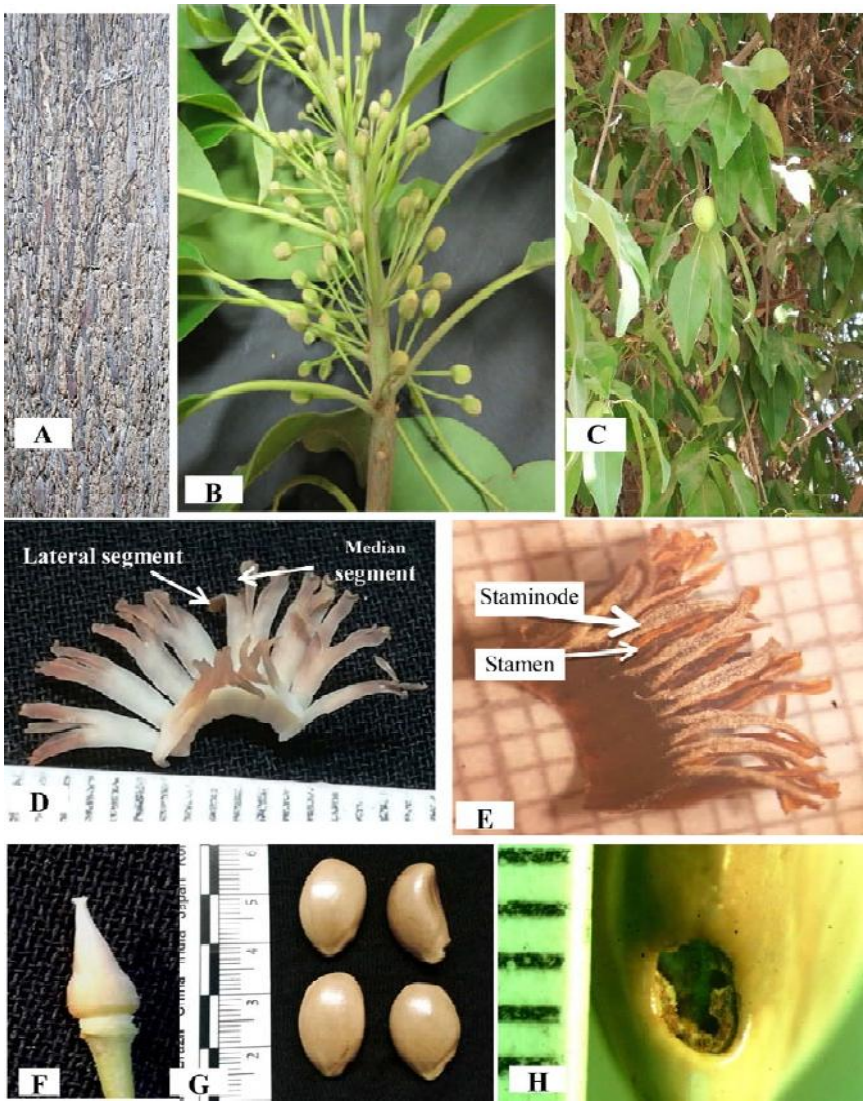


Fig. 3 : Field photographs of *Mimusops laurifolia* (Forssk.) Friis.

A: Trunk; B: Twig with leaves and inflorescence (flower buds); C: Twig with leaves, and fruit; D: Corolla opened with 3 corolla-lobes, showing median and 2-lateral segments; E: Corolla longitudinally sectioned and opened showing stamens and staminodes, ventral view; F: Gynoecium; G: Seed, lateral view; H: Seed with seed-scar, ventral view [Zoological Garden; *Abdelmohsen* s.n. (CAI)].

Global distribution: This species is restricted to the mountains around the Red Sea and the Gulf of Aden. It occurs in Northeast Tropical Africa (Djibouti, Ethiopia, Eritrea, and Somalia) and Arabian Peninsula (Saudi Arabia, N Yemen) in Temperate Asia. Records from Sudan (Northeast Tropical Africa), Uganda (East Tropical Africa), and Ancient Egypt are presumably due to introduction (Friis, 1981, 2003).

Taxonomic notes: In Orman (Giza) and Plant Island (Aswan), this species was regarded by earlier investigators as *Mimusops elengi* L. However, both species can be discriminated by floral and fruit characters. *Mimusops laurifolia* has blunt or rounded flower buds; lanceolate to oblong-lanceolate calyx-lobes with obtuse apex; shorter (6–7 mm long) flowers; corolla-tube longer than corolla-lobes; irregularly incised (divided) lateral segments; stamens with short (c. 0.5 mm long) filaments; short (c. 2.5 mm long) staminodes with acute apex; and green fruit with 1–4–seeded. In contrast, *Mimusops elengi* possesses acute flower buds; narrowly ovate calyx-lobes with acuminate apex; longer (8–12 mm long) flowers; corolla-tube shorter than corolla-lobes; entire

(undivided) lateral segments; stamens with longer filaments (1–3 mm long); longer (4–6 mm long) staminodes with toothed apex; and orange-red fruit with 1(–2)–seeded.

Uses: Fruit is edible; planted as shade or ornamental tree.

4. *Mimusops zeyheri* Sond., Linnaea 23: 74 (1850); Engl., Monogr. Afr. Pflanzenfam. 8:73 (1904). **"***Common red milkwood*"

Description: Small-to medium-sized, evergreen tree, up to 20 m high, with milky latex; crown much branched, spreading, and rounded; bark brown and reticulately fissured; bole straight, not buttressed. **Young vegetative parts** densely adpressed-pubescent, with ferruginous indumentum, soon glabrescent. **Leaves** coriaceous or thinly coriaceous, not clustered, broadest part at the middle, above or below and petiolated; blade elliptic to oblong-elliptic or obovate, sometimes lanceolate, (4–) 6–11 cm long, 2.0–5.5 cm wide, leaf blade 2–3 times as long as broad; apex acute to obtuse or commonly bluntly apiculate, rarely rounded or emarginate; base cuneate or acute; margin yellow, thickened, entire; upper surface dark green, glossy, with ferruginous hairs, later glabrescent, and with conspicuous vein reticulation; lower surface pale green, mat, with raised vein reticulation, more tardily glabrescent than above, with persistent ferruginous hairs on and along the midrib, indumentum sometimes becoming greyish; midrib prominent on both surfaces, lateral veins 10–15 pairs; petiole canaliculate, dilated at the base, 1.0–3.5 cm long, shorter than the leaf blade (leaf blade = 3–5 × petiole), adpressed-pubescent, with ferruginous hairs. **Inflorescence** 1–3 (–5, not in ours)–flowered; flowers 10–15 mm in diam.; flower buds 5–7 × 3–5 mm, 1.5–2.0 times as long as broad, acute or subacute, pedicelled; pedicel erect to ascending or commonly recurved, slender, sulcate when dry, 10–17 mm long, slightly accrescent in fruit, subequal or commonly shorter than the petiole (petiole = 1–3 × pedicel), adpressed-pubescent, with ferruginous indumentum. **Calyx** 7–10 mm long, not accrescent in fruit; calyx-tube c.1.0 mm long; outer calyx-lobes lanceolate or ovate-lanceolate, 8.0–9.0×3.0–4.0 mm, with paler margin, acute, dorsally ±3-nerved below, densely adpressed-pubescent, with ferruginous hairs outside and inside (less inside); inner calyx-lobes shorter, narrower, and paler, 6–7×2–3 mm, acute, dorsally with brown median groove, and with greyish-white hairs. **Corolla** white, 6-9 mm long, shorter to longer than calyx; corolla-tube 1–2 mm long; median segments elliptic to oblong-elliptic or lanceolate with a narrow basal attachment, 5–7 × 1 mm, acute or obtuse at the ± mucronulate apex, sometimes serrate-denticulate; lateral segments lanceolate, 4–6 mm long, entire (undivided), shorter to longer than the median segments, pluri–nerved, apex acute to obtuse or serrate-denticulate at the mucronulate apex. **Stamens** 4–5 mm long; filaments subulate, 1.5–2.5 mm long, glabrous; anthers lanceolate, 2.5–3.5×1.0–1.5 mm, with dark brown apiculate apex (apicula 0.3–0.5 mm long), hairy on and along the connective tissue; staminodes elongated deltoid to deltoid-lanceolate or lanceolate-subulate, either acute and shorter than stamens and corolla lobes or long acuminate-caudate and subequal or slightly longer than stamens and as long as the corolla lobes, densely pilose throughout outside except the paler tip. **Gynoecium** 7–11 mm long; ovary ovoid to globose, 2–3 × 1–2 mm, densely pilose; style yellowish, slender, 5–9 mm long, included or slightly exserted, glabrous; stigma reddish-brown, 0.3–0.5 mm in diam., hairy. Fruiting calyx clasped to the fruit or spreading; **fruit** green, turning into brittle yellow or orange when ripe, with orange pulp, ellipsoid to ovoid, 2.0–4.0 × 1.5–2.5 cm, 1.5–2 times as long as broad, with acute to obtuse or with shortly rostrate/beaked apex, usually crowned by the persistent style at least when young, glabrous, 1-seeded; **seed** obovoid, c. 18 × 9–10× 6–7 mm, glabrous; testa brown, shiny; scar basal, oblate (horizontal), 1.5 ×1.7– 2 mm. (Fig. 5).

Global distribution: It is widely distributed in South Tropical Africa (Angola, Malawi, Zambia, Mozambique, and Zimbabwe) northwards to Tanzania in East Tropical Africa, and southwards to Southern Africa (Botswana, Natal, and Transvaal).

In Egypt, *M. zeyheri* tends in the direction of var. *laurifolia.*

var. ***laurifolia*** Engler, Monogr. Afr. Pflanzen fam. 8: 73, tab. 27/C (1904).

Type: South Tropical Africa: Malawi, 1895, *Buchanan J.* 304, BM000925409, isotype, (BM, image!), (Fig. 5).

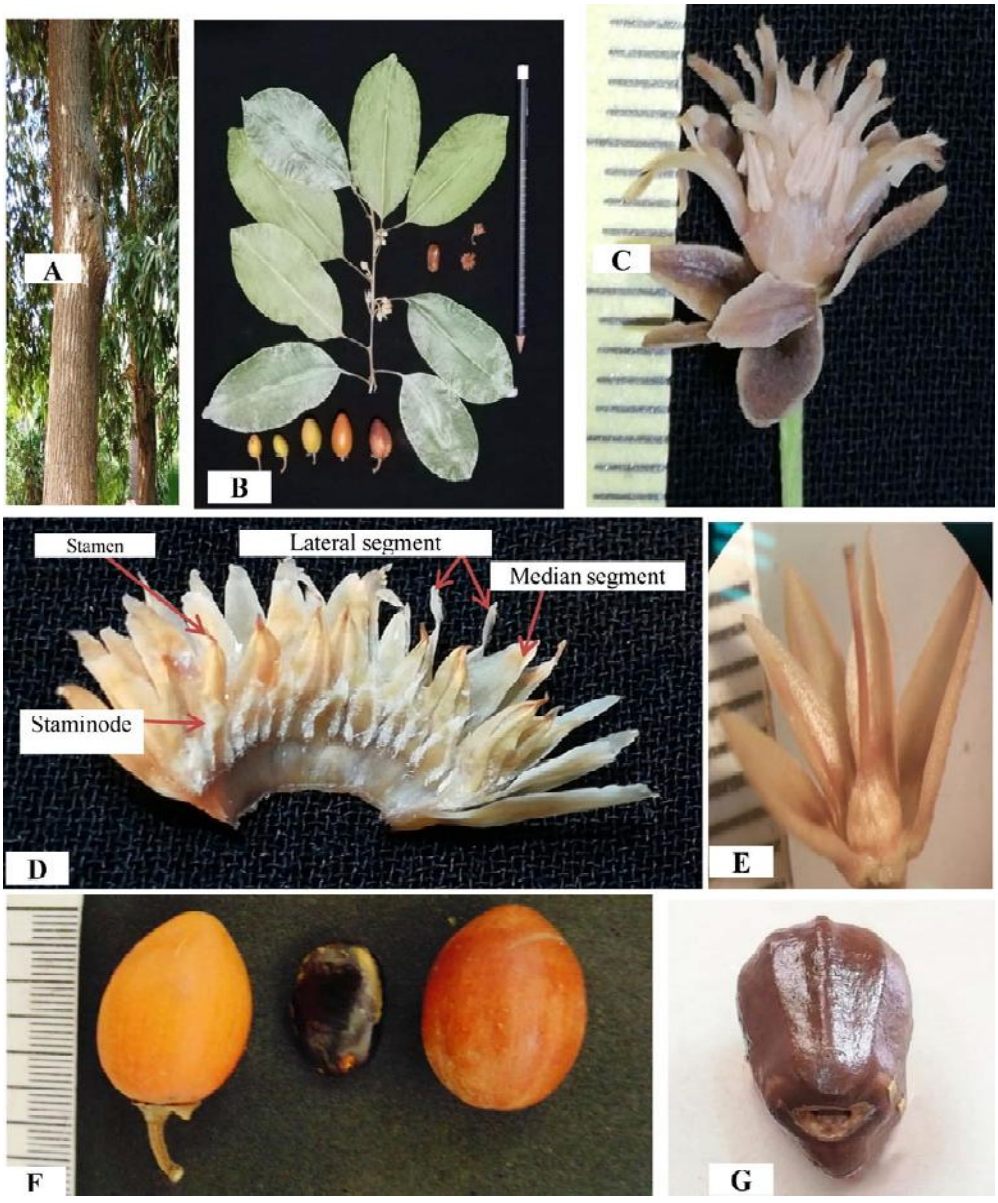


Fig. 4 : Field photographs of *Mimusops zeyheri* Sond.var. *laurifolia* Engl.

A: Trunk; B: Twig with leaves, inflorescence and fruits (below); C: Flower, lateral view; D: Corolla longitudinally sectioned and opened, ventral view; E: Corolla and one sepal removed, showing gynoecium; F: Fruit and seed, lateral view; G: Seed with seed-scar, ventral view [Zohriya Garden; *Abdelmohsen* s.n. (CAI)].

Petiole 2.0-3.5 cm long, 2-3 times as long as the pedicel; corolla-segments not serrate-denticulate at apex; staminodes deltoid lanceolate, 2–3 mm long, acute, shorter than stamens and corolla lobes (nearly half the corolla-lobes).

Taxonomic note: The specimens were misidentified as *M. obovata* Sond. since both have entire lateral segments. However, *Mimusops zeyheri var. laurifolia* Engl. has longer (2.0–3.5 cm) petioles [vs. shorter, less than 1 cm long]; flowering pedicels are shorter than the petioles [vs. longer than the petioles]; both median and lateral segments are different in shape and size [vs. subequal in shape and size]; and staminodes are shorter than stamens and corolla-lobes [vs. as long as stamens and corolla-lobes].

Phenology: Flowering season April, June-July, October; Fruiting season June, September-October.

Use: As ornamental tree.

