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Conserving Honey Bees with Forage Plant Mexican Creeper - *Antigonon leptopus*

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In this issue…

Hearty New Year Greetings from our Editorial Team to all the readers of JHS!

As the world is slowly coming out of glitches of pandemic, there is no other better way than celebrating 2021 as Year of Fruits and Vegetables as announced by United Nations Assembly to welcome the new year and recognize the importance of nutrition for better health. Fruits and Vegetables ensure the Nutritional Security to humankind. They play key role in addressing the malnutrition that is a major concern. We are proud that JHS creatins awareness of importance of fruits and vegetables by publishing the recent developments in research with respect to these crops.

Diversity of fruit crops and genetic resources available with respect to fruit crops are important for developing better fruit crop varieties. ***Sankaran and Dinesh*** *have reviewed the "Biodiveristy of Fruit Crops in India" in a very comprehensive way. There is diversity in Jasmine species.* ***Ganga et al.*** *carried out the palynological investigations and recorded the variability in pollen morphology in different species of Jasmine by documentating images using scanning electron microscope. Biodiversity can be linked to livelihood also. One such success story with tamarind selection 'Lakhamna' is being reported by* ***Kanupriya et al.*** *This tamarind selection has been identified from participatory breeding programme. It has a better pod characters and more preferred by consumers.*

Protected cultivation has seen greater momentum in last two decades. ***Adeniji et al.*** *identified the best varieties of tomato for polyhouse cultivation in Nigeria.* ***Rao et al.*** *selected two gladiolus hybrid selections IIHRG-7 and IIHRG-11 with red purple and red coloured flowers respectively. These hybrids have resistance to Fusarium wilt and suitable for cut flower and flower arrangement purposes.* ***Sankaran et al.*** *analysed the variance for 6 quantitative and 30 qualitative traits in mango in 400 genotypes and identified 18 clusters. Selected genotypes from specific clusters can be used in hybridization programme.*

The production aspects are important in perennial crops. It is crop management that needs to be prioritized for enhanced yield. ***Adiga et al.*** *have reviewed the research work carried in "Canopy Management in Cashew", providing the wholistic view of cultural operations to have a better crop. Use of soilless medium in nursery industry is gaining importance. Best suited potting mixture for mango stone graft of cv. Alphonso has been identified by* ***Lad et al.*** *They found that cocopeat + leaf manure + compost (1:1:2) as pot mixture provided better plant growth.*

Growing Chrysanthemum in pots is practiced in home and terrace gardens. The cultivar Kikiobiory is well suited for this purpose. ***Thakur*** *has studied the nitrogen requirement for this cultivar and has come out with the recommendation of 300 mg of N per pot applied*

twice in September and October in Punjab for best results. In another study, ***Singh and Bala*** *confirmed that use of benzyl adenine at 200 ppm helped in extended vase life of Chrysanthemum morifolium flowers.* ***Nair et al.*** *recorded that foliar spray of 30:20:20 NPK at weekly interval recorded more number of flowers of Dendrobium cv. Singapore White with significantly longer spikes.*

Crop production is directly influenced by pollinators. Decline in honey bee population is a serious concern and to conserve the pollinators community approach through ecosystem services is required. ***Rami Reddy*** *reports the benefits of having ornamental plant Mexican Creeper (Antigonon leptopus) as forage plant. This creeper attracted all the four species of honey bees studied. This creeper can be used as bioindicator of honey bee population.*

Aravindaraj et al. *have reported the honey dew secretion by Thrips palmi and analysed the composition of it. They had identified different sugars present in the honey dew secretion of Thrips. Thrips not only cause direct damage but act as vectors of many plant viruses. Management of diseases in perennial crops is a challenge. Phytophthora incited root infection in citrus needs concerted efforts.* ***Ingle et al.*** *have demonstrated that use of potassium salt of phosphonic acid could help in management of Phytophthora root rot in Nagpur Mandarin.*

Mushrooms can fill the gaps in nutritional security as they are rich in nutritive value. Iron deficiency is important issue to be addressed. Iron fortified oyster mushroom products have been developed by ***Pandey et al.*** *The bioavailability of iron from Arka Mushroom Fe-Fortified Rasam Powder has been confirmed. In another study, the amino acid profile of 18 isolates of oyster mushroom species belonging to 4 species have been documented by* ***Azeez et al.*** *Quantification of essential and non-essential amino acids has been reported. Nutritionally superior isolates can be selected from these isolates.*

The editorial team of JHS expresses the sincere efforts of reviewers who really complement the publication processes. All scientists and scholars can utilize the open access of JHS. Recently FAO has made JHS available through AGRIS. It is indexed by Redalyc, CABI_Hort and Scopus. All subscribers, scientists and scholars are requested to continue their support in publishing quality information in ***Journal of Horticultural Sciences****.*

S. Sriram
Editor in Chief

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Review

Biodiversity of Tropical Fruits and their Conservation in India

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ABSTRACT

India is one of the 12 mega biodiversity centres with 2 biodiversity hotspots which are the reservoirs of plant genetic resources. India stands at 7th place in the global agricultural biodiversity status. Among fruit and nut crops, there are about 117 cultivated species with 175 wild relatives of which only 25 species have been domesticated. Genetic resources conservation of fruit trees is intricate and complex as they are belonging to various genera and species which require specific climate. Hence, *in situ* and *ex situ* conservation can go simultaneously. The western ghat and North eastern India are centers of diversity for several important native fruits including Mango, Jackfruit and Citrus. Apart from the major fruit crops, India is home to several underutilized fruit crops. However, due to increased pressure on land use several of the wild types, which are a great source of genes governing useful traits, are disappearing. Thus, there is an urgent need to conserve them in both *in situ* and *ex situ* conditions. The genetic diversity and modes of conservation of tropical fruits are discussed in this paper.

Key words: Conservation, *Ex situ*, Fruits, GIS, Germplasm, *In situ*, Tropical, Varieties and Wild species

INTRODUCTION

India is one of the reservoirs of plant genetic resources which stand at 7th place in the globe in terms of richness of agricultural biodiversity. There are about 117 cultivated species of fruits and nuts with 175 wild relatives of which only 25 species have been domesticated for the use. Genetic resources conservation of fruit trees is intricate and complex in view of vast diversity of tropical, subtropical and temperate fruits germplasm belonging to various genera and species available in the country and consequent requirement of specific and complimentary conservation approaches encompassing both *in situ* and *ex situ* conservation. Plant genetic resources are of great importance as they form the basic raw materials to meet the current and future needs of crop improvement programmes. A wider genetic base, thus, assumes priority in plant breeding research aimed at developing new varieties for increased crop production (Paroda, 1991). This diversity comprises of native landraces, local selections, elite cultivars and wild relatives of crop plants. The collection and conservation of this diversity in a systematic manner is the primary responsibility of all plant genetic resources institutes/ centres. The mention of use and cultivation of fruits can be seen in epics like 'Ramayana'. Plant genetic resources are thus our heritage, which need conservation for posterity.

During the long period of domestication, utilization and cultivation, a wide array of fruit crop variability got generated by natural means and through both conscious and unconscious selection. Huge wealth of variability also got generated/adapted and diversified by crop introductions in the exotic environment or through migration of human population.

Although, humankind has used only about 5,000 plant species worldwide to meet food and other needs, this number is just a fraction of the total world flora. With population growth, we are increasingly dependent on most productive plants. Today, only about 150 plant species are important in meeting the food (calories) needs of humans worldwide. Hence, there is a greater dependence on fewer plant species; 20 to 30 species? in global context (Harlan, 1975). This gradually, has resulted in the loss of native genetic

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resources, which are otherwise essential as building blocks of genetic diversity. It is estimated that there are about 500 species of tropical fruit trees in Asia Pacific Oceania region, which include 30 families and 59 genera (Arora, 1998). In Southeast Asia alone, there are 120 major fruit species and 275 minor fruit species (Verheij and Coronel 1992). In Asia, 50-60 species belong to the most important indigenous fruits (Arora and Rao 1996). Citrus, mango, banana, rambutan, jackfruit, litchi and durian occupy 80% of total fruit production in the region.

WILD SPECIES AND DIVERSITY

The role of wild species in the fruit improvement programme is increasingly becoming important as the donor source for many of the disease and pest resistance. However, in most of the perennial trees, wild species or the indigenous germplasm has not been evaluated extensively either by morphological or by molecular means. Some geographical? areas may be richer in biodiversity than other areas, and some species may also have more variation than others in a particular area.

Conservation of germplasm is very important, because many species are becoming extinct and many others are threatened and endangered. The diversity of some fruits is well documented, while for others relatively little work has been done (Arora, 1994). Gaps in collections are found both between species and between regions. This is especially true for both underutilized species and wild crop relatives, where big gaps are noted. Kostermans and Bompard (1993) indicate that *Mangifera blommesteinii, M. leschenaultii*, *M. superba* and *M. paludosa* are in real danger of extinction. High genetic erosion has been noticed for jackfruit, *Citrus* spp. and *Litchi chinensis* in a survey carried out by the International Centre for Underutilized Crops (ICUC) and IPGRI (Haq, 1994). It is to be mentioned here that collection and utilization of wild species is not an easy task, as they require specific climate and do not so easily get acclimatized to the *ex situ* conditions on introduction.

BIODIVERSITY OF FRUITS

The concept of origin of cultivated plants was first put forth by A de Candolle and the geographic centres of variability were described by Vavilov. He identified Asia as a major centre with "Indian centre" of North East region as primary or secondary centre of origin for many crop plants. This region is centre of diversity for several important native fruits including mango, jackfruit and citrus. The plant genetic resources represent a sum of the diversity that come from wild species and primitive forms, accumulated through evolution and natural selection, plant introduction, migration and domestication and the material developed by artificial selection and breeding. The North Eastern region had remained isolated for a long time even today the accessibility is poor to many parts of this region. The wet tropics with rain forests, undisturbed environmental conditions and variable altitudes are some of the major reasons for genetic diversity.

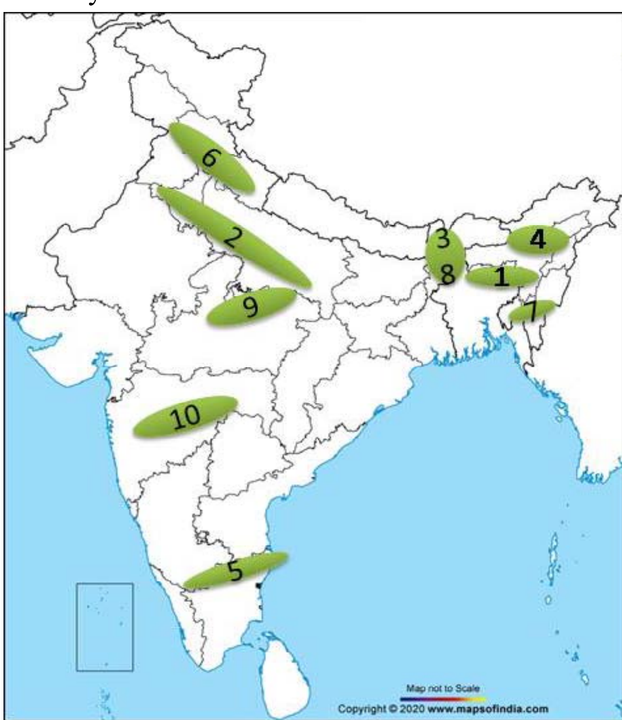

Fig.1. Distribution of fruit genetic resources in India

1. **North-eastern Himalayas-wild, semi-wild cultivated species**
2. **North-west- Semi-wild and cultivated types**
3. **South-centre - mostly cultivated types**

"Vast diversity in tropical and temperate fruits cultivated and wild - 109 species several wild, endangered and endemic species"

Biodiversity can be located both in the wild or in the backyard. Regarding many of the tropical fruit species, the variability can be traced in wild, wherein many species grow naturally even today viz., the occurrence of *Mangifera sylvatica* in the North-eastern parts of India or *M. andamanica* and *M. nicobariaca* in Andaman group of islands. In the wild

diversity was generated over a period mainly because of spontaneous mutants and the dispersal of seeds and seedling population. Seedling populations have been the source of diversity in the backyard as noticed in the case of fruits like mango and jackfruit. Diversity due to natural means has come about due to the seed dispersal as in pickling types of mango viz., Appemidi types in Uttara Kannada district of Karnataka or varietal wealth found in the Western Ghat regions.

CHARACTERISTIC FEATURES OF TROPICAL FRUIT TREE DIVERSITY

The main causes for the tropical fruit diversity in India be it mango or an underutilized fruit like jamun, whether in the wild or in the cultivated types have been;

1. the presence of high heterozygosity
2. cross pollination
3. seed propagation
4. absence of vegetative propagation in the earlier days
5. indiscriminate multiplication.

Unlike other crops, where there is a need to create variability, in tropical fruit species, it is the management of diversity, which is the more challenging task. In fact, in crops like mango, the varietal diversity itself is considered as a hindrance to the improvement (Naik *et al*., 1958).

Table 1. Main centres of diversity for fruits in India

Region	Species
Western Himalayas	*Elaeagnus hortensis*, *Ficus palmata*, *Fragaria indica*, *Moms spp.*, *Prunus acuminata*, *P. cerasiodes*, *P. cornuta*, *P. napaulensis*, *P. prostrata*, *P. tomentosa*, *Pyrus baccata*, *P. communis*, *P. kumaoni*, *P. pashia*, *Ribes graciale*, *R. nigrum*, *Rubus ellipticus*, *R. moluccanus*, *R. fruticosus*, *R. lasiocarpus*, *R. lanatus*, *R. niveus*, *R. reticulatus*, *Zizyphus vulgaris.*
Eastern Himalayas	*Fragaria indica*, *Morus* spp., *Myrica esculenta*, *Prunus acuminata*, *P. cerasiodes*, *P. cornuta*, *P. jenkinsii*, *P. napaulensis*, *Pyrus pashia*, *Ribes graciale*, *Rubus lineatus*, *R. ellipticus*, *R. lasiocarpus*, *R. moluccanus*, *R. reticulatus.*
North-eastern region	*Citrus assamensis*, *C. ichangensis*, *C. indica*, *C. jambiri*, *C. latipes*, *C. macroptera*, *C. media*, *C. aurantium*, *Docynia indica*, *D. hookeriana*, *Eriobotrya angustifolia*, *Mangifera sylvatica*, *Musa accuminata/M. balbisiana* complex, *M. manii*, *M. nagensium*, *M. sikkimensis*, *M. superba*, *M. velutina*, *Pyrus pyrifolia*, *P. pashia*, *Prunus cerasiodes*, *P. cornuta*, *P. jenkinsii*, *Ribes graciale*, *Rubus ellipticus*, *R. moluccanus*, *R. reticulatus*, *R. lasiocarpus*, *Myrica esculenta.*
Gangetic plains	*Aegle marmelos*, *Cordia myxa*, *C. rothii*, *Emblica officinalis*, *Grewia asiatica*, *Morus* spp.; *Phoenix* spp.; *Syzygium* spp.; *Zizyphus nummularia* and other species and *Manilkara hexandra* (more in north-western plains).
Indus plains	Meagre occurrence of *Syzygium*, rich variation in *Carissa congesta.*
Western peninsular tract	*Artocarpus heterophyllus*, *A. lakoocha*, *Garcinia indica*, *Diospyros* spp., *Ensete superba*, *Mangifera indica*, *Mimosops elengii*, *Spondias pinnata*, *Vitis* spp., *Zizyphus oenoplia*, *Z. rugosa*, *Rubus ellipticus*, *R. lasiocarpus*, *R. moluccanus.*

(Arora and Nayar, 1984)

Table 2. Wild relatives of some of the fruit crops

S. No.	Family	Species	Remarks
1	Anacardiaceae	*1. Mangifera andamanica* *2. Mangifera camptosperma* *3. Mangifera griffithi* *4. Mangifera nicobarica* *5. Mangifera sylvatica* *6. Semicarpus kurzii* *7. Spondias pinnata* *8. S. cytherea* *9. Bouea oppositifolia* *10. Dracontomelon dao* *11. Buchnania splendons*	Possess tolerance to biotic and abiotic stress
2	Annonaceae	1. *Annona muricata* L. (soursop) 2. *Annona reticulata* L. (bullock's heart) and 3. *Annona glabra* L	*A. glabra* is tolerant to salinity and could be suitably employed as a rootstock for other species of this group
3	Arecaceae	*1. Areca triandra* *2. Phoenix andamanensis* 3. *P. sylvestris* (L.) Roxb. *4. P. rupicola5.*	*P. paludosa* Roxb. All these five species are habitat of seashores
4 to	Clusiaceae	1. *Garcinia cowa* Roxb *2. Garcinia xanthochymus Hook.f* *3. Garcinia microstigma* *4. Garcinia speciosa* 5. *Garcinia dhanikhariensis* S.K.Srivast. 6. *Garcinia hombroniana* Pierre. 7. *Garcinia lancaefolia* Roxb. 8. *Garcinia andamanica*King. 9. *Garcinia brevirostris* Scheff. 10. *Garcinia cadelliana* King. 11. *Garcinia calycina* Kurz 12. *Garcinia cornea* Linn. 13. *Garcinia dulcis* (Roxb.) Kurz. 14. *Garcinia jelinekii* Kurz. 15. *Garcinia Kingii* Pierre ex Vesque 16. *Garcinia Kurzii* Pierre 17. *Garcinia lanessanii* Pierre. 18. *Garcinia mangostana* Linn.	About 36 species of *Garcinia* are reported be available in India of which 18 *Garcinia* species are found to exist in Andaman & Nicobar Islands. 6 species which are endemic to Andaman & Nicobar Islands ?? viz. *Garcinia andamanica* King. var. *andamanica*, *G. cadeliana, G. dhanikhariensis, G. kingii* Pierre ex. Vesque, *G. kurzii* Pierre. and *G. microstigma* Kurz.
5	Dilleniaceae	1. *Dillenia andamanica* C. E. Parkinson 2. *D. indica* L 3. *D. pentagyna* Roxb	Edible fruits are produced in all the three species.
6.	Ebenaceae	1. *Diospyrous blancoi* (velvet apple) *2. D. andamanica*	Fruit of *Diospyrus blancoi* has velvety surface and fragrant, cream-white flesh.
7	Euphorbiaceae	1. *Baccaurea sapida* (sapida) and 2. *B. ramiflora* (*khatta phal*)	*Fruits of B. ramiflora are rich in vitamin C.*

8	Moraceae	1. *Ficus carica* L. 2. *Ficus racemosa* L. 3. *Ficus hispida* 4. *Artocarpus heterophyllus* (jackfruit) 5. *A. altilis* (breadfruit) 6. *A. lakoocha* Buch.-Ham. (monkey jack) 7. *A. chaplasha* Roxb. (cham pedak)	*Artocarpus heterophyllus has 10 diversity centres in India. This is found in all states and it has multiple uses*
9	Musaceae	1. *Musa balbisiana* var. *andamanica* 2. *Musa paradisiaca* 3. *Musa indandamanensis* L. J. Singh 4. *Musa textilis* 5. *Musa sabuana*	Wild species of banana are rich in carotenoid content however the presence of seeds prevents the wider acceptability of the fruits.
10	Myrsinaceae	1. *Ardisia solanacea* Roxb. (Khaariphal) 2. *A. andamanica* Kurz.	These species are tolerant to salinity
11	Pandanaceae	1. *Pandanus andamanensium* Kurz 2. *Pandanus tectorius* Soland. Ex Parkinson 3. *Pandanus lerum* Jones ex Fontane var. *lerum* 4. *Pandanus lerum* var. *andamanensium* (Kurz.) D.C. Stone	Nicobari tribes extract the flour from the fruits and cake is prepared out of the flour. *Pandanus lerum* Jones ex Fontane var. *lerum*, and *Pandanus lerum* var. *andamanensium* (Kurz.) D.C. Stone are distributed in the swampy areas and *Pandanus tectorius* distributed in seashore.
12	Rhamnaceae	1. *Ziziphus glabrata* Heyne 2. *Ziziphus oenoplia* (L.) Mill var Oenoplia 3. *Ziziphus oenoplia* var *pallens* Bhandari & Bhansali	-
13	Myrtaceae	1. *Syzygium andamanicum* 2. *Syzygium hookeri* 3. *Syzygium kurzii* 4. *Syzygium sanjappaina* 5. *Syzygium manii* 6. *Syzygium claviflorum (wild jamun)* 7. *Syzygium aqueum (watery rose apple)* 8. *Syzygium samarnagense* 9. *Syzygium jambos* 10. *Syzygium malaccensis*	-
14	Myristicaceae	1. *Myristica andamanica* Hook.f. 2. *Myristica glabra* Blume 3. *Myristica glaucescens* Hook.f. 4. *Myristica irya* Gaertn. 5. *Myristica prainii* King 6. *M. elliptica* Wall ex. Hook. f. et Thoms. 7. *Knema andamanica* (Warb.) de Wilde ssp. *Andamanica*	*Knema andamanica* (Warb.) de Wilde ssp. *Andamanica, K. andamanica* (Warb.) de Wilde ssp. *nicobarica* (Warb.) and *Myristica andamanica* Hook.f are endemic to the

		8. K. andamanica (Warb.) de Wilde ssp. *nicobarica* (Warb.) *9. K. andamanica* (Warb.) W. J. de Wilde subsp. *peninsularis*	Andaman Islands.
15	Sapotaceae	1. *Manilkara littoralis* - Hindi - Sea Mohwa	Potential rootstock for Sapota
16	Menispermiaceae	*1. Haematocarpus validus*	Recorded from North Andaman. This crop has already been domesticated by a farmer in Diglipur area, North Andaman. The farmer has been identified as the custodian farmer
17	Vitaceae	*1. Vitis parviflora* *2. Ampelocissus barbata* (Wall.) Planchon *3. A. helferi* (Lows) Planchon *4. A. polystachya* (Wall) Planchon	*Vitis parviflora is being used in* grapes may be used in grape breeding programme as it has got reflexed stamen. Whereas the *Ampelocissus barbata* is used as a medicinal plant by the tribes of the Island.