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Original Research Paper

Assessing performance of horticultural farmers producer companies: Comparative case study

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ABSTRACT

Every year the horticultural sector of India faces huge quantity of food wastage due to lack of processing, value addition and post-harvest handling. Farmers Producer Company (FPC) can mitigate the loss through ensuring better value chain management. There are several horticulture based FPCs established in different parts of India. They have grown very fast and competing with agro-industries. The present study aimed to assess the performance of FPCs working in horticulture sector. The study was conducted in Maharashtra State of India by selecting three FPCs working in horticultural sector. Performance of these FPCs was assessed through Effectiveness Index developed for this study. Seven components *viz.* functional effectiveness, increase in income, increase in farmers share in consumers rupees, inclusiveness, sustainability of company, farmers satisfaction and empowerment were included in the index by following standard index forming protocol. Sahyadri Farms was found the best performing one among the selected FPCs, regarding effectiveness with a mean index score of 63.69 followed by Vasundhara Agro Producer Company Limited (50.20) and Junnar Taluka FPC Ltd. (41.29).

Keywords: Effectiveness, farmers' producer company (FPC), horticulture

INTRODUCTION

India is the second largest producer of fruits and vegetables in the world and produces 260 million tons of food grains. Despite this, India also faces huge post-harvest losses accounting for lack of proper handling practices and storage infrastructures. These post-harvest losses incurred due to inadequacies in storage and logistics account for Rs. 92,651 crores ($13 billion) per year.

According to the Committee on Doubling Farmers' Income, the proportions of produce that farmers are unable to sell in the market at the national level are 34 % and 44.6 %, for vegetables and fruits respectively and 40 % for fruits and vegetables together. This means, every year, farmers lose around Rs 63,000 crore for not being able to sell their produce for which, they have already made investments. It is also reported that only 10-11% of fruits and vegetables cultivated in India can be saved using cold storage facilities due to the expenses involved and lack of suitable facilities. Finance is another setback. To avert storage woes and lack of finance and liquidity; horticultural farmers are compelled to sell their produce immediately, within days of harvest, at any prevailing rate due to high perishability of horticultural crops. This covers distress sale and farmers do not realize the best price because of supply glut in the market. Farmers Producer Company may reduce this loss through improved value chain management. In India, the producer company concept has arisen as a new generation farmer's organization.

Fruits and vegetables are suitable sector which can provide 2-4 times higher income to farmers than cereals. Near about 23 per cent of total registered FPCs are working exclusively in horticulture and

many more are working in mixed approach *i.e.* combination of agricultural and horticultural crops as production options. FPCs can act as a potential driving force for agricultural and rural development. They are working as 'engines' of development that can uphold the pennon of rural development even ahead of local level, offering benefits to the rest of society (Blokland and Goue, 2007). In reality, FPCs have favorable position of scale economies applies to input purchases and accumulation, processing and marketing of the farmers produce in bulk. In both these cases, FPOs can bargain better prices. Through vertical and horizontal coordination as well as forward and backward linkage, FPCs work in value-addition processes which has not only enhanced their dealing power but also increased the share in consumers' rupee. FPCs have minimized the risk of farmers through promoting crop and livestock insurances. It has diminished the cost of information seeking, connecting smallholders to more complex market situation and making farmers acquainted with the competitive business environment through capacity building and empowerment.

There are several horticulture-based FPCs in Maharashtra which have grown very fast and competing with agro industries. The strategic and technological innovations in value chain, clear vision, strong planning and technical insight are the core factors which made the FPC a leader among all grapes exporting agencies. There is immense potential for FPCs to work similarly in the area of value-chain management, so that the huge amount of post-harvest losses can be saved and utilized for home consumption and exports. As the model is new, less studies have been carried out so far on assessing the performance of Farmers Producer Companies in horticulture. Therefore, the present study is aimed to assess the performance of FPCs working in horticulture sector.

MATERIALS AND METHODS

Study area: The study was conducted in Maharashtra State of India. The state is one of the pioneer states in India where the growth of Farmers Producer Companies is remarkably high. Three successful companies working in vegetable, fruits and overall horticulture and processing industry were selected from the state through purposive sampling based on five specific criteria *viz.* i. the FPC has been working for more than 5 years successfully; ii. it has a sizeable membership (more than 2000 members), iii. turnover has been more than Rs. 50 lakhs; iv. FPC has several reported success stories and v. it has a unique business model. The criteria based purposive sampling was useful to select an effective and functional companies working at ground level. Based on that three companies have been selected based on the growth. Junnar Taluka FPC Ltd. is a FPC in initial development stage and working in mainly vegetable sector. Vasundhara Agro Producer Company Limited was selected as a company working mainly in fruits and some vegetable crops at moderate stage of growth. Sahyadri Farms working both in fruits and vegetable was selected for the study as it has achieved a tremendous growth level. The data was collected from the members of their FPCs Pune and Nasik District of Maharashtra.

Operationalization of performance

In this research, we have operationalized the performance as how effectively the producer company carries out its functions. It is better related to organizational performance which indicates how successfully an organized group of people with a particular purpose perform a function. In an organization like Farmers Producer Company, it is important to take care of farmers' satisfaction, empowerment, increasing income of farmers, ensuring value chain management, functional easiness, inclusiveness etc. by combining all these, an Effectiveness Index was prepared which is used in this study.

Research design and survey instrument

In this study, an *Ex-Post Facto* research design was used. A semi-structured interview schedule was prepared. The interview schedule consisted of eighteen different socio-personal and socio-economic variables of respondents and an index was formulated to measure the effectiveness of horticulture-based producer company. The effectiveness index included seven components (1) Functioning efficiency, (2) Increase in income, (3) Increase in farmers share in consumers rupee (4) Inclusiveness, (5) Sustainability of Farmers Producer Company, (6) Farmers satisfaction and (7) Empowerment

The index was prepared based on the above-mentioned parameters and was calculated by the following equation.

$$E_{FPC} = (FE \times W_1 + I \times W_2 + FSC \times W_3 + Inc \times W_4 + S \times W_5 + FS \times W_6 + E \times W_7) / \Sigma^7_{i=1} \times 100$$

Where,

E_{FPC} = Indicated the effectiveness of the particular company

(1) FE = Functioning effectiveness, (2) I = Increase in Income, (3) FSC= Increase in farmers share in consumers' rupee, (4) Inc = Inclusiveness, (5) S = Sustainability of farmers producer company, (6) FS= Farmers satisfaction and (7) E = Empowerment

W_i is respective weight calculated based on Analytical Hierarchy Process (AHP) of experts rating to the seven components based on Saaty (2008) and Mukherjee *et al*., (2018c).

After consultation with the experts and reviewing a vast volume of literature, a rating scale was prepared for constructing the effectiveness index comprising the seven components. The effectiveness index was prepared following standard procedure. Twenty experts working in the top management for promoting farmers organizations were consulted and review of related studies were considered for constructing the index. The effectiveness index comprised of the seven following components.

(1) Functional effectiveness: A functional efficiency index with 1-5 point scale was developed to evaluate the functioning of FPCs. Ten most relevant dimensions were studied in this index measuring the functional effectiveness. Summation of the scores of 10 functioning variables used in the study yielded functioning score of a single respondent. The scores of members of a particular group were added together to get the functioning score of that FPCs. The index was calculated by dividing the actual score by the maximum possible score of functioning. A similar method was followed by Abadi (2010).

(2) Increase in income: Measurement of increase in income was calculated by outreaching the earlier income per year (*i.e.* before the intervention of the FPC) and the present income per year of the agricultural produce (*i.e.* after the intervention of the FPC).

(3) Increase in farmers share in consumer rupee: This was calculated by outreaching the earlier farmers share (i.e. before the intervention of the FPC) and the present farmers share of the agricultural produce (*i.e.* after the intervention of the FPC).

(4) Inclusiveness: The component inclusiveness was added as dimension in effectiveness to study how inclusive the companies were in including the backward class and poorest of the poor. The inclusiveness was studied by an index developed for the study including the category of farmers, caste, gender and financial class.

(5) Sustainability of the company: Sustainability of company is very much important. If a source of income is not sustained, it cannot provide livelihood security. The sustainability of FPC was measured by a schedule developed for the purpose. This included the growth trends of fixed and capital assets of company and most importantly the human resources were considered.

(6) Farmers satisfaction: The farmers satisfaction of the FPC services based on the selected dimensions was measured by an index developed for that purpose following the procedure given by Edwards (1957). This index consisted of 15 statements with 1-5 point of scale to which the respondents were asked to give their responses. The responses were averaged to get respondents satisfaction.

(7) Empowerment: Empowerment of farmers due to joining of FPC was measured by an index developed for the purpose following the procedure given by Edward (1957). This index consisted of 14 statements covering all aspects of empowerment with 1-5 point of scale on which the respondents were asked to give their responses.

The response of all seven components in this Effectiveness index were normalized by z transformation and then averaged. Similar methods were also followed by Mukherjee *et al*., (2011) and Nikam, (2013).

The weights for each component were assigned based on experts judgments using Analytical Hierarchy Process (AHP) depicted in Table 1 which indicates, the empowerment was weighted highest (eigen value = 0.26) followed by sustainability of producer company (eigen value = 0.20), members farmers satisfaction (eigen value = 0.17). Increase in income and share in consumers rupee was weighted next Eigen value 0.14 and 0.11 respectively. The consistency ratio of the AHP was 0.147 and consistency index 0.0991. The CI should be less than 0.1 which satisfies the result. The consistency index score indicated the consistency in judges' ratings.

Table 1 : Effectiveness index weight scores for various components of FPCs

Attribute	Functional efficiency	Income	Share in consume rupee	Inclusive-ness	Sustaina-bility	Satisfac-tion	Empower-ment	Eigen-value
Functional efficiency	1.0	0.33	0.40	0.50	0.29	0.31	0.25	0.05
Increase in income	3	1.00	1.50	2.00	0.67	0.80	0.50	0.14
Share in con-sume rupee	2.5	0.67	1.00	1.50	0.50	0.57	0.40	0.11
Inclusiveness	2	0.50	0.67	1.00	0.40	0.44	0.33	0.08
Sustainability	3.5	1.50	2.00	2.50	1.00	1.25	0.67	0.20
Satisfaction	3.25	1.25	1.75	2.25	0.80	1.00	0.57	0.17
Empowerment	4	2.00	2.50	3.00	1.50	1.75	1.00	0.26

Note: CR=0.147; CI=0.0991

Sampling and data collection

Focused group discussions (FGDs) and series of key informant interviews were carried out to identify the aspects of effectiveness. Additionally, previous effectiveness studies were also reviewed to prepare the survey instrument. The survey instrument was sent to experts for their comments and possible modification and improvement were done based on their recommendations. For easy understanding of the farmers, the instrument was translated in *hindi* (common language) and a pilot test of 20 farmers was done to further clarify the questions. In-depth interviews were conducted with key informants to ensure the triangulation of data. Proper care was taken to make the respondents comfortable and the unbiased recording of the data was ensured. The data were collected from 50 randomly selected members of the company but due to incomplete response some interview schedules were rejected. Finally, a sample of 34 respondents of Vasundhara Agro Producer Company; 37 respondents from Junnar Taluka FPC Ltd. and 38 respondents of Sahyadri Farms were considered for analysis.

Statistical analysis: Comparison of socio-economic characteristics of farmers across the company were done through non parametric tests. For the statistical analysis, the data were analyzed using MS Excel and SPSS 20 software.

RESULTS AND DISCUSSION

Profile of selected Farmers Producer Companies:

Assessment of effectiveness of FPCs starts with comparative profile study. It is important to understand the structural and functional difference of selected FPCs for better comparison as a case. The comparative profiles of selected FPCs are depicted in the Table 2.

Vasundhara Agri-Horti Producer Company Limited

Vasundhara Agri-Horti Producer Company Limited (VAPCOL) is a pioneering organisation functioning for the remuneration of farmers' family in tribal areas across various states of India. The company was established in July, 2004 with help of BAIF (Bharatiya Agro Industries Foundation) organization. Presently there are 48 producer groups consisting of 41,000 farmers from the state of Maharashtra, Gujarat, Rajasthan, Uttar Pradesh, Madhya Pradesh, and Chhattisgarh. The turnover of the company is estimated as Rs 17 crores.

Junnar Taluka Farmers Producer Company Ltd.

Junnar Taluka Farmers Producer Co. Ltd (JTFPC), promoted by Vegetable Growers association of India with support of Small Farmers Agribusiness Consortium (SFAC), (Ministry of Agriculture,

Table 2 : Comparisons of the profiles of selected Farmers Producer Company

Particulars of selected Producer Companies	Vasundhara Agro Producer Company Limited	Junnar Taluka FPC Ltd.	Sahyadri Farms
Year of Registration	2004	2013	2011
Promoting organization	BAIF	Veg. Growers Association of India	Own
Ownership model followed	Institutional	Individual	Individual
No. of members	41000 farmers of 48 producer groups	1600	1000
Area of operation	Maharashtra; Gujarat; Rajasthan; UP; Madhya Pradesh; Chhattisgarh	Pune, Maharashtra	Nasik, Maharashtra
Turnover (Rs. crores)*	17	5	500
Products marketed	Cashew, Mango and Amla value added products	All vegetables, pomegranate, grapes	All fruits and vegetables
Market landscape	National and international market	Local and Regional Markets	National and International markets
Service provided	Marketing, Financial assistance, Managerial support etc.	Supply of inputs, training of members, Value addition and marketing etc.	Financial assistance, crop insurance, Food processing, marketing, production improvement, Training etc.

Note : * approximate estimation

Govt. of India), is a registered Farmers Producer Company under the Companies act 1956. The company was established in the year 2009 is Pune, Maharashtra, with the hand holding of Vegetable Growers Association of India. This company is involved in crop production, crop protection and exploring marketing platform to the producer members in ameliorating the economic status by value addition to their produce.

The objectives of the company are collectivize the small vegetable growers, improve the standards of living through better use of improved technology of vegetable production, processing and marketing; minimize the environmental degradation while maintaining sustainable profits and provide consultancy in the field of horticulture especially for promotion of organic farming.

Sahyadri Farms

'Sahyadri Farms' is working as a Farmers Producer Company since 2011 in Nasik, Maharashtra. It is a 100 percent farmer's owned and professionally managed Producer Company. It is operationally sound with best use of production and processing technology. Today, the company is a leading exporter of grapes in India, exporting ~14 percent of the total export of grapes to Europe. There are more than 3000 farmers working day and night for the company. It is India's leading FPC which is producing, marketing and exporting of frozen vegetable, value added fruit products, *etc*. to Germany, USA, Norway and many other countries.

Socio-economic profile of FPC members

The socio economic profile of selected FPC members' from all three FPCs was studied for comparison. The results are presented in the Table 3.