(Sankaran *et al.,* 2014)

BIODIVERSITY OF TROPICAL FRUITS

1. MANGO

Mango is native to India. Mukherjee (1949,1985) opined that this genus might have originated in the region covering Burma, Siam, Indo-China and Malayan peninsula. The genetic diversity of mango available in India is very rich and at present more than one thousand vegetatively propagated varieties exists in the country. Clonal selection, selections from chance seedlings and breeding efforts have resulted in identification of many elite improved varieties of mango for commercial cultivation in the country.

All varieties in mango belong to one species *Mangifera indica*. Apart from *M. indica*, India is also reported to be the home of four other species viz., *M. andamanica*, *M. khasiana, M. sylvatica* and *M. camptosperma* (Mukherjee *et al.*, 1985). The species of *Mangifera* occur mainly as complex biotic community in tropical humid forests, sub-tropical rain forests and tropical dry forests/woodlands of Indo-Malayan biogeographic realm (Mukherjee, 1985).

The *Mangifera* germplasm can be classified under two categories;

1. Seedling races: This group includes both wild and cultivated types. Under this category the cultivated ones come under the monoembryonic types. The polyembryonic types are seen generally in the Western Ghats of Peninsular India.

2. Horticultural races: They include varieties, which when grown under different agro-climatic conditions and propagated vegetatively from the parent material have given rise to clonal variation Varieties like Alphonso, Dashehari and Langra are noticed to have clones resembling them in some of the morphological characters. Yadav and Singh (1985) opined that mango varieties of Northern and Southern regions belong to two different eco-geographic regions.

Centres of mango diversity and distribution in India

In India, seven centres of mango diversity have been recognized (Yadav and Rajan, 1993). These are the places where maximum diversity has been noticed for species as well as varietal diversity. They are:

1. Humid Tropical region-Manipur, Tripura, Mizoram and S. Assam
2. Chota Nagpur Plateau-Trijunction of Orissa, Bihar and Madhya Pradesh
3. Santal Paraganas in Bihar
4. South Madhya Pradesh adjoining Orissa and Andhra Pradesh
5. Dhar Plateau of Madhya Pradesh adjoining Gujarat and Maharashtra
6. Humid Tropical South Peninsular India
7. Andaman and Nicobar Islands

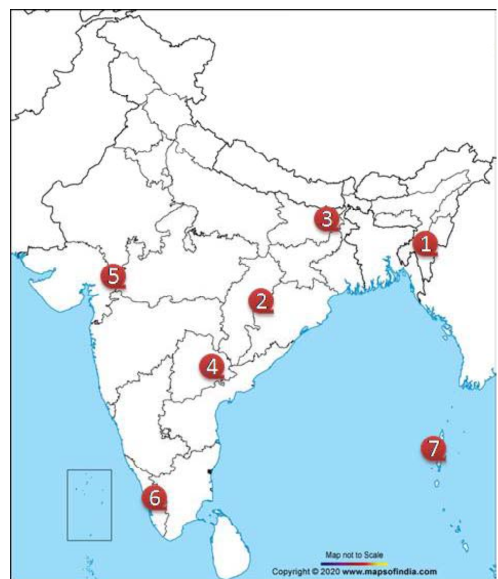

Fig. 2. Mango diversity centres in India

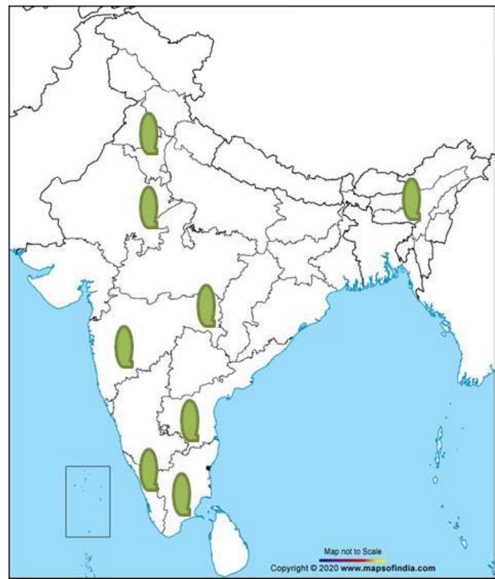

Fig. 3. Distribution pattern of citrus cultivars in India

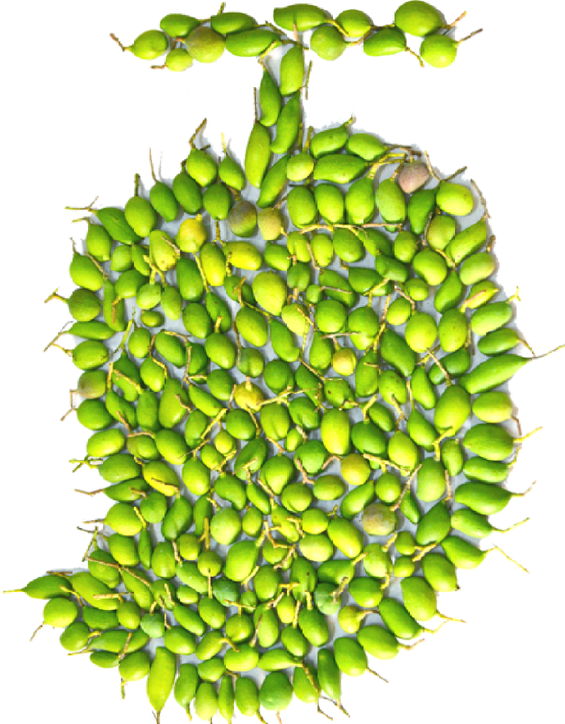

Fig. 2b. Appemidi Mango - an unique variety for pickle purpose

Fig. 2c. Diversity of mango germplam

Varietal diversity

In India about thousand varieties of mango are grown. Most of these varieties have arisen as chance seedlings. Each mango-growing region in India grows a different variety. Although, there are more than thousand varieties have been documented in India of which only twenty-five varieties are cultivated on a commercial scale in different states. Most of the commercial varieties have arisen as a result of

selection from seedling types for different fruit characteristics like colour, taste, flavour, size and bearing habit. Although, growth in mango is genetically controlled, the environmental interaction has brought about the change in growth pattern under different agro climatic conditions, which also has contributed for its biodiversity. In India, three main centres contributed to of the diversity of mango i.e., Lucknow - Saharanpur belt of Uttar Pradesh, Murshidabad area of West Bengal and Hyderabad area of Andhra Pradesh. Most of the varieties in these areas have specific fruit ?characteristics, require specific climate for optimum performance and have strong regional consumer preference.

2. CITRUS

The North East hilly region is rich in fruits, vegetables and flowers, especially orchids. It is considered as a centre of origin of Mandarins and few other citrus fruits. Sixteen species of Citrus, 52 varieties, and seven natural hybrids of Assam were described by Bhattacharya and Dutta as early as 1956. They also reported two species of sub genus *Eucitrus* viz., *C. indica* and *C. assamensis* and three species of sub genus of Papeda viz., *C. ichangensis, C. latipes* and *C. microptera* which grow at high altitudes. *C. indica* is considered to be the most primitive species of citrus and probable progenitor of cultivated species. Diverse forms of Pummelo, Sour Orange, Rough Lemon, Sour Pummelo, Adajamir Sweet Lime etc. are found in this region.

Varietal diversity

Mandarin orange is concentrated in Maharasthra (Nagpur, Amaravathi, Wardha and Yavatmal), North East region of India (Assam, Arunachal Pradesh and Meghalaya), limited area of Karnataka (Kodagu), Tamil Nadu (Nilgris, Palani and Shevroy hills) and Kerala (Wynad). Sathpura hills of Madhya Pradesh adjoining Vidharbha region of Maharasthra also grow good quality mandarins. Kinnow Orange, a hybrid of King X Willow Leaf Mandarin has recently spread in North West India, especially in Punjab, parts of Himachal Pradesh, Uttar Pradesh and Rajasthan. Cultivation of introduced varieties / hybrids also add to the varietal diversity by throwing spontaneous mutants over a period.

Sweet oranges are adapted well to arid tropics and sub tropics. They are commercially grown in Andhra Pradesh, Maharasthra, Punjab and parts of Tamil Nadu, Rajasthan and Utter Pradesh. in Andhra Pradesh Sweet orange cultivar Sathgudi is grown, whereas in Western and Central India sweet orange cultivar Mosambi is popular. In North Western India the cultivars Malta, Jaffa and Valencia are popular.

Acid lime is grown on a commercial scale in Tamil Nadu, Andhra Pradesh, Maharasthra and Karnataka states. Lemons are grown commercially only on a limited scale. Eureka lemon in some regions and Assam lemon in North Eastern India are popular varieties under cultivation.

3. BANANA

Bananas are one of the ancient fruits cultivated by man. It could be assumed that the fruit has evolved with the civilization (Krishnamurthi and Seshadri 1958) and found in Indus valley as early as in 327 B.C. Apart from its mention in Valmiki's Ramayana, it also finds a mention in Kautilya's Arthshastra and ancient Tamil classic Silappadikaram. These evidences suggested the early existence of banana in India. The wild *Musa acuminata* occurs in Assam, Burma, Siam, Indo-China, the Malayan peninsula and Archipelago and the Philippines.

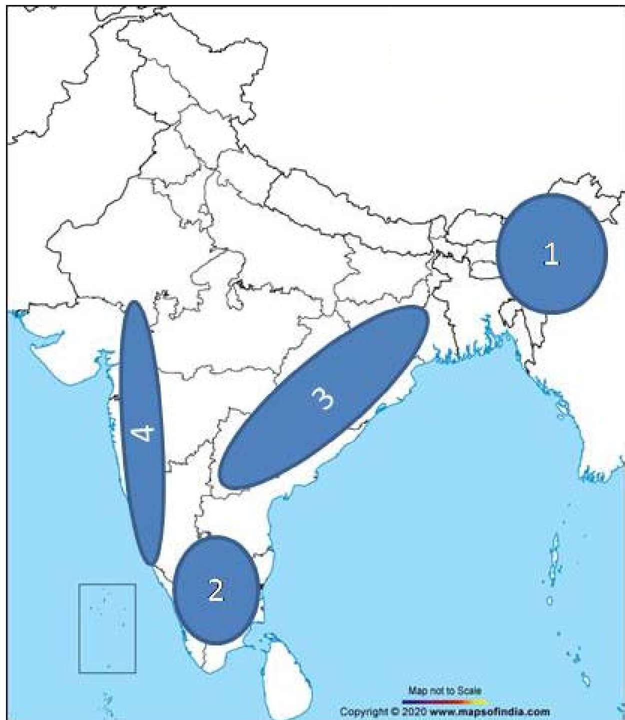

Fig 4. Distribution of banana cultivars in India

Table 3. Distribution of banana cultivars in India

Region/ State	Cultivars
Andhra Pradesh	Amrit Pani (Rasthali, AAB),Thella Chekkarakeli (AAA), Karpura Chekkarakeli (Poovan, ABB), Monthan (ABB), Robusta (AAA)
Assam	Bhimkol, Manohar (ABB), Chini Champa (AB), Malbhog (AAB), Jahaji (AAA), Bor Jahaji (AAA), Kanch Kol (ABB),
Bihar	Alpan (AAB), Chini Champa (AB), Basrai (AAA), Kothia/ Muthia (ABB), Bateesa (ABB), Malbhog (Rasthali, ABB).
Gujarat	Dwarf Cavendish (AAA), Lacatan (AAA), Harichal (AAA)
Karnataka	Dwarf Cavendish (AAA), Robusta (AAA), Poovan (AAB), Rasbale (AAB, Rasthali), Marabale (Pome, AAB), Monthan (ABB), Elekki Bale (AB, Ney Poovan)
Kerala	Nendran (ABB, plantain), Palayankondan (AAB, Poovan), Kunnan (AB), Rasthali (AAB), Monthan (ABB) and Red Banana (AAA)
Maharashtra	Basrai (Dwarf Cavendish, AAA), Robusta (AAA), Safed Velchi (AB), Monthan (ABB), Rajeli (Plantain, AAB)
Tamil Nadu	Virupakshi (Pome, AAB), Poovan (AAB), Rasthali (AAB), Nendran (AAB), Monthan (ABB), Dwarf Cavendish (AAA), Robusta (AAA), Peyan (ABB)
Bengal and Orissa	Champa (AAB), Morthaban (AAB, Rasthali), Amrit Sagar (AAA), Giant Grover (AAA), Lacatan (AAA), Monthan (ABB)

Singh, 1996

The northeastern region of India - including the states of Assam, Arunchal Pradesh, Tripura and Mizoram, lie at a point where *Musa balbisiana* from the Indian subcontinent meets *Musa acuminata* from Southeast Asia. These two species, as well as other wild relatives have mingled to form a distinctive concentration of genetic diversity, occurring in the semi-evergreen, sub-tropical forests of the hill slopes. Further sources of diversity occur in the valleys and plains where bananas are common as a backyard crop. The habitat of wild bananas is shared with tribal groups who practice a form of shifting cultivation. Unfortunately, a growing number of sites where wild bananas once grew are now denuded. Wild species found in North eastern part of India include *Musa acuminata, Musa balbisiana,* several species from the section *Rhodochlamys* as well as *Ensete glaucum*.

The diploid and triploid *acuminata* cultivars were taken by man to the native areas of *balbisiana*, which resulted in natural hybridization and formation of hybrid progeny with the genomes: AA, AAB, and ABB. It is thought that subsequent dispersal of edible bananas out of Asia was brought about by man. Secondary diversification with-in the groups of cultivated bananas are the result of somatic mutations.

Eumusa and *Rhodochlamys* are found in Assam region of India and Thailand whereas *Callimusa* and *Rhodochlamys* in Borneo, its surrounding

Table 4. Distribution of *Vitis* species in India

Species	Region	Salient Characters
V. riparia	North Westeran Himalayan region	Small berries, purple black in colour, cold hardy, early flowering
V .lanata	Himalayan region	Purple black berries known for crack resistance, and plant resistant to diseases
V. barbata	Parts of Assam, Khasi hills and Bengal	–
V. parviflora	North West Himalayas from Kashmir to Nepal	Small berries, delicately flavoured
V. tomentosa	Greater part of Deccan peninsula	–

The species *V. vinifera*, has not originated in India. However, it is very interesting to note that variety Thompson Seedless, which belongs to the species *V. vinifera,* and which is grown in several parts of the world as well as in India has contributed for the diversity in varieties; several mutants have come from the Thompson Seedless, Anab-e-Sahi and Kishmish Cherni grapes due to their extensive cultivation over the years.

5. GUAVA

Guava is an important fruit crop of India. It is said to have originated from tropical America. It is widely distributed all over the equatorial regions of tropical and sub-tropical climate. Guava is reported to have been introduced during Seventeenth century into India.

It has gained considerable prominence on account of its high nutritive value, availability at moderate prices, pleasant aroma and good flavour. It is one of the commonest fruits liked by the rich and the poor alike and is popularly known as the 'apple of tropics'. At present, it is grown throughout the length and breadth of the country from sea level to 1300 m altitude and is so much acclimatized that it appears to be native to India. The most important guava growing states are Uttar Pradesh, Bihar, Madhya Pradesh and Maharashtra.

The genus *Psidium* of Myrtacease family comprises of about 150 species of small trees and shrubs. About 20 species have edible fruits, of which the most cultivated is the common guava i.e., *Psidium guajava* L. It has been reported that the value of the wild *Psidium* species mainly lies in their utility as rootstocks for regulation of vigour, fruit quality and resistance to pests and diseases.

Varietal diversity

Guava is mainly a self-pollinated crop but cross-pollination is also common. This has resulted in large variability in the seedling population from which promising genotypes have been selected in different agro-climatic regions of the country. In India different workers in different regions have described guava varieties. The main centre of variability in guava has been the Allahabad area in Uttar Pradesh. The promising cultivars of different states are given as follows;

Table 5. Distribution of guava varieties in India

State	Cultivars
Andhra Pradesh	Allahabad Safeda, Anakapalli Banarasi, Chittidar, Hafsi (Red Fleshed), Lucknow-46, Sardar, Seedless, Smooth Green, Smooth White.
Assam	Amsophri, Madhuriam, Safrior payele.
Bihar	Allahabad Safeda, Chittidar, Hafsi (Red Fleshed), Harijha, Seedless.
Maharashtra	Dharwar, Dholka, Kothrud, Lucknow-24, Sardar,
Gujarat	Nasik, Seedless, Sindh.
Tamil Nadu	Anakapalli, Banarasi, Bangalore, Chittidar, Hafsi, Nagpur Seedless, Smooth Green.
Uttar Pradesh	Allahabad Safeda, Apple Colour, Chittidar, Red Fleshed, Banarasi Surkha, Sardar, Mirzapuri Seedless.
West Bengal	Behrampur and cvs. of Uttar Pradesh.

In India, due to seed propagation, varietal diversity is seen for guava, but species diversity is not observed.

6. PAPAYA

The papaya (*Carica papaya* L.) is one of the most important fruit crops valued for its rich nutrient content. It is a rich source of Vitamin A (2020 I.U), Vitamin B1 (40 mg), Vitamin C (46mg), protein (0.5%) and mineral matters (0.4%). Papaya is native to tropical America, its place of origin is said to be in southern Mexico and Costa Rica. It was taken to Manila by Spanish in the mid-16th century, reached Malacca shortly afterwards. It was introduced into India during 16th century. It is grown both in tropical and sub-tropical parts of the world. Wild diversity is not reported in India for papaya.

2.6.1.Varietal diversity

In India, varietal diversity is seen for papaya. The variability seen is more because of the open pollination and wide spread multiplication using these seeds. In papaya there are two basic types of varieties. Those varieties, which are dioecious, produce only female and male plants, and 'gynodioecious' that produce both female and hermaphrodite plants. Some of the varieties that are grown in different states are as follows;

Table 6. Distribution of papaya varieties in India

State	Cultivars
Andhra Pradesh	CO 2, CO 5, Sunrise Solo, Taiwanese lines
Bihar	Pusa Dwarf, Pusa Majesty, Pusa Nanha, Pusa Giant, Pusa Delicious and Ranchi
Karnataka	Coorg Honey Dew, Washington, Sunrise Solo, CO2, Surya and Taiwanese lines
Maharashtra	Washington, CO2, Pusa Delicious, Pusa Majesty, Ranchi and Taiwanese lines
Orissa	CO2, Coorg Honey Dew, Washington, Ranchi, Pusa Dwarf and Pusa Delicious
Tamil Nadu	C02, C03, CO4, CO5, CO6, CO7 and Coorg Honey Dew
Uttar Pradesh	Coorg Honey Dew, Pusa Dwarf, Pusa Delicious, CO3 and Barwani Red

islands, and Indonesia. *Australimusa* is largely found in Malayan islands, and Indonesia. It is also found in Assam, Indo-China, Malayan and Papua New Guinea, which is a primary centre of cultivated AA types. *M. balbisiana* occurred in Ceylon, India, Burma, Siam and Malaya where the A X B hybrids have evolved.

4. GRAPE (*Vitis* spp.)

European grape *V. vinifera* is considered to have originated primarily between Caspian and Black sea, and considered a hybrid between two American spp. *V. vulpine* and *V. labrusca*. It also resembles *V. parviflora* and *V. lanata* which are found in Himalayan region. This region may be considered as a secondary centre of origin. Native spp. resembling *Vitis lanata* and *V. palmata* grow wild in the northwestern Himalayan foothills. Indigenous varieties known as 'Rangspay', 'Shonltu White' and 'Shonltu Red' are grown in Himachal Pradesh even today.

Famous Indian medicine scholars, Sushruta and Charaka in their medical treatises entitled 'Sushruta Samhita' and 'Charaka Samhita', respectively, written during 1356-1220 BC, mentioned the medicinal properties of grapes. Kautilya in his 'Arthashastra' written in the fourth century BC, mentioned the type of land suitable for grape cultivation.

Cultivated grapes are believed to have been introduced into the north of India by the Persian invaders in 1300 AD, from where they were introduced into the southern parts of India (Daulatabad in Aurangabad district of Maharashtra) during the historic event of changing the capital from Delhi to Daulatabad by King Mohammed-bin-Tughlak. Ibn Batuta, a Moorish traveller who visited Daulatabad in 1430 AD, reported to have seen flourishing vineyards in south India.

7. SAPOTA

Sapota (*Achras zapota* L.) is a popular dessert fruit belonging to the family Sapotaceae. It is believed to have originated in tropical America, taken to Philippines by the Spanish and from there has spread to other countries (Purseglove, 1968).In India it is grown in the states of Andhra Pradesh, Gujarat, Karnataka and Orissa. About 30 varieties are reported in India at various places. Several locally grown genotypes identified include Bhuri patti, Morabba, Kali patti, Turi patti, Gole patti, Singapuri, Khabari and Chhumukia type. Among these, a genotype identified in Navsari, in Gujarat locally known as 'Morabba' bears fruits of bigger size and superior quality in comparison to Kalipatti, a local genotype grown in about 80% area of Gujarat. It is a selection from grafted plants collected from nursery located in Golwal. It may have originated as bud mutant and is now being propagated vegetatively. Another somatic mutation having desirable characteristics of the fruit was identified in Paria (Rai, 1995). Wild diversity is not observed for sapota, as it has been grown over the years by using grafts.