Table 3 : Socio-economic profile of members of different FPCs

Characteristics	VAPCOL (n=34)	Sahyadri Farms (n=37)	Junnar Taluka (n=38)
Age			
a. Young (18-35 years)	11 (32.4)	20 (54.05)	17 (44.7)
b. Middle aged (36-50 years)	6 (17.6)	9 (24.32)	16 (42.1)
c. Old (51-80 years)	17 (50.0)	8 (21.62)	5 (13.2)
Gender			
a. Male	16 (47.1)	32(86.5)	37 (97.4)
b. Female	18 (52.9)	5 (13.5)	1 (2.6)
Education level			
a. Middle schooling	18 (52.9)	8 (21.6)	21 (55.3)
b. Higher secondary	14(41.2)	16 (43.2)	13 (34.2)
c. Graduate	2 (5.9)	13 (35.1)	4 (10.5)
Family size			
a. Nuclear (up to 5)	10 (29.4)	12 (32.4)	10 (26.3)
b. Joint family (6 and above)	24 (70.6)	25 (67.6)	28 (73.7)
Farm Size			
a. Up to 1 ha	34 (100.0)	24 (64.9)	25 (65.8)
b. More than 1 ha	0 (0.0)	13 (35.1)	13 (34.2)
Social participation			
a. High	32 (94.1)	33 (89.2)	33 (86.8)
b. Low	2 (5.9)	4 (10.8)	5 (13.2)
Extension agency contact			
a. High	33 (97.1)	33 (89.2)	23 (60.5)
b. Low	1 (2.9)	4 (10.8)	15 (39.5)
Urban contact			
a. High	28 (82.4)	37 (100.0)	37 (100.0)
b. Low	6 (17.6)	0 (0.0)	0 (0.0)
Training experience			
a. Never	0 (0.0)	0 (0.0)	0 (0.0)
b. Once	0 (0.0)	0 (0.0)	0 (0.0)
c. Two and more	34 (100.0)	37 (100.0)	38 (100.0)
Members of Other Group			
a. No	0 (0.0)	5 (13.5)	17 (44.7)
b. Yes	34 (100.0)	32 (86.5)	21 (55.3)
Progressiveness			
a. Less	0 (0.0)	0 (0.0)	0 (0.0)
b. Moderate	0 (0.0)	0 (0.0)	0 (0.0)
c. High	0 (0.0)	3 (8.10)	0 (0.0)
d. Very high	34 (100.0)	34 (91.90)	38 (100.0)

Attitude towards the FPC			
a. Positive	34 (100.0)	36 (97.30)	38 (100.0)
b. Negative	0 (0.0)	0 (0.0)	0 (0.0)
c. Neutral	0 (0.0)	1 (2.70)	0 (0.0)
Annual Income			
a. 0-1 lakh	30 (88.2)	0 (0.0)	0 (0.0)
b. 1-2 lakh	4 (11.8)	0 (0.0)	16 (42.1)
c. 2-3 lakh	0 (0.0)	13 (35.1)	22 (57.9)
d. More than 3 lakh	0 (0.0)	24 (64.9)	0 (0.0)

Note: Figures in parentheses indicate percentage value

The Table 3 indicates that majority of the farmers were of young categories for Sahyadri farms (54.05%) and Junnar Taluka FPC (44.70%). In case of VAPCOL majority of the members were found much older and experienced than others. There was no significant difference in age groups recorded. Also, majority of the respondent members were male in both the cases of Sahyadri farms and Junnar Taluka FPC, but in case of VAPCOL, majority (52.90%) were female. A similar case was also recorded for level of education and family size. Majority of the VAPCOL farmers were small and marginal in nature having less than 1 hectare land holding. Although, in case of Sahyadri farms, it was found that 64.90 % of the farmers were marginal in nature where as 35.10 % had having land holding more than 1 hectare. In the vegetable based farmer producer company at Junnar Block 65.80 % of the farmers were marginal. Social participation is an important parameter of socioeconomic status. The highest social participation was recorded for VAPCOL farmers (94.10 %) followed by Sahyadri farms (89.20 %) and Junnar Taluka Farmer Producer Company (86.60 %). Similar case can also be seen in case of extension agency contact where, a majority of the VAPCOL farmers (97.10 %) had high level of extension agency contact followed by Sahyadri farms (89.20 %). For training experience it was found that all of the producer company members attended two and more trainings in their life time. Majority of them were members of other groups like self-help groups, co-operatives etc. The number is highest in case of VAPCOL because, it is following institutional model where several cooperatives combine to form farmers producer company, so apart from the membership in FPOs, the VAPCOL farmers were also associated in cooperatives. Sahyadri farm was developed from self help groups, that is why 86.50 % of the farmers had membership in other groups but the case is different for Junnar Taluka where, individual farmers associated with each other to form the company so, only 55.30 % farmers were associated with other groups. In case of progressiveness and attitude, it was found that majority of the farmers in all the groups were progressive in nature and have positive attitude towards FPCs. The increase in annual income was found to be the highest in case of Sahyadri farms, in which 64.90 % of the members where earning more than 3 lakh after joining Sahyadri farms whereas 35.10 % earn between 2 to 3 lakh per year. The majority of the farmers of Junnar Taluka (57.90 %) were earning 2-3 lakh and 42.10 % of them has enhanced their income up to Rs. 1 to 2 lakhs after joining the FPC. The VAPCOL is a association of very small farmers and it was found that the majority (88.20 %) had able to enhance income up to 1 lakh per annum after joining the company, while 11.8 % up to 1 to 2 lakh per annum.

Comparative effectiveness of selected Farmers Producer Companies

It is essential to assess the effective of FPCs working in the horticulture sector. Producer Company wise mean score of the components of effectiveness is depicted in Table 4. Functional efficiency wise, all the companies scored more than 4.5 out of 5, which is a quite high score. It indicated that the companies were well functioning. The highest score was obtained by Sahyadri Farms (4.55) as it has its own management team and qualified salaried staff. Functional efficiency wise the companies are nearly at par with each other.

Table 4 : Overall effectiveness of FPCs based on components mean score

Company	Functional efficiency	Increase in Income (%)	Increase in share in consumer's rupee (%)	Inclusive-ness	sustaina-bility	satisfac-tion	Empower-ment
VAPCOL	4.51	31.71	34.71	0.76	0.81	4.44	4.47
Sahyadri Farms	4.55	67.41	32.08	0.67	0.92	4.49	4.42
JTFPC Ltd.	4.52	32.29	32.18	0.75	0.69	4.37	4.44

As per the data, the highest percentage increase in annual income of members farmers before joining the company was observed in Sahyadri Farms (67.41%). The results showed that farmer's income had enhanced in a range of 32 to 67 per cent after joining Farmers Producer Companies.

Farmers share in consumer's rupee was another component, which indicates level of value addition. It was found that farmers share in consumers rupee had increased 32-35 per cent more than earlier. It is mainly due to the value addition at producer company level. The highest increase was found for VAPCOL (34.71 %) which was due to well-established marketing channel by the company. Beside this door to door picking and delivery to retail market and marketing efficiency has culminated the change.

Inclusiveness is another indicator used in this index to have a look on whether the companies are working with the poor and backward section of society or not. It was found all the FPCs were inclusive in nature. VAPCOL farms scored 0.76 out of 1 whereas Junnar Taluka FPC Ltd. scored 0.75 whereas in case of the Sahyadri Farms the members are already working in grapes and a large number of the members were rich before joining the FPC which is reflected in the lesser inclusiveness score (0.67). Sustainability of an organization is key factor in effectiveness. The big FPCs scored better in these parameters. In sustainability parameter, Sahyadri Farms, VAPCOL and Junnar Taluka FPC Ltd. got the index score 0.92, 0.81 and 0.69 respectively. Satisfaction of member farmers was high for all the FPCs. The score obtained by the companies was in the range of 4.37 (Junnar Taluka FPC Ltd.) to 4.49 (Sahyadri Farms). It indicates the farmers perceived level of satisfaction after joining Producer Company. Empowerment is another important parameter for effectiveness. The companies which empowered the member better were Vasundhara Agro Producer Company Limited (4.47) followed by Junnar Taluka FPC Ltd. (4.44) and Sahyadri Farms (4.42). The overall mean score of farmers satisfaction were more than 4.4 out of the scale of 5.

The effectiveness score of different Farmers Producer Companies are depicted in Table 5. The overall index score indicates that the Sahyadri Farms is the best among other regarding effectiveness with mean score 63.69 followed by Vasundhara Agro Producer Company Ltd (50.20) and Junnar Taluka FPC Ltd. (41.29). The reason behind this are that the companies are good in empowering their members, have a sustainable business venture, the members were highly satisfied with the performance of company and effective in enhancing farmers income.

To study the whether the companies significantly differ in effectiveness, one way ANOVA was conducted. The F value was 68.142 which were significant at 1 per cent level of significance. It is observed that the companies significantly differed from each other in effectiveness (Table 6).

Table 5 : Overall effectiveness of Farmer Producer Company

FPCs	Mean	SD	Range	Minimum	Maximum
VPCOL	50.20	10.64	46.31	27.67	73.98
Sahyadri Farms	63.69	12.33	52.98	34.69	87.67
JTFPC Ltd.	41.29	10.84	49.10	17.17	66.27

Table 6 : Effectiveness of FPCs (ANOVA)

Category	Sum of Squares	df	Mean Square	F	Sig.(p)
Between Groups	1.159	7	0.166	68.142	0.000
Within Groups	.693	285	0.002		
Total	1.852	292			

Effectiveness of any Farmers Producer Company depends on how better it is empowering farmers. How it is influencing the social, political, psychological and economic empowerment parameters of the rural community. Farmers Producer Company provided a platform for farmers to join together, involve together and work with groups. This enhanced farmer's interaction with different progressive farmers (Mukherjee *et al*., 2020). As per the experts rating, empowerment was weighted highest (0.26), the FPCs who ensured better empowering farmers through training and capacity building exercise in horticultural products gained major weightages. Sustainability of income was another important parameter realized to be the important in effectiveness of FPCs. It depends upon sales growth, membership growth, successful ventures made, profit growth, market linkages and several others factors. Farmer producer companies can play a more important role in sustainable agricultural intensification for smallholders, particularly by addressing the constraints like the size of landholding, access to credit, irrigation, and marketplaces (Reddy *et al*., 2020). Satisfaction of the producers are the next important index parameter which includes timeliness of inputs delivery, quality service, dividend distribution, income enhancement etc.

CONCLUSION

In this study, an attempt was made to measure the performance of horticulture based farmers producer companies with an effectiveness having seven components namely, functioning efficiency, increase in income, increase in farmers share in consumers rupee inclusiveness, sustainability of Farmers Producer Company, farmers satisfaction and empowerment. The component empowerment was weighted highest followed by sustainability of producer company members, farmers satisfaction and increase in income. Sahyadri Farms was the best among other regarding effectiveness with mean score 63.69 followed by and Vasundhara Agro Producer Company Limited. (50.20) and Junnar Taluka FPC Ltd. (41.29). The reason behind this may be that the companies are good in empowering their members, having a sustainable business venture, the members were highly satisfied with the performance of company and effective in enhancing farmers income. The three parameters, farmers empowerment, FPC sustainability and farmers satisfaction cumulatively contributing 63 % of index weights. To be effective, the horticultural FPCs need to focus on these three parameters most.

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Original Research Paper

Growth trend and potential of horticulture in Northeast India

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ABSTRACT

The Northeast region of India is endowed with diverse soil and agro-climatic conditions that are conducive to the growth of a large variety of temperate and tropical horticultural crops. Fruits, vegetables, and spices of the region are highly nutritious and have a market within and outside the country. The paper is an attempt to assess the potential of horticulture in the region. To gauge the state-wise and regional growth trend and variability in area and production of these crops during the period 2009-2019, Compound Annual Growth rates and Instability Index have been computed from secondary data. The study reveals a rising regional growth trend with low instability for the production of fruits and vegetables and moderate instability for spices. This indicates the possibility of sustainable development of horticulture in all the Northeast states through strategic planning. Fruits and spices of the region also have a market in Middle-East and neighbouring countries. However, lack of commercialisation, poor market intelligence, and linkages are impeding the growth of exports. To unleash the true potential of horticulture, it is imperative to develop infrastructure, modernise farming and establish seamless value chains with greater market integration.

Keywords: Export, GI tag, horticulture, linkages, sustainable development.

INTRODUCTION

The Northeast (NE) region of India is rich in biodiversity and home to a large variety of flora and fauna. The region, being a part of the Indo-Myanmar biodiversity hotspot (Myers *et al*, 2000), is one of the four hotspots present in the country. The states of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, and Tripura comprising the North-eastern region lie in the Eastern Himalayan Agro-climatic zone. The tropical and sub-tropical climate and the alluvial soil of the Brahmaputra and Barak plains and the temperate climate of the hills that have laterite and sandy soil, support a wide variety of plants in the region. The dense forest cover that spreads across 67.05% of the total geographical area (Aggarwal, 2020) generates large quantities of humus that add to the fertility of the soil. Out of approximately 15,000 species of flowering plants available in India, the Northeast region alone accounts for about 8,000 species and six out of the nine important vegetation types found in India (Hegde, 2000).The region enjoys a comparative advantage in terms of climate and soil.

The diverse climatic conditions of the region are conducive to the growth of a wide variety of tropical and temperate horticulture crops that are high on nutritional value (Sarmah and Deka, 2012). The good quality spices especially ginger and chilli of the region not only have high domestic demand but also have export potential especially in South and Southeast Asian countries (Das, 2016). Since demand for fruits and vegetables is highly income dependent, they account for a significant proportion of the total consumption expenditure of middle and high-income groups. Due to the enlargement of the market for these crops in recent years, the horticulture sector is fast emerging as an important sector providing lucrative income and employment to the farm sector and supporting a large number of agro-based industries (Nabi and Bagalkoti, 2017). Hence, the farm sector is witnessing a gradual shift in cropping pattern in favour of horticulture.

In 2014-15, ten horticulture products from the Northeast were given the GI registration, granting the region exclusive rights to produce them. These include Arunachal orange, Khasi mandarin, Tezpur litchi,

Kachai lemon, Indian wild orange popularly known as 'Memang narang', Tripura queen pineapple, Naga tree tomato, Assam Karbi Anglong ginger, Mizo Chilli, and Sikkim large cardamom (Kashyap, 2015).

Depending on the climate, altitude, and physiographic difference of land, the major fruits, vegetables and spices that are cultivated in different North-eastern states are:

State	Fruits	Vegetables	Spices
Arunachal Pradesh	Apple, Banana, Guava, Kiwi, Orange, Pear, Pineapple, Walnut	Bean, Bitter gourd, Brinjal, Cucumber, Muskmelon, Potato, Pumpkin, Radish, Sweet Potato, Tomato	Ginger, Large Cardamom
Assam	Banana, Guava, Jack fruit, Lemon, Litchi, Mandarin Orange, Mango, Papaya, Pineapple, Sapota	Bean, Brinjal, Brocolli, Cabbage, Capsicum, Carrot, Cauliflower, Colocassia, Cucumber, Gourds (different types), Knol-khol, Muskmelon, Pea, Potato, Pumpkin, Radish, Spinach, Sweet potato, Tapioca, Tomato, Yam	Black cumin, Black pepper, Chillies, Coriander, Cumin, Fennel, Fenugreek, Garlic, Ginger, Mint, Onion, Turmeric
Manipur	Banana, Lemon, Orange, Passion fruit, Peach, Pear, Pineapple, Plum	Bean, Cabbage, Cauliflower, Cucumber, Muskmelon, Pea, Potato, Tomato	Chillies, Garlic, Ginger, Hatkora, Onion, Turmeric
Meghalaya	Banana, Guava, Lemon, Mandarin Orange, Pear, Pineapple, Plum	Brocolli, Cabbage, Capsicum, Carrot, Cauliflower, Colocasia, Potato, Radish, Squash, Sweet potato, Tapioca, Tomato, Turnip	Bay leaf, Black pepper, Chillies, Ginger, Turmeric
Mizoram	Banana, Hatkora, Orange, Papaya, Passion fruit, Pineapple	Bean, Brinjal, Brocolli, Cabbage, Capsicum, Carrot, Cauliflower, Chow-chow, Cucumber, different types of Gourds, Knol-khol, Ladies finger, Muskmelon, Pea, Potato, Pumpkin, Radish, Tomato	Chillies, Ginger, Turmeric
Nagaland	Banana, Guava, Jack fruit, Lemon, Litchi, Mandarin Orange, Mango, Papaya, Pineapple, Plum, Pomegranate	Brinjal, Cabbage, Carrot, Cauliflower, Chow-chow, Colacasia, Ladies finger, Pea, Potato, Radish, Sweet Potato, Tomato	Black pepper, Cardamom, Chillies, Garlic, Ginger, Turmeric
Sikkim	Banana, Kiwi, Mandarin Orange, Papaya, Passion fruit, Pear	Bean, Brocolli, Cabbage, Cauliflower, Cucumber, Pea, Radish, Tomato, Turnip	Cherry pepper, Ginger, Large cardamom, Turmeric
Tripura	Banana, Ber, Guava, Jack fruit, Lemon, Litchi, Mango, Musambi, Orange, Papaya, Pineapple, Sapota	Bean, Cabbage, Capsicum, Carrot, Cauliflower, Cucumber, different types of Gourds, Parmal, Potato, Pumpkin, Radish, Sweet potato, Potato, Tapioca, Tomato	Black Pepper, Betel vine, Chillies, Ginger, Onion

MATERIALS AND METHODS

The study is an attempt to assess the performance of horticulture crops, i.e., fruits, vegetables, and spices of the NE region during the period 2009-10 to 2018-19. For this purpose, data pertaining to area and production of fruits, vegetables, and spices that are cultivated in the different Northeast states have been considered. The relevant data and information have been gleaned from the Indian Horticulture database of the National Horticulture Board (www.nhb.org.in), Agricultural Processed and Export Development

Agency (apeda.gov.in), annual reports of Horticulture Mission for Northeast and Himalayan States, Directorate of Horticulture of the Northeast states, published journals and newspapers.

Data collected has been analysed to estimate descriptive statistics such as mean and standard deviation and regression coefficients of the function $Y = ab^t u$, where Y represents the growth rate under study, a is the intercept; b is the regression coefficient; t is the time variable and u is the error term. The estimates have been used to compute the compound growth rates (CAGR) and Instability Index (II) which is used to examine the variability in area and production of agricultural crops. Since the Cuddy-Della Valle index (1978) corrects the coefficient of variation for overestimation of instability in long-term trends of time series data, it has been used in the present study.

Instability Index = $CV \times \sqrt{1-r^2}$, where CV is the coefficient of variation in percent and r^2 =RSS/TSS, i.e. the coefficient of determination of the trend regression that best fits the time series data.

The ranges of instability have been categorised as low instability = 0 to 15; medium instability = greater than 15 but less than 30; and high instability = greater than 30 (Sihmar, 2014). The data was analysed using the statistical software, SPSS 20.

RESULTS AND DISCUSSION

Trend analysis in area and production of fruits, vegetables, and spices for the period under study has been made using Compound Annual Growth Rate. Tables 1 (a) and (b) reveal that fruit cultivation can be adopted as a sustainable occupation in Assam, Manipur, and Meghalaya. These states have recorded a more than proportionate increase in the annual growth rates of production, as compared to the area under cultivation, with low instability indices. The success may be attributed to favourable soil and climatic conditions, use of high-quality seeds, better usage of technical know-how, and farm practices. For example, the per annum production growth rates of the major fruit crops during the period 2010-11 to 2017-18, such as banana in Assam (3%), mandarin in Manipur (6.7%), and pineapple in Meghalaya (6.3%) and the corresponding low instability index of 3.72%, 9.6%, and 5.87% respectively reflect the success of the strategies adopted in these states (Table 2).

Sikkim and Nagaland have registered 10.1% and 8% annual growth rates in the production with relatively lesser annual increase in land for growing fruits, production has been observed to be moderately unstable during the period. On the other hand, a 3.3% annual increase in the production in Mizoram and a 1.4% decline in the production in Tripura have been observed despite more land being brought under fruit cultivation. It can be opined that erratic climatic conditions and limited use of modern techniques of production may be the main reasons for the sluggish growth in fruit production in Mizoram, while lack of drip irrigation system and absence of farm mechanisation may be the factors responsible for poor production in Tripura.

A negative annual growth rate of production with high instability index of 48.03% indicates that fruit cultivation is risky in Arunachal Pradesh. Uncertain weather conditions, inadequate irrigation facilities, vagaries of monsoon, inferior farm inputs, and practices have resulted in severe fluctuations in the production of fruits. However, due to the recent surge in demand for kiwi, orange, and apple of the state, more land is being brought under cultivation and farmers are being induced to produce these crops.

During the period under study, the annual growth rate in fruit production has been almost double the growth rate in the area under cultivation in the region as a whole. Since the instability index for the entire region is 8.38% and 8.55% for area and production respectively, it may be inferred that barring Arunachal Pradesh, fruit cultivation is relatively stable and may be encouraged to augment employment and income in the farm sector.

Tables 3 (a) and (b) display the growth trend and instability index of vegetables during the period of study. The annual rate of growth of production of vegetables is found to be higher than that of the per annum growth rate in the area under cultivation in the states of Meghalaya, Nagaland, Sikkim, and Tripura. While the areas under cultivation and production are relatively stable in Meghalaya and Tripura, they are found to be moderately stable in Nagaland and Sikkim. Favourable agro-climatic conditions in Meghalaya and Tripura offer tremendous scope for the cultivation of

Table 1 (a) : State wise area in '000 hectares and production in '000 M Tonnes of fruit crops

Year	Arunachal Pradesh		Assam		Manipur		Meghalaya	
	A	P	A	P	A	P	A	P
2009-2010	72	107.9	117.3	1575.5	38.4	281.9	32.9	294.8
2010- 2011	72	107.9	137.5	1763.5	68.7	286.3	30.2	241.9
2011- 2012	85.1	308.9	142.8	1851.8	49.5	405.9	32.3	300.4
2012- 2013	86.86	312.24	150.71	2073.82	51.93	440.59	33.15	316.57
2013- 2014	89.09	321.26	144.68	2007.8	54.05	515.69	35.3	348
2014- 2015	90	331.4	155.51	2242.74	55.61	532.97	36.01	375.83
2015- 2016	66.21	306.27	145.71	2077.77	51.12	467.76	36.59	395.4
2016- 2017	48.71	124.38	142.89	2024.84	50.58	478.77	37.37	426.86
2017- 2018	48.13	125.7	147.26	2132.62	47.61	455.59	32.81	316.51
2018- 2019	48.13	125.7	167.2	2518.89	47.686	456.058	33.37	324.67
CAGR %	**-5.9**	**-1.3**	**2.2**	**3.8**	**-0.3**	**5.4**	**1.1**	**3.2**
R^2	0.49	0.006	0.54	0.75	0.004	0.501	0.24	0.35
b_1	0.941	0.987	1.02	1.04	0.997	1.054	1.011	1.032
II	**17.43**	**48.03**	**6**	**6.42**	**14.83**	**14.1**	**5.71**	**13.08**

A: area; P: production

Table 1 (b) : State wise area in '000 hectares and production in '000 M Tonnes of fruit crops

Year	Mizoram		Nagaland		Sikkim		Tripura		North East	
	A	P	A	P	A	P	A	P	A	P
2009-2010	27.1	328.3	30.8	223.7	12.2	18.5	36.9	573.8	367.6	3404.4
2010- 2011	27	211.5	18.2	151.3	17.5	25.8	40.8	643.9	411.9	3432.1
2011- 2012	43.7	257.7	33.7	347.7	13.4	22.5	54.5	644.4	455	4139.3
2012- 2013	49.68	292.95	37.23	275.95	14.65	24.02	60.12	697.87	484.33	4434.01
2013- 2014	57.55	343.9	40.56	411	16.02	24.05	63.38	786.35	500.63	4758.05
2014- 2015	66.14	386.62	40.56	411	17.59	26.42	67.27	563.5	528.69	4870.48
2015- 2016	55.01	330.28	37.05	374.13	17.53	23.48	75.74	854.05	484.96	4829.14
2016- 2017	62.56	339.05	39.19	388.49	18.55	25.56	57.84	559.92	457.69	4367.87
2017- 2018	63.19	340.51	39.19	380.52	19.36	54.9	53.75	547.52	451.3	4353.87
2018- 2019	62.911	335.6	39.5	380.52	19.55	55.45	51.39	525.42	469.74	4722.31
CAGR %	**10**	**3.3**	**5.2**	**8**	**4.5**	**10.1**	**3.7**	**-1.4**	**1.8**	**3.2**
R^2	0.73	0.32	0.39	0.51	0.69	0.61	0.25	0.07	0.27	0.52
b_1	1.1	1.033	1.052	1.08	1.045	1.101	1.037	0.986	1.018	1.032
II	**14.73**	**12.94**	**15.05**	**18.45**	**8.44**	**27.67**	**18**	**16.51**	**8.38**	**8.55**

A: area; P: production

Table 2 : State wise area in '000 hectares and production in '000 M Tonnes of select fruit crops

Year	Assam (Banana)		Manipur (Mandarin)		Meghalaya (Pineapple)	
	A	P	A	P	A	P
2010- 2011	47.6	723.6	3.5	27.7	9.7	86
2011- 2012	49.1	745.3	4.7	28.7	10.6	112.9
2012- 2013	51.51	837.02	5.02	32.64	10.82	109.39
2013- 2014	50.81	857.72	5.2	41.2	11.31	117.77
2014- 2015	51.28	865.67	5.35	43.06	11.59	124.6
2015- 2016	51.1	882.71	4.91	43.34	11.58	123.13
2016- 2017	49.27	854.85	4.81	42.91	12.16	140.95
2017- 2018	53.08	913.27	4.46	39.89	12.37	144.73
CAGR %	**0.9**	**3**	**2.1**	**6.7**	**3.2**	**6.3**
R^2	0.427	0.781	0.152	0.706	0.93	0.855
b_1	1.009	1.03	1.021	1.067	1.032	1.063
II	**2.59**	**3.72**	**11.06**	**9.6**	**2**	**5.87**

A: area; P: production

a wide variety of vegetables throughout the year. Improvement in irrigation facilities through the introduction of drip irrigation and sprinkler irrigation, protected cultivation of vegetables, provision of modern farm implements and tools, and other facilities to the farmers under the Technology Mission for Integrated Development of Horticulture have enabled the states to make remarkable progress in the production of vegetables. For instance, the low instability index of 0.38% for potato production in Meghalaya and 6.11% for brinjal production in Tripura over the period 2013-14 to 2017-18 indicates the success of the strategies applied in stabilising the production of the crops grown in the states. On the other hand, peas production in Sikkim has been moderately unstable, though the annual rate of growth has been an impressive 15.4% (Table 4). Hence, to stabilise production a re-engineering of the strategies adopted is required with greater farm support to the cultivators.

Despite a decline in the annual growth rate in the area under production by 0.65% in Arunachal Pradesh, the state has recorded an increase of 6.1% per annum in the production of vegetables. Improvement in irrigation facilities, expansion of protected cultivation system to avert the impact of harsh weather conditions, and adoption of organic farming to maintain soil fertility are some of the measures that may be undertaken to secure higher stability in both the area under cultivation and production of vegetables.

Assam, Manipur, and Mizoram have been found to have brought more land under cultivation of vegetables but have not been able to increase production commensurately. Among these three states, Mizoram's vegetable production has been the least, growing at 2.7% per annum compared to an 11.1% annual increase in the growth rate of the area under cultivation. Moderate instability has also been observed in both the area under cultivation and production. To accelerate growth in the production of vegetables, a more integrated approach has been adopted by the government. Farmers are being encouraged to adopt commercial farming of focus crops such as tomato and cabbage. High yielding variety seeds, superior quality of farm inputs, modern tools and implements, irrigation facilities, and facilities for post harvest handling of produce are being made available for the purpose. The slow growth of production in comparison to the area under cultivation in Manipur may be attributed to the use of inferior quality seeds and other farm inputs, lack of irrigation facilities, and incidence of pests and diseases.

Though Assam is the major vegetable producing state in the region with low instability (5.5%) in the area under cultivation, yet the state has recorded a

negligible per annum increase of 0.6% in production with moderate instability (17.29%) during the period. The small-sized scattered holdings in the state are a major hindrance in adopting large-scale production using modern technology. Frequent floods, use of low-yielding variety seeds, and other planting materials are also some of the factors that are impeding the growth of vegetable production.

The region as a whole has witnessed 4.4 % and 2.9% annual growth rates in area and production of vegetables respectively with a low instability index. Except for Arunachal Pradesh, more land is being brought under the cultivation of vegetables and there has been an increase in production as well. This is an encouraging trend as farmers can diversify and adopt multi-cropping which will yield sustained income throughout the year besides making the region self-sufficient.

It can be observed in Tables 5 (a) and (b) that Arunachal Pradesh and Sikkim have recorded stable growth in the area under cultivation and production of spices during the period. Spice cultivation, thus, appears to be a viable alternative in the farm sector and should be encouraged in these states. For example, one of the major spices of Sikkim is ginger. Table 6 reveals that ginger production has registered a 2.1% per annum growth rate during the period 2013-14 to 2017-18 with a low instability index of 1.37%.

Spice cultivation also appears to have very good prospects in Assam. The state has recorded 9.1% per annum growth in the area under spice cultivation and a 19% annual growth rate in production. The average yield of the major spices, i.e., ginger, turmeric, chilli, and black pepper has increased over the years in the state (Borah, 2020). However, the instability index of 20.29% and 27.86% for the area under cultivation and production respectively indicate moderate instability which can be mitigated through strategic planning and correct policy interventions. For instance, the soil and climatic conditions of the eastern part of Assam are conducive for black pepper farming. Hence, the cultivation of the spice may be extended to the tea gardens of the area as well to augment production and secure stability.