Biodiversity of underutilized fruits

In India various native fruits, such as aonla (*Emblica officinalis)*, bael fruit (*Aegle marmelos*), jackfruit (*Artocarpus heterophyllus*), jamun (*Syzygium cuminii*), karonda (*Carissa congesta*), Kokum (*Garcinia indica*) and phalsa (*Grewia subinaequalis*) with lot of diversity in a wide range of agro-ecological situations throughout the tropics, subtropics and temperate regions, which could be grouped as underutilized. Some of these fruits yield juice with excellent flavour, which can be converted into blended beverages and these could play an important role in meeting the demand for nutritious,

pleasantly flavoured and attractive natural food of high therapeutic value. Encouraging local people to produce these fruits can help in uncontrolled harvesting from the wild under check and conservation of various species in their native habitats where they perform best.

8. JACKFRUIT

Artocarpus is a genus of small to large evergreen trees, distributed from Sri Lanka and India to South China and through Malaysia to Solomon Islands. Nine species are recorded in India. The spp, *A. heterophyllus* Lam. is grown for their edible fruits, and *A. chaplasha* Roxb., *A. hirsutus* Lam. and *A. lakoocha* Roxb., are important timber trees.

A. chaplasha Roxb is distributed in the moist deciduous and evergreen forests of the sub-Himalayan tracts from Nepal eastwards to West Bengal, Assam and Tripura. In West Bengal and Assam, it occurs in moist types of mixed deciduous and evergreen forests. In Andaman and Nicobar Islands it is an important constituent of evergreen and deciduous forests.

A. cummunis J.R. & G. Frost, commonly known as bread fruit is found mainly in Westcoast and Western Ghats, Wynad, in the Nilgris, Lower Plains, the Courtallam hills and the Annaimalais. There are two distinct varieties in this species. One is a seeded type and the other entirely seedless. The seeded types are found in a wild state in its native and are of little economic value. It is not useful in culinary preparations but the seeds, which resemble chestnut, are relished when roasted or boiled.

A. heterophyllus Lam. commonly called as jackfruit is one of the most popular fruitsof South India. The tree is indigenous to the evergreen forests of the Western Ghatsat altitudes of 450 - 1200m, but seen growing throughout other hotter parts of India too. Because of seed propagation, the existing population of jack comprises innumerable trees differing from each other in fruit characters of shape, size and quality.

A. hirsutum Lam is commonly found in the evergreen forests of Western Ghats from Konkan southwards, is common in North Kanara and Kodagu in Karnataka to Kerala where it is an important tree. It requires heavy rain fall, not less than 174 cm annually and thrives well on lateritic soils at the foot of the Ghats. The tree can stand shade, but thrives best with a fair amount of light. It does equally well in the open and withstands exposure to sun after the first few years.

A. lakoocha Roxb, is commonly known as monkey jack. In its wild state it is chiefly found in the moist or deciduous forests along the banks of streams and along the site of moist ravines. It thrives best in deep laterite soils and generally comes to bear after about eight years. It is commonly cultivated throughout the greater part of India as a shade or ornamental tree. It is perhaps one of the foremost among neglected but useful trees. It is distributed in evergreen, semi-evergreen and moist deciduous forests upto an altitude of 1800 m in eastern and northern India. On the west coast it is found from Konkan southwards to Kerala, and in Tamil Nadu. It is also found growing in many localities in Andaman Islands.

9. AONLA

Aonla or Indian gooseberry (*Emblica officinalis* Gaertn.) is considered as a wonder fruit for health-conscious population. It is being grown in India for more than 3500 years. Sushruta, the father of ancient medicine (during 1500 BC-1300 BC), has mentioned about its usefulness in 'Ayurveda' in detail. It belongs to family Euphorbiaceae and is one of the important indigenous fruits of Indian subcontinent. In different parts of India, it is known by different vernacular names such as Amla or Aonla in Hindi (Pathak, 2003). The plant and fruit of aonla are regarded as sacred by 'Hindus' and have great mythological significance.

The aonla tree is native to tropical Southeast Asia, particularly central or southern India, Pakistan, Bangladesh, Sri Lanka, Malaya, Southern China and to Mascarene Islands. Seedling trees are of common occurrence in the mixed deciduous dry forests of India, ascending from sea level (western and Eastern Ghats, Aravali and Vindhya hills) to 1300 m above sea level, from northwest Himalayas (Jammu & Kashmir, Himachal Pradesh, Uttranchal) to eastern Himalayas in Assam, Meghalaya, Mizoram, Manipur and Tripura (Pathak, 2003). The natural distribution of wild aonla is found on the Himalayas, Chota nagpur, Bihar, Orissa, West Bengal, North Circars, Deccan, Karnataka and in WesternGhats (Rawat and Uniyal 2003).

In India, the homeland of aonla, domestication first began in Varanasi (earlier known as Benaras) district of Uttar Pradesh with the initiative of Maharaja of Kashi. Banarasi, a superior genotype was selected from the wild aonla trees available in large number in the nearby Vindhyan hills. Authentic information

regarding its cultivation dates to 1881-82 in the Pratapgarh district of Uttar Pradesh.

The wild aonla germplasm is mostly confined in the mixed forests with sloppy topography and sometimes even difficult to approach. A rich genetic diversity of aonla exists in northeastern region of India, particularly in lower Assam, Maghalaya, Mizoram and Tripura (Yadav *et al.,* 2001). Aonla grows abundantly in the forest of Khasi and Garo hills of Meghalaya and locally known as "Sohmylleng" (Pandey *et al.,* 1993). The natural population of aonla in west Khasi hills (Nongkhyllum, Rajaju, Khonjoy area) of Meghalaya warrants *in situ* conservation, which may even be declared as gene sanctuary for this species (Hore, 1998). Mizoram is homeland of wild aonla and star gooseberry (*Phyllanthus acidus*), which has potential as dwarfing rootstock for aonla. Wild Star gooseberry trees are found in forests of Kolasib, Thingdawl and Champhai in Mizoram. Madhya Pradesh forests have rich diversity of aonla. Jharkhand and adjoining areas of Chhatisgarh have rich diversity of aonla in the native forest. The important sites in Jharkhand are Lali forest near Ranchi, Dalma range of Jamshedpur, Theo Ghat forest of West Singhbhoom, Tiamara valley area between Ranchi and Jamshedpur, Ramgarh area of Hazaribagh, Parasnath hills of Girideeh, Kodemera and Jaomi areas of Bihar border, Simdega and Netarhat forest areas of Gumla, Belta forest of Daltonganj, Palamu and Garhwa of Jharkhand and adjoining areas of Sarguja and Ambikapur districts of Chha tisgarh and Sahdol district of Madhya Pradesh. The Belta forest (Daltonganj), Netarhat range in Gumla and Dalma range of Jamshedpur has comparatively high plant population of aonla in the natural habitat. In western and eastern ghats, three species of *Phyllanthus emblica, Phyllanthus indofisheri* and *Phyllanthus acidus* are of common occurrence. A wild strain of aonla grows in the Himalayas up to an altitude of 1600 m asl. The fruits of wild aonla are relatively smaller. In the mid Himalayas wild aonla is distributed right from western to eastern Himalayas including Nepal (Pathak, 2003).

10. BAEL

Bael (*Aegle marmelos*) is native to India and cultivated throughout the South East Asia and East Indian Archipelago. The genus consists of 2 to 3 species. It is found in UP, Bihar and West Bengal. Some important types selected in different regions are UP: NB 1, NB5, NB6: Bihar: Etawah Kagzi, Sewan Large, Mirzapuri and Deoria.

11. BER

Out of the 50 reported species nearly 18 to 20 are native to India. A resume of species availability in different locations in India are given below :

Species	Location
Ziziphus apatala, Z. *funiculosa*, Z. *incurva*	North-Eastern hills
Z. *mauritiana*, Z. *nummularia*	All over the drier tracts, particularly in North-West India and UP
Z. *oenoplia*, Z. *rugosa*	Throughout India except in drier tracts, particularly in Central and Eastern India
Z. *vulgaris*	North-Western Himalayas
Z. *rupicola*	Central and Eastern India
Z. *xylocarpus*	MP and peninsular region

There are more than 100 named varieties in ber and areas rich in variability have been identified in several places in UP, Rajasthan, Haryana, Gujarat, MP, Maharashtra, AP, Karnataka and Tamil Nadu. However, some popular cultivars are Umran, Gola Reshmi and Illaichi.

12. CUSTARD APPLE

The genus *Annona* contains some 120 species originating from warm countries but few important species became integral part of the Indian culture bearing the names of great heroes of the epic Ramayana. Some important species and their natural distribution are given below:

Species	Common name	Varieties	Location
A. squamosa	Sweet sop, Sharifa, Sitaphal	a) Green types: Balanagar, Mammoth, British Guinea, Washington-95,Barbadose seedling, Arka Sahan, (An F1 hybrid) (b) Red types: Red Sitaphal	Low and medium elevations throughout tropics
A. cherimola	Lakshmanphal	Cherimoyar	Cooler places in India
A. atemoya		Pinks mammoth, Bradley, Keller, Page, African Pride, Island Gem	Adopted to colder climate and alkali soils
A. reticulata	Ramphal or Bullock's Heart	Used mostly as a root stock	
A. glabra	Pond apple	Root stock	Flooded areas
A. muricata	Sour soup, Hanuman Phal	Root stock	Mountain regions of India
A. mantna	Mountain soursop	Used in breeding programme for quality	
A. purpurea	soncoya	Used as resistant source for fruit cracking	
A. scleroderma	Eoshto	Used in breeding programmes for thick hard shell	

13. FIG

The original home of origin of fig (*Ficus carica)* is South Arabia. There are four horticultural types in this crop viz., Smyrna, Capri, Sanpedro and Adriatic. This crop has very narrow range of diversity in India and there is a scope for introducing exotic germplasm. However, there are wild relatives found in India and some of them are given as under:

Species	Common name	Distribution
F. auriculata	Timla	Bihar,Orissa, Khashi hills, Manipur
F. benghalensis	Banyan	All over Inedia
F. benjamina		
F. carica	Fig	Uttar Pradesh, Rajasthan, Andhra Pradesh, Maharashtra, Karnataka
F. elastica	Indian Rubber tree	Assam and Khasi hills
F. glomerata	Cluster fig	
F. hispida		Throughout India
F. krishnae	Krishna's fig	
F. lucescens		North India, MP and W peninsula
F. palmata		N.W India and Rajasthan
F. religiosa	Peepal tree	All over India
F. rumphii		Punjab, MP, Assam
F. samicordata		Punjab, Assam, Bengal, Khasi hills and Manipur