While production has been moderately stable in Manipur and Tripura at 19.88% and 18.54% respectively, it has been relatively high in Nagaland (36.96%). On the other hand, fluctuations in climatic conditions, inferior planting materials, and plant

Table 3 (a) : State wise area in '000 hectares and production in '000 M Tonnes of vegetables

Year	Arunachal Pradesh		Assam		Manipur		Meghalaya	
	A	P	A	P	A	P	A	P
2009-2010	4.2	38.5	255.2	4569.9	19.9	221.8	44.3	415.8
2010- 2011	4.2	38.5	260.1	2925.5	22.2	236.5	41.8	356.5
2011- 2012	6.3	83.5	266	3045.6	20.8	200.3	39.5	385
2012- 2013	1.5	37.6	278.7	3415.1	21.7	219.8	40.5	403.4
2013- 2014	1.4	35	281.4	3031.9	25.2	271	43.6	515.3
2014- 2015	1.7	41	337.94	4647.79	27.58	288.44	44.6	534
2015- 2016	4	33.01	317.51	3821.71	34.36	316.51	47.5	494.88
2016- 2017	1.75	14.42	300.75	3329.58	59.4	369.86	49.5	523.42
2017- 2018	2.58	16.68	300.17	3292.88	45.3	342.11	49.11	519.67
2018- 2019	2.58	16.58	324.13	4060.14	45.281	341.692	49.84	531.88
CAGR %	**-0.65**	**6.1**	**2.7**	**0.6**	**12.3**	**6.7**	**2.3**	**4.4**
R^2	0.152	0.684	0.676	0.011	0.814	0.817	0.681	0.693
b_1	0.935	1.061	1.027	1.006	12.3	1.067	1.023	1.044
II	**48.74**	**11.22**	**5.55**	**17.29**	**18.1**	**6.7**	**4.73**	**8.16**

A: area; P: production

Table 3 (b) : State wise area in '000 hectares and production in '000 M Tonnes of vegetables

Year	Mizoram		Nagaland		Sikkim		Tripura		North East	
	A	P	A	P	A	P	A	P	A	P
2009-2010	10.6	179.1	10.4	78.3	28.7	147.7	32.5	446.9	405.8	6098
2010- 2011	17.4	115.6	10.7	79.4	23.9	120.9	36	532.3	416.3	4405.2
2011- 2012	37.4	221.1	33	222.6	25	127.7	34.2	552.6	462.5	4796.4
2012- 2013	39.3	236.68	26	207.7	25.6	132.5	45.1	754.1	478.4	5406.88
2013- 2014	42.87	260.16	38.6	492.4	26.1	134.5	47.7	780.5	506.87	5520.76
2014- 2015	44.1	273.74	38.55	492.37	26.12	134.92	35.57	606.08	556.16	7018.34
2015- 2016	45.1	272.5	43.53	494.61	20.25	106.94	46.48	793.24	558.73	6333.4
2016- 2017	47.02	283.84	47.17	464.62	25.54	190.72	46.68	817.94	577.81	5994.4
2017- 2018	36.2	171.01	46.21	561.61	38.42	229.1	45.94	795.68	563.93	5928.74
2018- 2019	34.6	177.16	46.21	561.61	38.8	231.39	45.53	791.13	586.971	6711.582
CAGR %	**11.1**	**2.7**	**17.8**	**25.7**	**3.4**	**6.2**	**3.8**	**6.1**	**4.4**	**2.9**
R^2	0.429	0.078	0.722	0.782	0.242	0.455	0.53	0.684	0.913	0.366
b_1	1.111	1.027	1.178	1.257	1.034	1.062	1.038	1.061	1.044	1.029
II	**25.95**	**24.62**	**21.83**	**25.19**	**18.94**	**21.38**	**10.18**	**11.22**	**3.82**	**1.112**

A: area; P: production

Table 4 : State wise area in '000 hectares and production in '000 M Tonnes of select vegetables

Year	Meghalaya (Potato)		Sikkim (Peas)		Tripura (Brinjal)	
	A	P	A	P	A	P
2013- 2014	18.43	3.52	2.02	9.27	3.52	53.56
2014- 2015	18.47	3.71	2.02	9.27	3.71	56.25
2015- 2016	18.56	3.65	2.05	8.85	3.65	70.87
2016- 2017	18.9	3.68	2.46	10.62	3.68	79.8
2017- 2018	18.91	3.62	4.1	17.7	3.62	77.88
CAGR %	**0.7**	**0.5**	**17.5**	**15.4**	**0.5**	**11.6**
R^2	0.883	0.14	0.701	0.618	0.14	0.881
b_1	1.007	1.005	1.175	1.154	1.005	1.116
II	**0.42**	**1.86**	**19.56**	**18.65**	**1.86**	**6.11**

A: area; P: production

diseases may be some of the factors responsible for the negligible (0.7%) annual growth rate in the production in Mizoram.

Despite a decline of 6.8% in the annual growth rate of the area under cultivation in Meghalaya, production has increased at the rate of 12.2% per annum. But both the area under cultivation and production has recorded high instability. However, production of two of the major spice crops i.e., turmeric and chilli cultivated in Meghalaya and Nagaland respectively have registered positive per annum growth rates in production during 2013-14 to 2017-18. The instability index for turmeric is 3.72% for the area under cultivation and 6.10% for production, while that of chilli is 3.97% and 3.61% respectively (Table 6). The low instability indices of the two crops suggest that

with the use of climate and disease resilient planting materials, better irrigation facilities, and adoption of organic farming and modern technology stable production of spices can be achieved in these states.

The region as a whole has witnessed a lower annual growth rate in production (3.4%) compared to the area under cultivation (7.6%). Spice production is found to be moderately unstable at 20.17%. This indicates that the region is yet to realise its true potential in Spice production. However, the organically produced spices of the region, namely, the Lakadong turmeric having high curcumin content, the less fibrous Nadia ginger, and the highly pungent Bird's eye chilli and King chilli have niche markets both within and outside the country. With the recent interventions by the Spices Board and the implementation of various development schemes of the Horticulture Mission for North East and Himalayan Region (HMHEH), the region is poised to become the organic 'spice hub' of the country and generate huge exportable surplus.

Export potential

Being strategically located with an international boundary of 5182 kilometers, the Northeast region is the gateway to India's connectivity with South - East Asia and ASEAN countries. Despite the presence of lucrative markets in the neighbouring countries of Bangladesh, Nepal, Bhutan, and Myanmar, the region has a negligible share in the total horticulture exports from India. Khasi mandarins, pineapples, gingers, and chillies grown in the region have a good market in Middle East countries like Saudi Arabia, UAE, Qatar, Oman, and Bahrain (APEDA, 2016). Tables 7 (a) and (b) indicate that during the period 2011-12 to 2015-16, fruits and spices have predominantly been exported from the region. Tomato is the only vegetable that has been exported every year with occasional inclusion of peas, cabbage, radish, etc. It is observed that though orange and ginger constitute the major share of the export basket of Assam, the share of tomato has also increased over the years. On the other hand, ginger and betel nut have fetched higher foreign exchange for Meghalaya during the period. However, Tripura's exports have been mainly fruits and that of Manipur have been vegetables. With the increase in the number of commodities exported from the region in 2015-16, it may be inferred that horticulture exports are gradually picking up. The major exports during the year were ginger, orange, and apple for Assam, dry chilli for Manipur, and orange for Tripura. Though

Table 5 (a) : State wise area in '000 hectares and production in '000 M Tonnes of spices

Year	Arunachal Pradesh		Assam		Manipur		Meghalaya	
	A	P	A	P	A	P	A	P
2009-2010	7.63	43.34	27.37	18.55	8.89	7.84	17.41	72.01
2010- 2011	10.1	61.6	89.2	222.1	10.5	24.1	16.8	71.4
2011- 2012	10.1	61.6	93	261.6	10.5	24.1	74.8	16.85
2012- 2013	10.17	64.27	96.66	287.5	10.47	24.14	74.81	17.5
2013- 2014	10.17	64.27	93.08	279.14	10.47	24.14	83.88	17.5
2014- 2015	10.17	64.27	98.6	321.03	10.47	24.14	17.5	83.88
2015- 2016	11.44	68.72	100.53	333.69	10.47	24.14	18.37	90.26
2016- 2017	11.44	68.72	119.99	291.3	10.47	23.14	18.61	92.16
2017- 2018	11.44	68.72	101.6	302	10.5	23.1	18.7	92
2018- 2019	11.64	71.29	103.24	312.61	10.61	23.99	18.18	91.7
CAGR %	**3.5**	**3.7**	**9.1**	**19**	**1**	**6**	**-6.8**	**12.2**
R^2	0.696	0.605	0.4	0.362	0.302	0.248	0.09	0.203
b_1	1.035	1.037	1.091	1.19	1.01	1.06	0.932	1.122
II	**6.28**	**7.79**	**20.29**	**27.86**	**4.14**	**19.88**	**76.82**	**46.19**

A: area; P: production

Table 5 (b) : State wise area in '000 hectares and production in '000 M Tonnes of spices

Year	Mizoram		Nagaland		Sikkim		Tripura		North East	
	A	P	A	P	A	P	A	P	A	P
2009-2010	22.67	80.63	7.22	38.62	26.58	41.73	3.96	12.1	121.73	314.82
2010- 2011	21.4	110.5	7.5	38.5	24.4	52.4	5.8	18.1	185.7	598.7
2011- 2012	20.6	115	9.8	39.2	24.4	54.4	5.7	18	248.9	590.75
2012- 2013	22.47	59.62	9.77	39.16	26.56	60.08	5.69	18.04	256.6	570.31
2013- 2014	22.47	59.62	9.77	39.16	32.06	55.8	5.69	18.04	267.59	557.67
2014- 2015	23.3	65.72	9.77	39.16	34.08	61.14	5.69	18.04	209.58	677.38
2015- 2016	24.57	68.89	15	119.25	29.46	64.78	5.69	18.04	215.53	787.77
2016- 2017	24.81	97.2	15.69	105	32.25	66.58	5.69	18.04	238.95	762.14
2017- 2018	27.7	100.9	9.9	64.8	32.3	66.6	6.6	32.4	218.74	750.52
2018- 2019	27.66	100.93	9.95	67.26	32.54	69.05	6.15	30.22	219.97	767.05
CAGR %	**3**	**0.7**	**5.3**	**10.8**	**3.4**	**4.7**	**3**	**7.8**	**7.6**	**3.4**
R^2	0.789	0.006	0.391	0.484	0.636	0.829	0.451	0.64	0.654	0.2
b_1	1.03	1.007	1.053	1.108	1.034	1.047	1.03	1.078	1.076	1.034
II	**4.68**	**24.89**	**20.77**	**36.96**	**7.48**	**5.78**	**8.75**	**18.54**	**11.3**	**20.17**

A: area; P: production

Table 6 : State wise area in '000 hectares and production in '000 M Tonnes of select spices

Year	Meghalaya (Turmeric)		Nagaland (Chilli)		Sikkim (Ginger)	
	A	P	A	P	A	P
2013- 2014	2.17	12.53	5.82	41.9	9.3	52.11
2014- 2015	2.17	12.53	5.82	41.9	9.3	52.11
2015- 2016	2.54	15.86	5.4	40.08	10.03	54.99
2016- 2017	2.61	16.63	6.01	44.86	12.3	55.9
2017- 2018	2.65	16.5	5.98	44.5	12.3	55.9
CAGR %	**6**	**8.7**	**0.9**	**1.9**	**8.7**	**2.1**
R^2	0.852	0.814	0.102	0.403	0.864	0.852
b_1	1.060	1.087	1.009	1.019	1.087	1.021
II	**3.72**	**6.10**	**3.97**	**3.61**	**5.35**	**1.37**

A: area; P: production

ginger, betel leaf, and betel nut were the major exports from Meghalaya, lesser quantities of tomato, bay leaf, and orange fetched higher revenue in the international markets. This reflects the high export potential of commodities produced in the region. With the availability of better market intelligence and export linkages, the marketable surplus of horticulture crops of the region can fetch higher revenue in terms of foreign currencies.

SWOT analysis

Strength

The Northeast region produces a large variety of temperate and tropical fruits, vegetables, and spices that are of good quality and high on nutrition. The region exhibits a rising trend in growth rates with low instability in area and production of fruits and vegetables and moderate instability in spice

Table7 (a) : Export of horticultural commodities through various land custom stations (LCS) in NER (2011-2012 to 2015-16)

State	Commodity	2011-2012		2012-2013		2013-2014	
		Quantity (MT)	Value in INR	Quantity (MT)	Value in INR	Quantity (MT)	Value in INR
Assam	Ginger	5040.29	83754578	16655	220796080	-	-
	Tomato	2.99	35976	6.596	98986	25.9	322000
	Onion	-	-	-	-	612	17252000
	Orange	6297.144	139224050	257186	89176570	1230.7	16307000
	Apple	-	-	-	-	-	-
	Mango	-	-	-	-	110	1548000
	Banana	-	-	-	-	-	-
	Pomegranate	-	-	-	-	-	-
	Grapes	-	-	-	-	4.25	112000
	Citrus	117.96	1307629	157.863	2132166	840.35	14424000
	Betel leaf	88.38	3109925	60.481	2927246	-	-
	Betel Nut	-	-	337	2304937	-	-
Manipur	Dry Chilli	-	-	-	-	-	-
	Peas	-	-	-	-	620	22315000
	Other Vegetables	-	-	-	-	81.41	5633000
	Dry Grapes	-	-	-	-	-	-
	Betel Nut	-	-	48	1728000	-	-
Meghalaya	Betel leaf	-	-	-	-	-	-
	Ginger	-	-	852	11458575	-	-
	Bay leaf	-	-	-	-	-	-
	Tomato	-	-	-	-	13.17	5000
	Cabbage	-	-	-	-	-	-
	Radish	-	-	-	-	-	-
	Orange	-	-	-	-	-	-
	Betel Nut	144	973742	337	2304937	10	103000
Tripura	Banana	11.44	49538	6	30229	4	24000
	Apple	-	-	-	-	-	-
	Pomegranate	-	-	-	-	-	-
	Orange	0.11048	132577	-	-	-	-
	Citrus	0.4651	533548	44.148933	2457024	-	-
	Litchi	-	-	-	-	-	-
	Grapes	-	-	-	-	-	-
	Ginger	5.51	201821	7.781	34364	-	-
	Vegetable seeds	-	-	-	-	-	-

Data Source: APEDA

Table 7 (b) : Export of horticultural commodities through various land custom stations (LCS) in NER (2011-2012 to 2015-2016)

State	Commodity	2014-2015		2015-2016	
		Quantity (MT)	Value in INR	Quantity (MT)	Value in INR
Assam	Ginger	6770.044	112944962	5239.693	94137239
	Tomato	35.534	498000	36.13	686880
	Onion	-	-	-	-
	Orange	811.732	10151500	1774.495	29642547
	Apple	-	-	401.421	10094384
	Mango	6.784	115000	17.996	354428
	Banana	-	-	3	16456
	Pomegranate	-	-	16.23	391059
	Grapes	-	-	2.763	69075
	Citrus	1.82	27000	-	-
	Betel leaf	11.244	592000	26.403	1401851
	Betel Nut	-	-	-	-
Manipur	Dry Chilli	-	-	448	33600000
	Peas	200	74	-	-
	Other Vegetables	-	-	-	-
	Dry Grapes	-	-	-	-
	Betel Nut	-	-	268.14	44243100
Meghalaya	Betel leaf	-	-	844.87	197543.55
	Ginger	1589.5	283.77146	1413.08	25820237.07
	Bay leaf	-	-	48.98	186105.86
	Tomato	-	-	175.23	1282832.68
	Cabbage	-	-	4.25	24545.88
	Radish	-	-	1.9	12250.5
	Orange	-	-	18.7	181844.39
	Betel Nut	36.5	4.10676	763	9348646
Tripura	Banana	21	109000	2.50	14723
	Apple	-	-	22.09	568783
	Pomegranate	-	-	5.5	115444
	Orange	-	-	388.826	7782638
	Citrus	-	-	-	-
	Litchi	0.5	8100	-	-
	Grapes	0.5	8700	-	-
	Ginger	-	-	-	-
	Vegetable seeds	-	-	0.15	76645.43

Data Source: APEDA

production. This indicates that the horticulture sector offers high scope for expansion and sustainable development. Majority of the crops that can have a good market in the neighbouring countries of Bangladesh, Nepal, Bhutan, and Myanmar, can be commercially produced for export. A large number of highly nutritious fruits and vegetables that are exclusive to the region can also be promoted. If farmers are given incentives to diversify into the production of horticulture crops through dissemination of modern farming techniques, provision of irrigation facilities, cheap credit, storage, and marketing facilities, then the hitherto underutilized sector can generate higher employment and income, both in domestic and foreign currencies, for the region.

Weakness

Despite the comparative advantage the region has in terms of soil and climate, yet the sector awaits commercialization. Traditional methods of cultivation with excessive dependence on monsoon result in less than the optimum yield of many horticulture crops, thereby generating a less marketable surplus. Table 8 shows the poor availability of storage and packaging facilities in the region. The absence of such facilities reduces the waiting time significantly and farmers are forced to sell their produce in the domestic markets at a lower price. Establishment of large number of cold storages, setting up of modernized cold chains, establishment of integrated packaging houses and processing units, greater market linkages will induce farmers to diversify to horticulture to reap higher returns.

Opportunity

Close proximity to South and South-East Asia opens up opportunities of not only trade in primary horticultural products but also offers a market for processed products that will generate higher foreign revenue. The existing market for horticulture produce especially in the Middle East can be leveraged to enter into markets of the developed countries of the West. Hence, all efforts should be made to expand the market by increasing the production of both primary and processed horticulture products that comply with international quality standards and have a competitive edge in the export market. Development of the horticulture sector in the region will boost the volume of trade and accelerate the growth and development of the predominantly agrarian economy.

Threat

If cold storages and food processing units are not established across the region, then the neighbouring countries, especially the rapidly developing Bangladesh, will import the fruits, vegetables, and spices from the region and make value additions by processing them to produce a large range of products

Table 8 : Available infrastructure in Northern as on 31.03.2018

State	Cold Storage		Cold Chain Projects		Pack House	
	Number	Capacity (MT)	Number	Capacity (MT)	On Farm Pack House	Integrated Pack House
Arunachal Pradesh	2	6000	1	3983	16	0
Assam	37	163258	31	160250	25	0
Manipur	3	7100	1	1600	187	7
Meghalaya	4	8200	1	5000	459	0
Mizoram	3	3971	1	3471	82	0
Nagaland	4	7350	1	5000	437	1
Sikkim	2	2100	1	100	134	2
Tripura	14	45477	5	24027	7	0

Data Source: National Horticulture Board, National Horticulture Mission, Directorate of Marketing and Inspection (DMI) up to 2009, Ministry of Food Processing Industries (MOFPI), MIDH

that will have a lucrative market in South-East Asia and the ASEAN. To augment export demand and expand the market share, it is imperative to increase productivity, establish processing units that can produce a wide range of customized processed products based on market demands and quickly upgrade the value chain to take full advantage of early entry into the export market.

CONCLUSION

The Northeast region is strategic for greater integration with South - East Asia and ASEAN countries. The development of the economic corridor will boost the volume of trade and flow of foreign exchange into the country. Horticulture crops of the region being highly nutritious, rich in minerals, vitamins, and dietary fibers can constitute a significant part of the agro-based export basket and propel the growth of the region. Being a labour-intensive activity, horticulture has huge potential to provide gainful employment opportunities in the agrarian sector and improve livelihood. Development of the sector will generate backward and forward linkages that will encourage investments in food processing units, cold storages, and packaging units thereby yielding higher income via the multiplier effect. To unleash the true potential of the sector, the state governments of the region should develop rural infrastructure, adopt a development strategy that is more resilient to variations in climate and pests, organize capacity building programs on scientific methods of cultivation and provide farm inputs at subsidized rates to induce cultivators to adopt horticulture on a commercial basis. The establishment of a seamless value chain with efficient market linkages can transform the sector into a driver of growth for the region.

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Short Communication

Diversity assessment of Nerium accessions for growth and flower yield

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ABSTRACT

Thirty nerium accessions were evaluated for growth and flower yield. Each accession had specific vegetative and flowering traits, among them ACC- 19 (Rasipuram pink double) recorded the maximum plant height (236.84 cm) and flower yield per plant (333.09g). ACC-2 (Panamarathanpatty white single) recorded the maximum number of primary branches (6.80), leaf area (33.61 cm^2), early flower bud initiation (90.47), flower bud length (3.40), number of inflorescences per plant (24.17), number of flowers per plant (10.67) were maximum in ACC- 12. Accessions 12 (Rasipuram pink single) displayed profuse blooming and long-lasting blooming characteristics, which made them an excellent choice for commercial cultivation and landscaping.

Keywords : Commercial, evaluation, flower, genotype, landscape and nerium

Nerium (*Nerium oleander* L.) is an evergreen shrub belongs to the Apocynaceae family native to Northern Africa and the Mediterranean region. Globally, it is well acclaimed as ornamental due to its abundant and long-lasting flowering habit and for its heat, salinity and drought tolerance capacity (Adome *et al.*, 2003). *Nerium oleander* L. is one of the important ornamental flowering shrubs which finds a place in all gardens. This ornamental shrub is suitable for commercial cultivation all over the tropical region. The nerium is used as loose flowers for religious purposes, garland making and worship in home and temples. In addition, they are preferred for growing as shrubs in the garden along a boundary wall to mask some areas of lawn. In recent days, nerium has great demand in landscape architecture for the beautification of home gardens, industrial gardens, public gardens, road dividers in highways, railway stations, airport surroundings and historical monuments. The ornamental plant market is extremely dynamic and demands constant novelties. To meet such needs, advances in genetic improvement programs aligned with the demands of consumers are crucial. These flowering plants exhibit considerable diversity with respect to growth habits, flower colors, shape, size and color patterns. These flowers are relatively easy to grow, begin flowering as young plants, continue to produce flowers throughout the year. The proper selection of nerium cultivars is critical for success and expected to increase yield by enhancing the number and size of flowers. Cultivars that respond well in local climatic conditions protect themselves from the depredation of insect, pest and diseases and as result, vigorous growth occurs to face the seasonal hazards. The selection of suitable cultivars depends on the purpose for which crop has to be grown (i.e.) used for loose flowers, ornamental shrubs and pot culture and also adaptability to specific growing places.

The present study was conducted at the Department of Floriculture and Landscaping, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore. The experiment was laid out in a randomized block design with two replications and thirty genotypes as treatments. Five plants from each genotype for each replication were randomly selected for recording observation on growth, flowering and flower yield parameters. The variability among nerium accessions is presented in Fig.1. The data collected under field experiment in randomized block design subjected to analysis of variance (ANOVA) using AGRES 3.01 and AGDATA software. The mean values of the treatments were compared using LSD at 5 per cent level of significance.

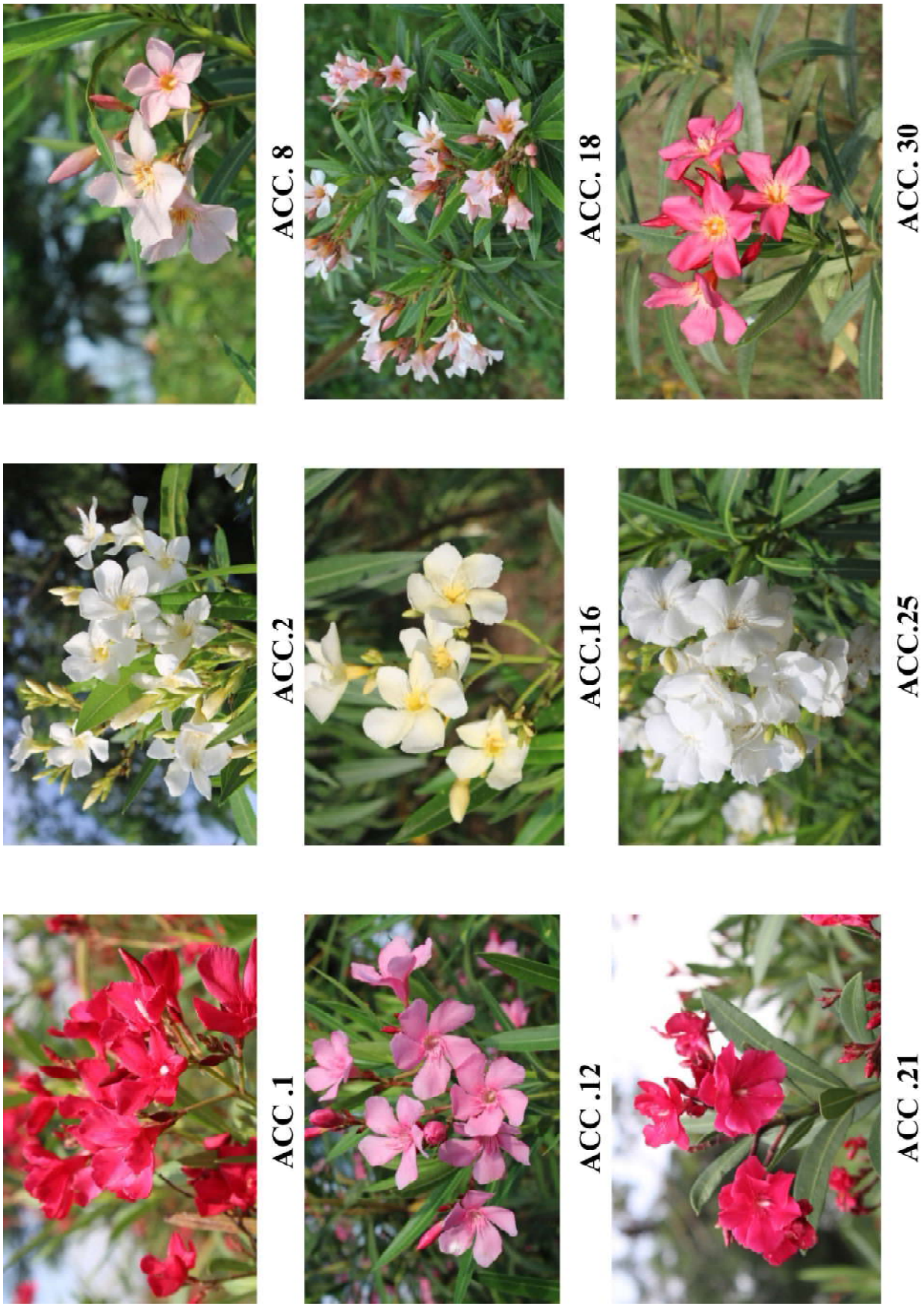

Fig. 1 : Diversity in flower colour of different nerium accessions

The vegetative growth was measured in terms of plant height (cm), number of primary branches, leaf length (cm), plant spread and leaf area (cm^2). Significant differences were observed in plant height during crop growth of nerium accessions at 12^{th} month after planting. The plant height ranged between 74.63 to 236.84 cm. Acc.19 recorded the maximum plant height of about 236.84 cm and it was on par with Acc.20 (234.67 cm) and minimum plant height (74.63 cm) was recorded in Acc.18. The variation in plant height among the accessions could be due to genetically controlled factors, which varies among the genotypes as well as influenced by the growing environmental conditions. This result was in accordance with Sharova *et al*. (1977) and further reported that the increased plant height in certain accessions might be associated with the rapid meristematic activity, probably due to rapid cell division and elongation during the growth period. Similar variation in plant height among cultivars was also observed in Nerium (Rajiv *et al*., 2018), Crossandra (Bhosale *et al*., 2018; Priyanka *et al.*, 2017) .With respect to the number of branches per plant, Acc.2 recorded a maximum number of primary branches (6.80) which was on par with Acc.3 (6.58 Nos.). Whereas, Acc.18 recorded the minimum number of primary branches (3.65). Increased number of branches leads to the production of more leaves which in turn enhances the yield of flowers by increasing the source and sink relationship. A similar trend was noticed by Chowdhuri *et al.* (2016) in different China aster genotypes, Gupta *et al.* (2015) in Dahlia; Ramachandrudu and Thangam (2010) in Crossandra. Among the accessions highest leaf length was recorded in Acc.9 (27.80 cm) followed by Acc.11 (24.79 cm). The lowest leaf length was registered in Acc.18 (8.89 cm). The highest leaf area was observed in Acc.12 (33.61 cm^2) which was on par with Acc.16 (33.39cm^2). The lowest leaf area was observed in Acc.18 (9.38 cm^2). The differences in the length and leaf area might be due to the genetic influences of the genotypes and this variability may be associated with adaptability to the climatic conditions (Costa *et al*., 2009). Similar observations were made by Pal *et al*. (2018) in Balsam, Priyanka *et al.* (2017) in crossandra and in hibiscus (Seeruttun and Ranghoo-Sanmukhiya, 2013). Significant results were obtained for plant spread in different nerium accessions. Acc.3 recorded maximum plant spread 152.18 cm (N-S) and 156.77 cm (E-W) which was on par with the Acc.12 (151.36 cm and 153.66 cm) and the minimum plant spread was recorded with Acc.18 (76.28 cm and 79.47 cm). An increase in plant spread might be due to the production of more number of branches and by the genetic nature of the plant. Variation in plant spread is due to additive gene effects (Vidalie *et al*., 1985). The data related to flowering and flower yield parameters of different Nerium accessions are presented in (Table.1). The number of days taken for flower initiation varied significantly among the accessions. The earliest flower buds appeared in Acc.12 (90.47 days), while Acc.24 recorded the maximum number of 115.75 days. The difference in flower initiation indicated that supplementary dry matter accumulation during favorable climatic conditions might be the reason for earliness. Similar results were obtained in china aster (Rai and Chaudhary, 2016) and chrysanthemum (Srilatha *et al.*, 2015).