14. SYZYGIUM

This genus *Syzygium* comprises about 1000 species of evergreen trees and shrubs; most of them are tropical in origin. Jamun is found in Western Ghats and very extensively in the tropical region. The diversity found is due to the high heterozygosity and seed multiplication. Some of the species are described below:

Species	Common name	Distribution
S. aqueum	Watery Rose-apple, Fruits edible	A small tree distributed in Assam and Meghalaya
S. arnottianum	Produces edible fruits	Western Ghats, The Nilgris, Palni and Anamalai hills
S. aromaticum	Clove, dried flower buds are of commercial importance	Evergreen trees cultivated in Tamil Nadu and Kerala
S. claviflorum	Fruits are acidic and edible	Andamans
S. cumini	Java plum, Jamun, Jambu	Throughout India
S. fruticosum	Wild Jamun	Avenue tree
S. jambos	Rose-apple	Many parts of India
S. mappaceum	Grown as ornamental plant	Assam, Meghalaya, Arunachal Pradesh and Tamil Nadu
S. samarangense	Wax Jumbu, fruits edible	Andamans and many parts in India
S. zeylanicum	Aromatic fruits are edible	Maharashtra, Karnataka, Orissa, Kerala and Andamans

15. POMEGRANATE

Pomegranate (*Punica granatum*)is an ancient fruit, which originated in Persia, Afghanistan and Baluchistan naturalized in Western India very early. Its wild forms are found in lower hills of Himachal Pradesh. Most of the pomegranate types cultivated in India are of seedling origin and thus providing a wide range of variability with respect to fruit shape, size, and mellowness of seed, aril colour, rind colour, sweetness and acidity of juice. Some popular varieties in different regions are furnished below:

Region	Variety/type
Maharashtra	Ganesh, Super Bhagwa, Solapur Lal, Mridula, Aaraktha,G-137,P-23, P26, Muskat
Karnataka	Ganesh, Ruby, Bassein Seedless
Gujarat	Dholka
Rajasthan	Jodhpur Red, Jodhpuri White
Tamil Nadu	Yercaud, Vellodu, Kabul Red, CO-1

Apart from the above-mentioned fruit species, there are several other species of fruits for which considerable diversity exists in the wild and conservation of such fruits needs to be carried out both *in situ* as well as in *ex situ.* There is also a need to work out the diversity using molecular means, so that the concept of 'core collection' can be practiced effectively.

CONSERVATION OF TROPICAL FRUIT TREES

Conservation of plant genetic resources is undertaken at genotype, gene pool, species and ecosystem level using diverse approaches. Plant genetic resources conservation is possible using in situ and ex situ approaches wherein each approach extends further options depending on the biological status, propagation method and population size of the species. Vast genetic diversity of underutilized fruits represents varied germplasm of wild, semi-wild species, genetic stocks, cultivars, farmers selections etc. requiring application of more than one method of conservation. It is, therefore, emphasized that a complementary conservation strategy (Rao, 1998; Rao and Sthapit, 2013), involving the use of more than one relevant

approach (in situ and ex situ) would be the best option for achieving safe conservation of these underutilized fruit species facing severe threat of extinction. There is big challenge to protect and conserve wild and semi-wild species of several major and minor fruits. Most of the wild species of these fruits occur in the protected areas and buffer zones of forest reserves and National parks. More over regeneration capacity and population size of some of the species is highly inadequate which is a matter of further concern and there is a probability of these being pushed to rare and endangered category (Malik *et al.,* 2006). Due to various developmental projects and changing climate these areas have become highly vulnerable and there is an urgent need to protect and collect the existing important plant diversity for safe ex-situ conservation. Coordination with forest department and joint programmes with Ministry of Environment, Forest and Climate Change is imminent to collect the germplasm and to ensure suitable in situ conservation measures.

In situ conservation of tropical fruit tree species is one of the most important aspects in the overall conservation of fruit diversity (Dinesh, 2001). It is well known that many of the species of mango, when introduced to other areas do not perform well or die. It is observed that the Mangifera andamanica, M. camptosperma and M. griffithi when introduced to mild tropics could not survive (Prakash, 2001). It is to be mentioned here that inspite of innumerable problems that are faced in the ex situ conservation, it is still advantageous to maintain them in the field gene bank, as it keeps the biodiversity of a particular species safe when plants are destroyed in the wild. Hence, to rationalize the concept of core collection was introduced, which in a limited set represents the genetic spectrum in the whole collections (Brown, 1989). It is proposed that landraces should be preserved for future generations as they harbour a diversity of interesting traits for future breeding work, for developing new farming systems and moreover, reflect the cultural identity of certain groups of people (Altieri and Merrick, 1987).

Until recently, germplasm conservation of crop landraces, as well as of their wild relatives, relied on ex situ methods (i.e., the conservation of biological material outside its natural habitat, UNCED 1992), mostly in germplasm banks. More recently in situ (on-farm) conservation (i.e. the conservation of biological diversity in its natural habitat) has been proposed as a conservation strategy which allows evolutionary processes to continue rather than being halted as occurs in ex situ conservation (Frankel *et al.,* 1995; Maxted *et al.,* 1997).

POLLEN STORAGE AS A MEANS TO CONSERVE DIVERSITY IN TROPICAL FRUIT TREES

Genetic conservation through pollen storage is desirable for a variety of horticultural plant species, since pollen is known to transmit important genetically heritable characters. Pollen is a product of genetic recombination and can provide a reliable source of nuclear genetic diversity at the haploid stage. Fruit tree pollen is generally required to be stored for controlled crossings, either to achieve a desired breeding objective, or to overcome a constraint involved in commercial fruit production.

Gene pool conservation at the haploid stage can, therefore, be effectively accomplished through pollen, which can provide a rich source of nuclear genetic diversity. A major emphasis on research needs include pollen storage (Arora and Rao, 1995) for citrus, mango, rambutan, jackfruit, durian and litchi. Pollen cryopreservation research has been recently recognized by IPGRI (IPGRI, 1995). As one of the gene pool components in an integrated PGR conservation programme, pollen can serve as an alternative or additional ex situ conservation method. Alexander and Ganeshan (1993) have extensively reviewed pollen storage research in fruit crops. Grout and Roberts (1995) have elaborately described the methodology involved in pollen cryopreservation. Hoekstra (1995) has assessed the merits and demerits of pollen storage for genetic resources conservation.

One of the main lacunae so far in the survey and collection of tropical fruit tree species has been the non-adoption of 'Geographic Information System' (GIS) tools, with the result that many of the regions with rich diversity were excluded. However, the use of Geographic Information System tools of late has helped in avoiding duplication of surveys and carrying out surveys with fair degree of accuracy.

These tools can also help in isolating genotypes with exceptionally good characteristics.

GIS TOOLS AND PREPARATION OF DISTRIBUTION MAPS

Geographic Information System (GIS) is widely used in management of natural resources. Presently GIS is being widely used in mapping biodiversity by different organizations. GIS is a database management system with specific functions to handle spatial data, i.e., latitude and longitude. Many applications of GIS have been developed for commercial purposes or for specific management purposes, for example, Atlas, MapInfo for Windows, Arc/Info, etc. for commercial use, and GRID, FloraMap, DIVA, etc. for specific purpose of mapping biodiversity. For mapping biodiversity and its assessment for tropical fruit tree species, software such as FloraMap and DIVA which were developed by the International Potato Centre (CIP) and International Centre for Tropical Agriculture (CIAT) for research purposes. GIS has two kinds of software, viz. rector-based system and raster-based system. The rector-based system stores geographic data as points, while the raster-based system stores data as grid cell. For mapping genetic diversity, rector-based system is popularly used. Using DIVA and FloraMap maps have been generated for fruit species like *Mangifera* and *Citrus*.

BIODIVERSITY ASSESSMENT USING MOLECULAR TECHNIQUES

With the advent of molecular biology techniques, DNA-based markers have replaced enzyme markers in germplasm identification and characterization. Because of its plasticity, ubiquity and stability, DNA is the ideal molecule for such analysis in fruit crops (Lalitha Anand, 2001). Although, molecular markers have been used to create extensive linkage maps for many annual plants (Helentjaris 1987; Bernatzky and Tanksley 1986; McCouch et al., 1988), few attempts have been made for their use in fruit crops. Genetic analysis and breeding of woody perennial fruit species can be complicated by many factors including long periods of juvenility, high ploidy levels, lack of described Mendelian markers and so on (Rowland and Levi, 1994). Hence, the task of developing molecular marker-based genetic maps is both challenging and important.

The basic premise is that variation in the nucleotide sequence of DNA can be exploited to produce characteristic fingerprints. The systems available are:

1. Restriction fragment length polymorphism (RFLP)
2. DNA amplification fingerprinting (DAF which comprises AFLP- amplified fragment length polymorphism / RAPD- random amplified polymorphic DNA)
3. Microsatellites / Simple Sequence Repeats (SSRs)
4. Cleaved amplified polymorphic sequences (CAPS).

SUMMARY

Collection, characterization and conservation of genetic resources of important tropical fruit ?? species such as *Mangifera* species, Citrus species, *Annona squmosa* (Custard apple), *Aegle marmelos* (Bel), *Artocarpus heterophyllus*, *Buchanania lanzan* (Chironjee), *Capparis decidua* (Ker), Carissa carandus (Karonda), wild and semi-wild *Citrus* species, *Cordia myxa* (Lasoora), *Embolica officinalis* (Aonla), *Garcinia* spp., *Grewia asiatica* (Phalsa), pau *Manilkara hexandra* (Khirni), *Phoenix sylvestris* (Date sugar palm), *Salvadora oleoides* (Pilu), *Syzygium cumini* (Jamun), *Tamarindus indica* (Tamarind) and *Ziziphus* spp. (Ber) has been undertaken. Several underutilized fruit species are propagated through seeds as vegetative propagation methods are hardly available. Presently many ex-situ conservation approaches have been suggested for long-term conservation depending on propagation method and seed storage behavior of these under utilized species. Successful cryopreservation protocols have been developed for seeds, embryos and embryonic axes in several non-orthodox difficult-to-store seed species and more than 2000 accessions have been successfully cryo-stored at National Cryo gene bank. However, there is still need to establish and strengthen field gene banks and clonal repositories for conservation and utilization of germplasm and to facilitate farmers with elite planting material of these important indigenous fruits.

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Review

An overview of canopy management in cashew (*Anacardium occidentale* L.)

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ABSTRACT

Being a tree crop of commercial importance, the productive performance of cashew is greatly influenced by how best its canopy is architectured for harnessing maximum benefits in terms of yield. The initial training is crucial for the development of photosynthetically efficient canopy in cashew as in other perennial fruit trees. Pruning of dead wood and crisscross branches can alone increase the yield by 30-40 per cent. The dwarf rootstocks also play a role in manipulating the canopy in cashew, wherein, canopy containment and yield were influenced by such rootstocks. By resorting to soil application of growth retardants like paclobutrazol, cashew canopy could be successfully contained to suit high density planting system. The studies on planting geometry has indicated the advantage of high density planting in enhancing profitability of cashew orchards in the initial years of plantation. The advantages of rejuvenation as well as top working techniques are also discussed in this paper.

Key words: Canopy management, PBZ, Planting geometry Pruning, Rootstocks and Training,

INTRODUCTION

Cashew, being a commercial plantation crop of India with high export demand, is confronted with an issue of low productivity which has necessitated the processing industries to import raw nuts from other countries to meet the demands of domestic and overseas consumers of Indian cashew. The lower productivity is attributed to various reasons like larger area under senile plantations of seedling origin, poor management practices, etc. Under management practices, management of canopy plays a significant role in cashew production and productivity.

The canopy management comprises of its components like training and pruning, use of growth regulators, selection of rootstocks/variety, etc. Canopy in a fruit yielding tree refers to its physical composition comprising of stem, branches, shoots and leaves. Canopy density is determined by the number and size of the leaves. Canopy architecture is determined by the number, length and orientation of the stem, branches and shoots. Canopy management refers an interpretation of physiology of light penetration and interception which are critical components of overall tree productivity. The main controlling factors are amount of incoming radiation and percentage of radiation intercepted by tree canopies. The productivity of fruit crops depends on several factors, management of canopy architecture being the most important one (Goswami *et al.,* 2014).

PRINCIPLES OF CANOPY MANAGEMENT

Fruit trees produce fruits regardless of human intervention. However, it is important to manage fruit tree canopies to optimize the balance between vegetative growth and fruit production, and also for easy inter-cultural operations such as spraying, ploughing interspaces and fruit picking at manageable heights.

The basic concept in canopy management of a perennial tree is to make the best use of land and the climatic factors for an increased productivity in three dimensional approaches. Canopy management includes a range of techniques to alter the position and the number of leaves, shoots and fruits in space which determines, to a large extent the plant geometry

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structure including spatial distribution of leaf area and leaf orientation.

Orchard architecture largely depends upon orchard production system which is a combination of variety, rootstock, tree spacing, training and pruning. These factors strongly interact to develop a specific production system and determined yield, fruit quality and longevity of the trees. In perennial fruit crops, cultural practices like nutrition, irrigation, planting density, rootstocks training system, pruning and growth retardants can be used as potential means to alter the shoot vigor, size and shape of the canopy and the microclimate at the canopy and thereby increase yield and quality (Vandana *et al*., 2017).

The cashew is a vigorous evergreen perennial woody plant having long juvenility and high heterozygosity. Canopy development in cashew is a seasonal and continuous process. However, the varieties which are available nowadays are of semi vigorous to vigorous type. Canopy management has a direct influence on plant vigour which ultimately influences the cashew nuts yield. The manipulation of the canopy by training and pruning, plant growth inhibitors and dwarfing rootstocks plays an important role in management practice to regulate vegetative growth, flowering and yield in fruit trees (Srilatha *et al.*, 2015).

The response to pruning depends on age, growth habits, tree vigour, varieties, location and cultivation practices of cashew. Heavy pruning promotes excessive vegetative growth and often reduces the yield due to the dense canopy with reduced flowering (Balamohan *et al.*, 2016). The application of plant growth retardants such as paclobutrazol (PBZ) has been found useful in the manipulation of vegetative growth, vigour and yield of cashew (Meena *et al.,* 2018; Babli *et al.*, 2019). The optimized application of PBZ helps to obtain maximum benefit with minimum undesirable impact on food and environmental safety aspects (Kishore *et al.*, 2015).

In cashew canopy development, two types branching exists, in which one is intensive and the other is extensive (Saroj *et al*., 2014). In high yielding trees more than 60 per cent intensive branches are seen whereas low yielders possess less than 20 per cent intensive branches. The 'intensive shoot' grows to a length of about 25- 30 cm and ends in a panicle, while in the 'extensive type', the shoot grows to 20-30 cm length and rests. Concurrently, in the intensive type that tends to give bushy appearance to the tree, 3 to 8 lateral shoots come up below 10-15 cm of the apex and few of these laterals may also bear panicles. On the other hand, in the 'extensive type', a bud sprouting 5-8 cm below the apex gives rise to further growth which continues for two or three years without giving flowers and results in spreading tree habit.

The training and pruning in tree crops affect the quantity of sunlight intercepted as tree shape determines the presentation of leaf area to incoming radiation. An ideal training strategy focuses around the arrangement of plant parts, especially, to develop a better plant architecture that optimizes the utilization of sunlight and promotes productivity. Light is critical to growth and development of trees and their fruits and productivity. The green leaves harvest the sunlight to produce carbohydrates and sugars which are transported to the sites where they are required – inflorescence, buds, flowers and fruits. Better light penetration into the tree canopy improves tree growth, productivity and fruit quality. The density and orientation of planting also impact light penetration in an orchard. Generally, in close planting, quicker shading becomes a problem. An east-west row orientation results in more shading as compared to the western and southern orientation of trees. The strong bearing branches tend to produce larger fruits. The problem of a fruit grower is initially to build up a strong and balanced framework of the trees, then equip them with appropriate fruiting. Obviously, pruning in the early years has to be of a training type to provide strong and stocky framework with well-spaced limbs or any other desired shape. Some of the basic principles in canopy management are maximum utilization of light, avoidance of built-up microclimate which is congenial for pest and disease infestation, convenience in carrying out the inter-cultural operations, maximizing productivity with quality fruit production and economy(Singh, 2010).

TRAINING OF YOUNG CASHEW PLANTATION

In case of the new plantations with the grafts, the plants should be trained in the early years i.e., 2-3 years so as to provide better plant architecture which

facilitates the easy inter-cultural operations. Training indirectly assists in ease of other operations such as weeding, manuring and pest and disease management (Satpathy, 1988). The lateral shoots arising from rootstocks need to be removed periodically till 2-3 years. This will assure the proper growth of the scion portion of the grafts. The grafted plants should be shaped by removing the branches and water-shoots growing from the main stem up to a height of 0.75m to 1.0m from the collar region during first 3-4 years. Besides, weak and interlocking branches should also need to be removed. After the age of 4-5 years, in tall type of cashew plants the main trunk may be de-topped at a height of 4-5 m from the ground region. This will ensure a round globular canopy which helps to harvest maximum sun light for photosynthesis. Severe pruning of the young grafts may be avoided as it may extend the juvenility and the pre-bearing period of the plants (Nayak *et al*., 1996). In general, two types of training systems are being practiced in cashew, a) Modified leader system and, b) Open center system.

a) Modified leader system

In this system, cashew grafts are allowed to grow as single stem up to a height of 75 to 100 cm by removing side sprouts. Then lateral branches are allowed to grow at desirable direction by de-topping. De-topping height varies from 2.5 to 4 m depending on spacing. Under normal spacing (8m x 8m), de-topping at 4 m from ground level is recommended. Whereas, for high density planting (5m x 5m), de-topping at 2.5 m from ground level is recommended. Removal of crisscross branches and trimming of branches has to be resorted to get dome shape canopy and the same should be maintained in later years by imposing mild pruning. This kind of canopy helps in reducing weak shoots and water shoots development. Modified training system is suitable for both normal and high-density planting system.

b) Open center system

Cashew grafts are allowed to grow straight up to 50-60 cm from ground level. The terminal growing point is pinched off to form lateral branches. The branches are regulated to grow in four directions at equal distance. Because of fast vegetative growth, the canopy spreads rapidly. To avoid this, canopy center needs to be opened up once in a while to support more light interception to the interior plant parts. This encourages flowering at inner and outer surface of canopy and thus increases the yield (Nayak *et al*., 2019).

PRUNING IN THE ESTABLISHED PLANTATIONS

The trees which have not received any training and pruning in the initial years grow haphazardly and resulting in canopies without desirable shape and size. Besides, the development of deadwood, inter-mingling of branches with neighboring trees, crisscross branches, development of water shoots etc. will bring down the productivity of the tree (Nayak *et al*., 1996).

Deadwood/dry branches

The dead wood/dry branches develop mainly because of the effect of shade on lower branches caused by overlapping of the upper branches. Deadwood will be an additional burden to the plants. Furthermore, the dead and decaying woods may invite the entry of pathogenic organisms or saprophytic growth which may spread further in due course of time.

Crisscross branches

The lower branches remain crawling on the ground for want of space and sunlight, where the plants are not trained or pruned in the initial years. Similarly, the branches at higher level also grow haphazardly in search of sunlight resulting in irregular canopy architecture.

Intermingling of branches

The problem of entangling of branches starts after 10-12 years in regularly spaced (8x8 m) plantations. The exterior branches get entangled with neighboring trees as a result, only a portion of canopy (crown portion) remains exposed to sunlight. Such a development inside the plantation is a hindrance to the regular intercultural operations and general maintenance of the orchard.

Water shoots/sprouts

Water-shoots are vegetative shoots which are extraordinarily vigorous growing from dormant buds at higher points on main stem in upright direction. They grow at the expense of parent branches from which they arise. They are erect in growth and much thicker in size than the normal branches and bear much longer and coarser leaves. These branches

outgrow the rest of the neighboring drooping branches. If water shoots are not removed in time, they soon cover the center of the canopy and obstruct sunlight.

Frequency of pruning

The old trees with deadwood, crisscross branches, water- shoots and inter mingling branches should be pruned at least once in 2-3 years (Khan *et.al*., 1987). Pruning can be taken up in dormant season i.e., at least 2-3 months earlier to productive flushing. All the types of unwanted growth mentioned before are to be pruned off. However, the plant should have a better look and structure after pruning. This can be achieved using one's discretion and experience in pruning and orchard management.

Leader shoot pruning

Cashew trees enter a brief resting period after the harvest of the crop (May - June) and it continues up to next productive flushing season (September - November). The flushes or flower bearing twigs are known as lateral shoots. These shoots usually form the terminal portion of a leader shoot which will give a single shoot (lateral) from its terminal bud. If the terminal bud is disturbed by means of pruning the dormant lateral buds will sprout resulting in a greater number of lateral shoots per unit area. This will result in increased number of productive inflorescences.

Pruning the leader shoots can be taken up at least 2-3 months (July to August) before flushing. In a tree about 50-60% of the leader shoots may be headed back to one-third of their original length. A pair of leaves may be retained while pruning wherever possible. While pruning, the leader shoot should be of a pencil thickness and should not have turned to ash color before taking up pruning.

In Bhaskara variety of cashew, leader shoot pruning was not useful and the number of flowering laterals was drastically reduced. However, pruning of lateral shoots to 25per cent in the month of September was very effective in enhancing flower production and nut yield (Anon. , 2019).