Significant differences were observed in flower weight, the maximum flower weight (0.94 g) was recorded by Acc.20 which was statistically on par with Acc.19 (0.90 g) and the minimum flower weight (0.15 g) was recorded in Acc.18. The variation in flower weight might be primarily determined by the size of the flower head and number of whorls of the variety, which may be influenced by the inherent characteristics of the particular cultivar and the environment. Similar variation was also observed in China aster (Rai and Chaudhary, 2016) and Chrysanthemum (Talukdar *et al.,* 2003) With respect to flower diameter, the Acc.20 recorded maximum flower diameter (5.15 cm) followed by Acc.19 (5.13 cm). The minimum flower diameter was recorded in Acc.18 (2.49 cm). With regard to flower bud length, it was observed that Acc.12 (3.40 cm) recorded maximum flower bud length, which was statiscally on par with Acc.14 (3.34 cm) and Acc.28 (3.30 cm). Minimum flower bud length was recorded by Acc.18 (2.62 cm). Number of inflorescences per plant and number of flowers per inflorescence varied significantly among the accessions which directly influenced the yield of the plant. The number of inflorescences per plant ranged from 7.17 to 24.17. The highest number of inflorescence (24.17 Nos.) was recorded in Acc.12 followed by Acc.3 (24.04) and Acc.14 (23.0 Nos.), while the lowest number of inflorescences per plant was recorded in Acc.24 (5.34). Number of flowers per inflorescence ranged from 3.87 to 10.67. The highest number of

Table 1 : Evaluation of nerium accessions for flowering parameters

Accession No.	Days taken for flower initation (days)	Single flower weight (g)	Flower diameter (cm)	Flower bud length (cm)	Number of inflore-scences per plant	Number of flowers per inflore-scences	Yield (g/Plant)
Acc. 1	100.82	0.27	4.79	3.08	16.73	9.58	197.33
Acc. 2	96.17	0.24	4.86	3.08	18.12	8.89	183.75
Acc. 3	97.53	0.27	4.80	3.29	24.04	10.09	262.52
Acc. 4	109.89	0.30	4.89	3.20	11.17	9.51	151.57
Acc. 5	97.28	0.24	4.03	2.88	14.67	10.67	171.02
Acc. 6	94.37	0.27	4.97	3.38	10.83	8.83	140.63
Acc. 7	114.08	0.24	4.62	3.12	14.67	9.00	172.17
Acc. 8	107.83	0.21	4.86	3.26	11.83	8.50	120.89
Acc. 9	113.98	0.25	4.91	3.20	12.17	8.83	135.47
Acc. 10	104.73	0.27	4.46	3.12	14.67	9.35	136.05
Acc. 11	113.58	0.23	4.39	3.14	13.50	9.89	172.61
Acc. 12	90.47	0.29	4.80	3.40	24.17	10.67	265.37
Acc. 13	98.10	0.23	4.84	3.26	10.00	7.51	115.65
Acc. 14	93.87	0.27	4.74	3.34	23.00	10.00	258.33
Acc. 15	98.89	0.23	4.60	3.26	9.33	8.33	126.23
Acc. 16	109.09	0.24	4.13	3.26	12.00	8.00	127.87
Acc. 17	120.12	0.23	4.42	3.06	9.83	7.67	134.03
Acc. 18	91.14	0.15	2.49	2.62	8.13	6.30	98.87
Acc. 19	100.63	0.90	5.13	3.00	18.83	4.83	333.09
Acc. 20	101.41	0.94	5.15	2.96	17.98	4.67	329.49
Acc. 21	104.37	0.67	4.26	2.94	15.42	4.33	281.29
Acc. 22	103.65	0.24	4.84	3.18	12.51	9.67	148.01
Acc. 23	95.82	0.27	4.59	3.24	14.67	8.67	160.12
Acc. 24	115.75	0.67	4.56	2.92	9.17	4.33	191.02
Acc. 25	120.89	0.57	4.17	2.90	11.93	4.83	209.45
Acc. 26	104.13	0.24	4.79	3.14	12.00	9.33	136.81
Acc. 27	119.82	0.50	4.20	2.85	11.31	3.85	193.33
Acc. 28	92.00	0.29	4.99	3.30	14.67	9.17	216.18
Acc. 29	106.79	0.70	5.08	2.88	10.17	4.17	290.45
Acc. 30	98.37	0.25	4.87	3.00	9.35	8.90	156.78
Mean	103.85	0.36	4.61	3.11	13.90	7.95	187.21
SE(D)	4.37	0.02	0.18	0.13	0.60	0.35	7.83
CD (p=0.05)	12.66	0.05	0.52	0.36	1.74	1.02	22.71
CV (%)	5.95	6.74	5.54	5.71	6.12	6.24	5.92

flowers per inflorescence (10.67) was recorded in Acc.5 and Acc.12 followed by Acc.3 (10.09). Acc.27 (3.85) recorded the lowest number of flowers per inflorescence. Number of inflorescences per plant and number of flowers per inflorescence, this might be due to the transport of photosynthetic assimilates to the developing floral buds which might be triggered by the amount of endogenous growth regulators in the flower (Halevy, 1987). Variations in the number of flowers per plant are related to recurrent blooming habit due to their genetic makeup (Manjula, 2005). The variation in the number of flowers may be due to the genetic nature of the cultivar and also the effect of agro-climatic conditions. The varietal differences for yield potential may also be due to attributed additive gene effect. This was in accordance with the findings of Prashanta *et al*. (2016) in tuberose and Ramachandrudu and Thangam, (2010) in crossandra. Flower yield per plant per year showed significant differences among the Nerium accessions. The highest flower yield was recorded by Acc.19 (333.09 g) followed by Acc. 20 (329.49g) and the lowest flower yield per plant per year were recorded by Acc.18 (98.87 g). The variation among the accessions with respect to flower yield might be due to increased flower size with a number of whorls in nerium. Further, being a genetic factor, variations were expected among the accessions of nerium. The higher yield might be due to increased morphological parameters *viz.,* plant height, more branches and leaf area which attributes in production of more photosynthates resulting in greater accumulation of dry matter which in turn leads to the production of more flowers per plant. Similar results were observed in crossandra (Ramachandrudu and Thangam, 2010), Priyanka *et al.* (2017), Rose (Shahrin *et al.*, 2015) and China aster (Tirakannanavar *et al.*, 2015).

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Short Communication

Phenotypic trait association studies in brinjal upon drought stress

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ABSTRACT

Eggplant is popularly known as poor man's vegetable. With respect to present situation of climatic challenges, fruit yield of eggplant is reduced due to drought or moisture stresses. In view of this condition, an experiment was aimed to study character association between yield and yield components in eggplant. The resultant outcome from correlation analysis computed among nine eggplant characters indicated that traits like plant height and total plant length at harvesting, fruit length and number of fruits per plant significantly correlated with fruit yield per plant. Whereas, traits like plant height and total plant length observed at harvesting stage, number of days for flower initiation, number of primary branches, fruit length and average fruit weight were significantly associated with fruit yield per plant under moisture stressed condition.

Keywords: Brinjal, drought, fruit yield, moisture stress and phenotypic correlation.

Brinjal is one of the most important solanaceous vegetables next to tomato, potato and chilli. Brinjal is enriched with high net content of nutrients like carbohydrates, proteins, and edible good fats, along with some minerals, vitamins, antioxidants and secondary metabolites. Eggplant is basically originated from India during 300 B.C. to 300 A.D. and distributed all across the country. Brinjal is economically grown as annual crop though it is a perennial plant. Eggplant mainly bears gradient violet big solitary flower but some cultivars or species bears clustered inflorescence with variable tinge color with five petals, five sepals, five stamens and variable length of stigma *i.e.,* long styled, medium styled and short styled flowers.

Eggplant is hardy crop and even sustains prolonged stress periods but many studies have been reported there was decrease in fruit yield upon increased moisture deficiency. In eggplant upon increased drought there would be sequential decrease in fruit length, circumference, width, average fruit weight, plant height, days for flower initiation and increased number of fruits and branches (Faizan *et al.,* 2021c) which would be drought susceptible traits. Whereas, increased leaf chlorophyll, membrane stability index, relative tissue water, epiculticular wax, root length, volume and number of secondary roots would be drought tolerant character for genotype selection (Faizan *et al.,* 2021b) more over upon drought induction cytological and molecular changes will also occur like certain gene expressivity (Faizan *et al.,* 2021a).

Screening genotypes based on particular trait or a character can be done on its genetic values like phenotypic and genotypic coefficient of variance, broad sense heritability as well genetic advance over mean that would help breeder to understand or find out material genetic variability and study influence of environment over trait exhibition while selecting elite genotypes. As fruit yield is a dependent trait majorly governed by additive gene action with the association of different traits. Therefore, it was directed that association studies of yield and yield components is an elementary protocol to find out elite genotypes upon correlated trait or character. Character association or correlation analysis is an appropriate statistical method to quantify the degree, range and explain nature of relationship sharing between two variables based on its intensity of association.

Our primary aim was to study the effect of moisture stress on physiological, root, yield and yield components and evaluation of genetic values present in research incurred material for experiment. In addition to these, in this experiment we are aiming to exhibit yield component association or relationship towards fruit yield.

Country wide collected fifty eggplant genotypes (Table 1) were sown in portray after treating with carbendazim and etiolated for three days and after 30 days of sowing seedlings were transplanted into pots. Experiment was designed with factorial completely random design which includes two factors *viz.,* (a) drought conditions (Normal moisture condition/control

Table 1. List of eggplant genotypes used in the present experiment

S. No.	Genotype	Source of collection
1.	Pusa Upkar	IIVR, Varanasi Uttar Pradesh
2.	Arka Kranti	
3.	Bhagyamati	
4.	Pusa Ankur	
5.	Pusa Bindu	
6.	Punjab Sadabahar	
7.	Aruna	
8.	Shobha	
9.	Swarna Manjari	
10.	CH-215	
11.	Jawahar Brinjal-8	
12.	Jawahar Brinjal-69	
13.	R-2580	Vegetable Research Station Kalyanpur, Uttar Pradesh
14.	R-2594	
15.	R-2591	
16.	Malapur Local	
17.	L-2232	
18.	R-2581	
19.	L-2230	
20.	M4	College of Horticulture, Mudigere
21.	M21	
22.	M17	
23.	Mattigulla	
24.	Ramdurga	
25.	Melavanki	
26.	M19	
27.	Very Green Long	Zonal Research Station, Chianky, Palamu, Jharkhand
28.	IIHR-322	
29.	Pant Samrat	
30.	IIHR-7	
31.	Long Green	
32.	Swarna Pratibha	
33.	Swarna Mani	
34.	Early Round Market	Hiriyur Local Collection (Chitradurga, Karnataka)
35.	Rampur Local	
36.	Hebbal Gulla	
37.	Round Green	
38.	IC354140	NBPGR, New Delhi
39.	IC90785	
40.	IC99676- Long	
41.	IC99676- Round	
42.	IC90691	
43.	IC354597-Round	
44.	Suvarna GP098	Suvarna Seeds Pvt. Ltd.
45.	Vijaya ARBH98	Vijaya Seeds Pvt. Ltd.
46.	CO-2	TNAU, Coimbatore, Tamil Nadu
47.	*S. macrocarpon*	College of Horticulture, Bangalore
48.	*S. indicum*	
49.	*S. torvum*	
50.	*S. mammosum*	

Fig. 1. Heatmap for comparative mean performance of eggplant genotypes over growth and yield parameters.

X_1- Plant height @ 90 DAT (cm), X_2- Total plant length @ 90DAT (cm), X_3- No. of days for flower initiation, X_4- No. of primary branches/plant, X_5- Fruit length (cm), X_6- Fruit circumference (cm), X_7- Ave. fruit weight (g), X_8- No. of fruits / plant, X_9- Fruit yield (g/plant); S- Moisture stress condition, n- Normal moisture conditions.

Table 2. Estimates of phenotypic correlation coefficients for 12 different characters in eggplant genotypes under normal moisture and moisture stress

Trait	Moisture condition	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9
X_1	r_n	1.000	0.768***	0.299***	0.161*	-0.332***	-0.196*	-0.16*	-0.009	-0.372**
	r_s	1.000	0.669***	0.181*	0.043	0.019	-0.163*	-0.308***	0.145	-0.294***
X_2	r_n		1.000	0.267***	-0.032	-0.323***	-0.17*	-0.177*	0.004	-0.318***
	r_s		1.000	0.315***	0.096	-0.132	-0.243***	-0.373**	0.17*	-0.412***
X_3	r_n			1.000	-0.074	-0.371***	0.053	-0.07	0.061	-0.152
	r_s			1.000	0.057	-0.144	-0.029	-0.17*	0.127	-0.316***
X_4	r_n				1.000	-0.062	-0.083	-0.149	0.141	-0.044
	r_s				1.000	-0.386***	0.025	-0.194*	0.158	-0.263***
X_5	r_n					1.000	0.118	0.258***	0.017*	0.409***
	r_s					1.000	0.089	0.165*	-0.282*	0.272***
X_6	r_n						1.000	0.651***	-0.426**	0.147
	r_s						1.000	0.527***	-0.481**	0.141
X_7	r_n							1.000	-0.507**	0.138
	r_s							1.000	-0.592**	0.392***
X_8	r_n								1.000	0.533***
	r_s								1.000	0.036
X_9	r_n									1.000
	r_s									1.000

* - Significance @ 0.5 (r>0.16), ** - Significance @ 0.01 (r>0.209), *** - Significance @ 0.005 (r>0.228), *** - Significance @ 0.001 (r>0.266) ; $r_{n:}$ correlation for normal moisture plants; r_s : correlation for moisture stressed plants.

X_1- Plant height @ 90 DAT (cm), X_2- Total Plant Length @ 90DAT (cm), X_3- Number of days for flower initiation, X_4- Number of primary branches/plants, X_5- Fruit length (cm), X_6- Fruit circumference (cm), X_7- Average fruit weight (g), X_8- Number of fruits per plant, X_9- Fruit yield (g/plant).

and moisture stress condition); (b) 50 eggplant genotypes with three replications. Moisture stress was induced for about 15 days during two critical stages of eggplant *i.e.,* flower initiation and fruit initiation stage. Furthermore, drought level was monitored by tensiometer regulated at 85 centibars. Upon experimentation, traits like number of days taken for flower initiation, plant height, total plant length, number of primary branches per plants, fruit length, fruit circumference, average fruit weight, number of fruits per plant, fruit yield per plant were recorded. Phenotypic correlation coefficient was done for moisture stress (r_s) and normal moisture condition (r_n) with the help of WINDOWSTAT *V.7.2*.

The phenotypic correlation coefficient was calculated by using mean data (Fig. 1) obtained from fifty eggplant genotypes after analyzing for variation. Significant variation was observed for all the eight traits except for the number of days for flower initiation.

Plant yield is a complex trait and direct selection for this character based on genetic estimates alone is not enough. Fruit yield is dependent on various other indirect component traits like plant height, number of branches, fruit length, fruit circumference, average fruit weight, *etc.* An acquaintance on the relationship between these traits helps in attaining the improved yield. A phenotypic correlation coefficient is an important appliance for the breeder which helps in selection of genotype for a complex trait through the selection of simpler traits. In this aspect, several studies reported significant relationships among the different pairs of the assorted characters of eggplant (Abd-El-Hadi *et al.,* 2004, Melad *et al.,* 2005). The phenotypic correlation of coefficient for both normal moisture (r_n) and moisture stress condition (r_s) has been presented in Table 2.

Fruit yield per plant in normal moisture has recorded a significant association with four traits *viz*., negative association with plant height at harvesting stage (r_n= -0.372), total plant length at harvesting stage (r_n= -0.318) and positive association with fruit length (r_n= 0.409) and number of fruits per plant (r_n= 0.533). Whereas, in case of moisture stress condition, six characters *viz*., negative association with plant height at harvesting stage (r_S=-0.294), total plant length at harvesting stage (r_S= -0.412), number of days for flower initiation (r_S= -0.316), number of primary branches (r_S= -0.263) and positive association with fruit length (r_S= 0.272) and average fruit weight (r_S= 0.392) had significant correlation with fruit yield per plant.

Under normal moisture condition, fruit yield per plant had a non-significant association with number of days for flower initiation, number of primary branches per plants, fruit circumference and average fruit weight. Whereas, under moisture stress condition fruit circumference and number of fruits per plant are non-significantly associated with fruit yield per plant. Under normal moisture, fruit yield per plant had significant association with plant height and total plant length at harvesting, fruit length and stage number of fruits per plant. This explains that fruit yield per plant increases upon increase in degree of the traits and these traits are having strong inherent association with fruit yield per plant.

However, under moisture stress, plant height and total plant length at harvesting stage, number of days for flower initiation, number of primary branches, fruit length and average fruit weight showed significant association with fruit yield per plant. This explains that throughout moisture stress fruit yield increases upon decreased rate plant height and total plant length at harvesting stage, number of days for flower initiation and number of primary branches. Whereas, fruit length and average fruit weight increased upon moisture stress condition this because of material which incurred for experimentation constitutes of maximum drought tolerant germplasm.

The positive significant association between fruit length, average fruit weight and number of fruits per plant with fruit yield per plant is in conformity with the findings of Kranthi and Celine (2013); Singh and Kumar (2004); Nayak and Nagre (2013); Akter and Rahman (2019). However, the negative significant correlation between plant height and total plant length at harvesting stage, number of days for flower initiation and number of primary branches with fruit yield per plant is similar with the resulted reported by Gobu (2015); Dhaka and Soni (2014); Thirumurugan (1997), Reddy (2003) and Murugavel (2006).

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Short Communication

First report of *Lasiodiplodia theobromae* causing leaf spot on *Flacourtia montana,* a wild edible fruit tree of Western Ghats, India

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ABSTRACT

***Flacourtia montana* J. Graham wild edible fruit tree, endemic to the Western Ghats, India was found infected with leaf spot disease. Based on morphological characteristics, molecular analyses (ITS and LSU) and pathogenicity, the pathogen was identified as *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Botryosphaeriaceae).This is the first report of *L. theobromae* causing leaf spots on *F. montana* from Western Ghats, India.**

Keywords: *Flacourtia montana*, *Lasiodiplodia theobromae,* leaf spot, Western Ghats

Flacourtia montana J. Graham (Salicaceae), is a wild edible fruit tree, endemic to Western Ghats, India, commonly known as Mountain Sweet Thorn and distributed in evergreen and semi-evergreen forests at an altitude upto 1000 m asl. It is a potential fruit-bearing tree as the fruits are rich in reducing and non-reducing sugars, proteins, essential micro and macronutrients (Mundaragi *et al*., 2015, 2017). The fruits were good source of natural antioxidants, so can be used as a functional food and for pharmaceutical applications (Chand and Azeez, 2021). The commercially viable wine prepared from the fruit contains major phenolic acids and had free radical scavenging property (Mundaragi *et al*., 2019). Traditional practitioners residing in the Kattunaikka tribe in Wayanad Wildlife Sanctuary, Kerala, use the bark decoction for liver disorders (Ratheesh *et al.,* 2011). The leaves possess hepatoprotective, anti-inflammatory and antioxidant activities (Joshy *et al*., 2016).

Lasiodiplodia theobromae (syn. *Botryodiplodia theobromae*), is a ubiquitous pathogen associated with the dieback of woody trees and horticultural crops in tropical and subtropical regions. The pathogen could cause severe damage to various tissues (twigs, bark, vascular tissue and fruits) of affected plants and lead to economic loss (Pavlic *et al*., 2007). The study aimed to identify and characterize the causative agent associated with the leaf spot of *F. montana* based on morphological, molecular and pathogenicity studies.

Infected leaves of *F. montana* showing typical symptoms of leaf spot diseases were collected from Vazhachal forest areas (N 10° 17.028' E 076° 37.726'; ± 272m asl), Vazhachal Forest Division, Thrissur Dist., Kerala during February-March 2021 (Fig.1a). The initial symptoms of this disease appear as very small rust brown zone that gradually increases from 5 to10 mm in diameter, changing from circular to elliptical lesions on the leaves (Fig. 1b & 1c). Gradually lesions enlarge and coalesce; causing diseased leaves to become blighted (Fig. 1d).

Infected leaves were cut into small pieces; surface sterilized with mercuric chloride for 1 min, washed in sterile distilled water, placed on potato dextrose agar (PDA) plates and incubated at 27°C for 7 days. After incubation, morphologically distinct colonies were selected, purified and used for further studies (John *et al*., 2021). Colony morphology including colour, shape and growth rate was determined after 7 days of incubation on PDA at 25°C in darkness. Slide cultures were prepared by agar cubes and incubated at 25±2°C for 3-5 days, until adequate growth and conidiogenesis had occurred. After incubation, the slide culture and fungal morphological structures were observed

under an Olympus SZX2 stereomicroscope and Leica DM2000 LED compound microscope with a DS-5Mc camera.

Pathogenicity test was evaluated on healthy *F. montana* leaves using the universal protocol (Koch's postulates). It was performed by inoculating actively pathogens to healthy leaves and the fresh leaves without inoculation sprayed with distilled water served as control. The inoculated leaves were maintained at 27°C for 7 to 12 days in a plastic box with wet sterile filter paper for the observation of disease symptoms (John *et al.*, 2021).

Genomic DNA was extracted from the pure cultured fungal plate isolate (KFRIMCC315) using the NucleoSpin ® Plant II Kit (Macherey-Nagel). The internal transcribed spacer (ITS1–5.8S-ITS2) region of rDNA was amplified using the universal primers ITS-1F & ITS-4R and larger subunit (LSU) using the universal primers LROR & LR7 to verify the identity of the fungus (White *et al.,* 1990). The PCR product was electrophoresed in a 1.2% agarose gel with a 2-log DNA ladder marker. The PCR amplified product was purified by gel ExoSAP-IT (GE Healthcare) and subjected to direct sequencing using BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufacturer protocol. Obtained Sequences (ITS & LSU) were compared with those from the Genbank database using the BLAST software on the NCBI website. The multiple sequence alignment was performed by using the Clustal W program for the KFRIMCC315 sequence along with 3 sequences of other isolates of the *Lasiodiplodia theobromae* and *Diplodia alatafructa* and *Aspergillus candidus* as outer group (Thompson *et al.,* 1994). Phylogenetic tree was constructed by MEGA6 software using the maximum likelihood method with a bootstrap of 1000 replicates (Tamura *et al.*, 2013).

Cultures of the isolates grew and spread as velvety, effuse white on PDA after 7 days (Fig.1e). The isolated fungal pathogen was observed with white-grey fluffy mycelia (Fig. 1f) and become brownish black with age (Fig. 1g). Blister like fruiting bodies was produced on PDA after 20-25 days (Fig.1h). The hyaline and cylindrical pycnidial paraphyses were observed. Conidia were ellipsoidal with a broadly rounded apex and thick-walled, contents granular. Initially, the conidia were hyaline and aseptate (Fig.1i), and then become dark brown with 1-septa (Fig.1j&1k). The average size of the conidia is 24-27×10-12µm (n=20). From these morphological characteristics, we concluded that the isolated fungal species belonged to the genus *Lasiodiplodia*. A reference specimen (KFRIMCC315) was deposited in the culture collection of the Plant Pathology Department of Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala.

After the molecular study, the obtained sequences of the present pathogenic fungus (ITS-365 bp, and LSU-661bp) were deposited in the Genbank as MZ707764 and MZ707765 respectively. The sequence was analyzed through BLAST homology search the against NCBI database (https://blast.ncbi.nlm.nih.gov/Blast) and showed 100% similarity with the ITS and LSU sequences of *L. theobromae* isolates originated from different crops (Table 1). In the phylogenetic tree, the representative isolate KFRIMCC315 (Dot marked) was placed within the same clade comprising reference strains of *L. theobromae* (Fig. 2 a&b). Therefore, the pathogenic fungus was correctly and authentically identified as *L. theobromae* based on both morphological and molecular characteristics.

For the pathogenicity test, control leaves remained symptomless and healthy (Fig. 3a), while inoculated leaves shows brown, necrotic, margins hairy with yellow hallow circular spots after 6-7 days of inoculation (Fig. 3b & 3c). The initial lesions were observed after three days. The symptoms on the inoculated plants were similar to those observed in the infected plant in the field. Fungi re-isolated from lesions developing on the inoculated leaves were found to be morphologically and microscopically identical to the original isolates used for inoculation studies thus fulfilling Koch's postulates. The experiment was performed in triplicate. The results revealed that *L. theobromae* was found as causal agent of leaf spot of *F. montana.*

Based on morphology, molecular analyses and pathogenicity, the pathogen was identified as *Lasiodiplodia theobromae* from leaves of *F. montana.* The Literature survey indicates that there was no record of *L. theobromae* on *F. montana* from all over the world (Farr and Rossman, 2021).

L. theobromae is a pervasive pathogen belonging to the family Botryosphaeriaceae associated with approximately 500 hosts including perennial fruit and

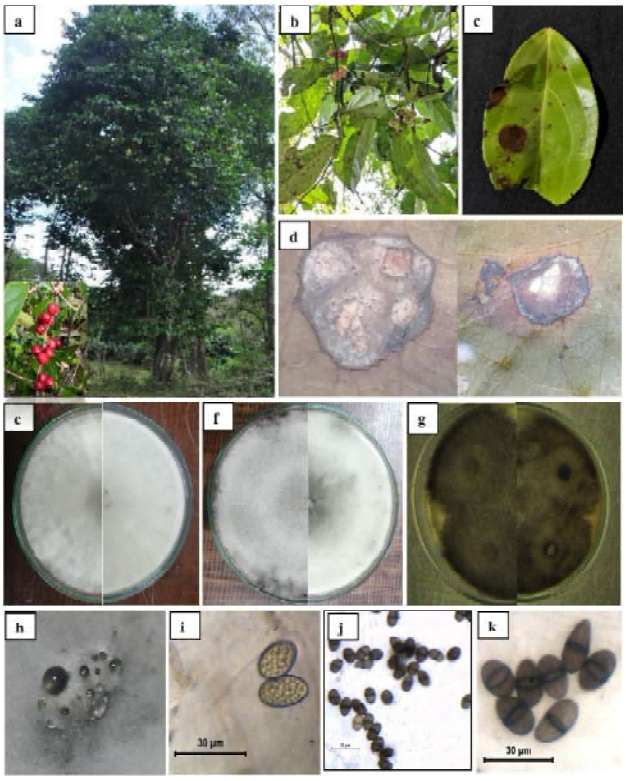

Fig. 1 (a) : *Flacourtia montana* habit (inset) Close up of ripened fruits (b) Infected leaves (c) Upper surface infected leaf (d) Enlarged view of infected portions (e) Upper and lower view of 7-day-old colony on PDA (f) Upper and lower view of 30-day-old colony on PDA (g) Upper and lower view of more than one month -old colony (h) Fruiting body (i) Immature and hyaline single celled conidia (j) & (k) Mature, brown and septate (2 celled) conidia. Scale bar = 20µm and 30µm

nut trees, vegetable crops, and ornamental plants (Punithalingam, 1980) and occurs also as an endophyte (Rubini *et al*., 2005; Mohali *et al.,* 2005). It causes damage to several crops and trees in India including root rot and collar rot disease of physic nut in India (Latha *et al*., 2009); peduncle blight of tuberose in India (Durgadevi *et al*., 2019); leaf spot of *Parthenium* (Kumar *et al.,* 2000); dieback of cocoa (Kannan *et al.,* 2019); immature nut rots in cashew (Prathibha *et al*., 2017); tip blight disease of *Dracaena fragrans* (Banerjee *et al.,* 2017); top dying disease of *Rauvolfia serpentina* (Dadwal *et al*., 2011).

The accurate identification of Botryosphaeriaceae species is necessary to determine the global distribution of these pathogen contribute to develop effective disease management strategies, because these species differ considerably in their interactions with different hosts and environmental conditions (Britton and Hendrix, 1986) Denman *et al*., 2003.

In the current study, based on morphological characteristics and molecular analyses and pathogenicity, the pathogen isolated from *F. montana* was identified as *L. theobromae.* This is the first report of *L. theobromae* causing leaf spots on *F. montana* from India. Since, the plant is ecologically and economically very important, the leaf spot caused by *L. theobromae* is of great concern. Therefore, the early detection and appropriate remedy against this pathogen is necessary to protect the plants from this disease.

Table 1 : Isolates of *Lasiodiplodia theobromae, Aspergillus candidus, Diplodia alatafructa* retrieved from Genbank.

Species name	Isolate	GenBank accession number	
		ITS	LSU
*L. theobromae**	KFRIMCC315	MZ707764	MZ707765
L. theobromae	MRR-153	MT075447.1	N/A
L. theobromae	MRR-130	MT075443.1	N/A
L. theobromae	MRR-126	MT075440.1	N/A
L. theobromae	L3	N/A	MN181372.1
L. theobromae	N/A	N/A	KC442316.1
Aspergillus candidus	ATCC1002(ITS) TUMS1390(LSU)	NR_077149.1	JQ846017.1
Diplodia alatafructa	CBS 124931	NR_111416.1	N/A

*From this study

Fig. 2a : Phylogenetic tree based on ITS sequences

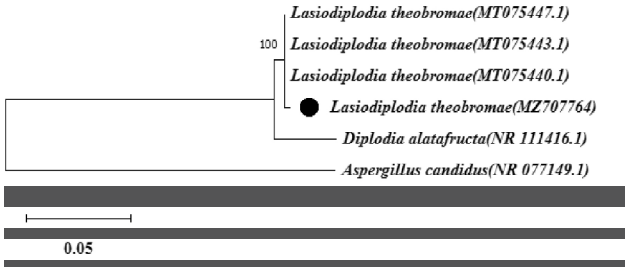

Fig. 2b : Phylogenetic tree based on LSU sequences

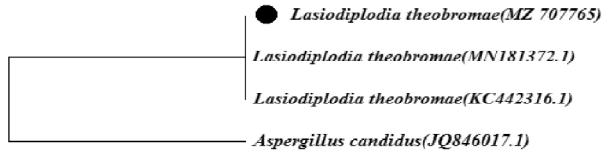

Fig. 2a, b : Phylogenetic tree representing the genetic relatedness of *Lasiodiplodia theobromae* with other isolates of *Lasiodiplodia theobromae*. *Aspergillus candidus, Diplodia alatafructa* is used as out group retrieved from Genbank, inferred by the maximum likelihood method using the ITS (2a) and LSU (2b) sequences. The robustness was evaluated with 1,000 bootstrap replicates. Our isolates are shown in bold dot mark.

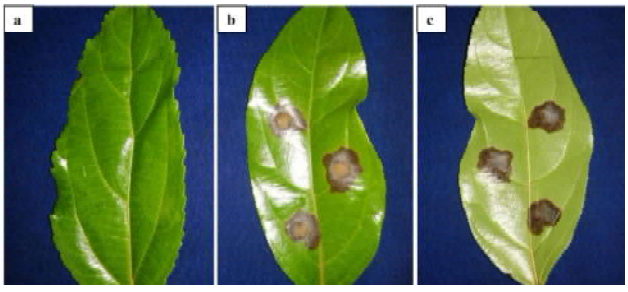

Fig. 3 (a) : Uninoculated leaves; (b) & (c) upper and lower view of leaves inoculated with fungal mycelium disc.

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