Yield increase in pruned trees

The past season leader shoots can produce only one lateral from its terminal. Pruning enhances the production of lateral shoots; thus, the yield can be increased. Pruning intensity and time varies for different specific agro-climatic regions. Pruning of dead wood and crisscross branches can increase yield by 30-40% (Khan *et al*., 1987). Leader-shoot pruning doubled the yield in cashew (Mohan and Room Singh, 1988). Results of pruning on 28-year-old trees revealed that trees with three branches pruned recorded the highest number of panicles/m^2 (39), highest number of flowers/panicle (588.70) and fruit-set to an extent of 14.42%, while unpruned trees recorded only 7.75% increase in yield (Panda, 1990). Under Jhargram conditions, pruning of leader-shoots during July enhanced the number of productive laterals, increased the number of bisexual flowers per panicle, fruits per panicle and yield per tree (Chattopadhyay and Ghose, 1994). Pruning treatment increased the number of laterals/leader but did not affect duration of flowering and harvest (Mohan and Rao, 1995).

Effect of the pruning in different shoots in two varieties namely, BPP-4 and BPP-6 was conducted at the Cashew Research Station, Bapatla, Guntur district (AP). The shoots were decapitated back to 5 cm in mid-July, mid-August and mid-September months of the leader shoots, lateral shoots and leader as well as lateral shoots pruned separately and different growth parameters on individual trees were studied. In response to the pruning, the variety BPP-4 performed better as compared to BPP-6. The production of flowering shoots and nut yield as influenced by the cultivar, level of pruning and time of pruning that a moderate incremental growth with large number of flowering shoots could be obtained by pruning the leader shoot in mid-August under local agro-climatic condition. The study further indicated that the vigorous cultivar BPP-4 and off-season production cultivar BPP-6 performed well during a rainy year compared to the dry year which was associated with prolonged dry spell and delayed rains in August-September months. Another important observation from the study indicated that the off-season cultivar of cashew needs essentially the pruning of the leader shoot in mid-August so as to avoid the off-season flowering and to increase productivity in the normal season. Pruning of leader shoots in mid-July was found to be beneficial during both the years of study to produce higher tree yield of nuts (Prasannakumar *et al*., 2015).

ROLE OF ROOTSTOCKS

Rootstocks play a very important role in propagation of plants. It may modify form or stature and adopt a variety to a soil in an incompatible climatic condition and also build up the resistance to biotic and abiotic stresses meanwhile increase the production and productivity. Rootstocks play a very important role in improving production, canopy architecture, flowering and fruiting quality and tolerance to stress. Although, lot of advancements was made in rootstock research of other fruit crops, such works on cashew is very limited.

The root system of the young dwarf cashew is one very well-developed main root that branches many times and can grow-up to 10 m or more in deep sandy soils. Lateral roots develop in the upper soil layers between 15 and 32 cm deep. The length of the superficial roots may reach twice the diameter of the crown in dry-land conditions (Barros, 1995). When irrigated the lateral roots are concentrated around the wet area of soil. The characteristics of the tap and lateral roots are of importance in relation to the fertilization of cashew (Crisóstomo *et al*., 2007).Great variation exists in the depth of the main root and distribution in depth and length of the lateral roots due to the effects of topography, soil texture, stoniness and the presence of a hardened soil layer on the development of the cashew root system (Falade, 1984).

Using dwarfing rootstocks offers the possibility to manipulate tree vigour, better anchorage, nutrient uptake, tolerance to biotic and abiotic stress, as well as yield and productivity without increasing input costs (Webster, 2004). Rootstock selection is a critical tool for the management of vegetative and reproductive growth of scion in perennial fruit crops, which are propagated by grafting or budding. Numerous studies have shown that they offer the advantage of rootstocks in the cultivation of tropical and temperate fruit crops on aboveground tree growth and yield (Balamohan *et al*., 2016; Webster, 2004; Nibolkar *et al*., 2016). Very limited studies have been investigated on cashew to select suitable rootstocks to modify scion vigour and increase productivity. The preliminary results reported by Adiga *et al*. (2014), provided the background information for the performance of vigorous cashew cultivars as influenced by dwarf rootstocks. The dwarf accession, NRC-492 could be used as a rootstock to induce semi dwarfism with a higher nut yield. Although cashew is a scion dominant species, the effect of rootstock is reflected in terms of stionic combination in particular, to control the plant vigour of the plant.

Different rootstocks differentially influence the morphology of grafted cultivars, including tree height, trunk cross-sectional area (TCSA), internodal length and yield. In one of the studies, Janani *et.al.* (2020) reported that VRI-3(scion)/ Taliparamba-1(rootstock) had low vigour based on lower means of tree height, plant volume, TCSA and canopy spread. The stionic combinations of VRI-3/ NRC-492 recorded the highest cumulative nut yield of 16.77 kg/tree (five seasons of cropping). This showed the possibility of manipulating cashew nut productivity through rootstock. Based on the observations on growth and yield of various stionic combinations, it was revealed that NRC-492 could be used as a rootstock to induce semi dwarfism with a higher nut yield. However, in Brazil, the different rootstocks tried for dwarf cashew clones could not influence the yield and nut weight in cashew (Paiva *et al.,*2004).

USE OF GROWTH RETARDANTS

The canopy management by pruning in later stages of growth often affects orchard life and performance of trees. High density planting system (HDP) has been attempted in cashew to obtain early benefits in terms of yield during initial years of planting. Under HDP, maintenance of tree and canopy growth becomes important due to closer spacing and shading of canopy of trees. In cashew, due to non-availability of dwarf clones and dwarfing rootstocks, use of growth retardants like paclobutrazol (PBZ) assumes importance. Hence, a study was aimed to evaluate the morpho-physiological responses of cashew to PBZ treatments under field trials (Meena *et al*., 2018; Babli *et al.,* 2019). The PBZ treatments resulted in reduced vegetative growth and enhanced reproductive growth with most striking responses of PBZ @3 g a.i./tree treatment. PBZ treatments altered cashew tree physiology by modifying tree size, canopy growth, internodal length, branching pattern and overall ground coverage of the tree. Higher total leaf chlorophyll content, better photo assimilation and enhanced leaf photosynthesis contributed in inducing early flowering and development of more flowering panicles with perfect flowers. Enhanced fruit set and

increased number of nuts/m^2 canopy contributed in yield increment. Regression analysis showed leaf pigments, nut number and number of inflorescences as the most contributing traits for yield enhancement under PBZ. These findings highlight the exploitation of morpho-physiological traits for better canopy growth and yield maximization by PBZ in cashew under the HDP.

PBZ treatments are effective in arresting vegetative growth and promoting reproductive growth of cashew. The PBZ treatments altered cashew tree physiology through reduction in vegetative growth, enhancement of flowering, production of more fruits and more fruit set due to efficient distribution of photosynthates, enhanced total leaf chlorophyll contents and increased leaf photosynthesis. These ultimately resulted in enhanced nut yield. Therefore, the findings may provide useful insights on finding solutions to tackle low productivity of cashew by proper regulation of endogenous growth hormones that can relate to enhanced nut yield. In addition, these findings may also throw light on induction of the desired physiological effects in cashew trees that can help in modifications of canopy growth and tree vigour. These in turn can be exploited well under the HDP system to harness early benefits with enhanced yield in cashew.

ROLE OF PLANTING GEOMETRY

In India, the established processing capacity of raw nuts is around 15-20 lakh tonnes, where the domestic contribution is around 7-8 lakh tonnes. In the recent years, there is an increase in the domestic demand for cashew. Thus, India has been importing nearly half of the raw cashew nuts processed in the country mainly from the African countries at the cost of Rs. 8839 crores annually (Anonymous, 2017). Of late, the import possibility from many of these African countries is dwindling, as these countries have setup their own processing facilities and also the competition for import of nuts from these countries by the major cashew processing and exporting countries like Vietnam is increasing. The major cause for deficit of raw nuts for processing by Indian cashew industries is the low productivity (720 kg/ ha). It is mainly due to large area of old senile orchards, low plant population per unit area, poor canopy management and non-adoption of improved package of practices. In recent times, demand for cashew in both domestic and international market is growing every year. In India, cashew consumption has increased by about 5.5 times in the last decade and is expected to grow further in the future. It has been estimated that the domestic demand for raw cashew nut is about 50 million MT or more by 2050 (Saroj and Nayak, 2016).

Hence, to meet this huge demand for cashew there is an urgent need for increasing the productivity per unit area. This can be achieved easily by the adoption of ultra and high-density planting systems. In recent times, there is a shift in farmers' perception from production to productivity and profitability which can be achieved through accommodating a greater number of plants per unit area. Studies on high density planting systems in fruit crops such as guava, mango and cashew have been shown to be more economical compared to the traditional planting system (Yadukumar *et al.,* 2001, Bal and Dhaliwal 2003, Sousa *et al.,* 2012, Gaikwad *et al.* (2017). Efforts have been made to standardize the high-density planting in cashew (Rejani *et al*., 2013), and mango (Gunjate *et al*., 2009) and some pruning techniques for improving nut yield in cashew (Mohan and Singh 1988, Kumar *et al.,* 2015, Murali *et al.,* 2015). In a long-term experiment on standardizing the planting geometry for 9 popular cultivars of cashew under west coast conditions of Karnataka, Adiga *et al*. (2014) found that the spacing requirement varied with varieties for optimum performance with respect to yield. They found that planting density of 500 plants per hectare was associated with highest cumulative nut yield as against planting density of 200 plants per hectare. The variety Vengurla-4 which exhibited highest leaf area index (1.80) was also associated with highest nut yield of 3.60 tonnes per hectare in the sixth harvest. The results of these studies have revealed that closer planting will help in increasing the productivity. However, the responses of the varieties to the pruning varied. Therefore, it is very much essential to identify varieties suitable for ultra and high-density planting which respond to pruning. Study revealed that interaction effect of varieties by spacing was observed for most of the growth and yield related characters except plant height and nut traits. Though the unit cost of establishment and maintenance for the first decade was high under high density planting system, the net income expected from high density planting (625

plants/hectare) was 130 to 150 per cent higher than normal density (200 plants/hectare) planting system (Yadukumar *et al*., 2003).

CANOPY REJUVENATION

About one third of plantations owned by cashew development corporations are old and senile and has contributed to lower productivity in the country. The rejuvenation of such plantations can address the issue of low productivity. The crux of canopy rejuvenation lies in the art of exploiting the existing root system of such senile trees to enhance canopy efficiency through severe pruning in case of named varieties or through top working if the plantation is of non-descript varieties or low yielding seedling origin trees.

The technology envisages beheading of trees, allowing juvenile shoots to sprout and taking up *in situ* grafting with scions of high yielding trees. This technology can offer 3-4-fold increase in cashew production in a short span of time. The increased yield of 5-10 kg/tree/year ensures sustained in come to the farmers (Khan *et al.,* 1986). The extent of growth in top worked trees at 5 years was on par with 17-year-old trees apart from 5-fold increase in nut yield (Kumar, 1990). The height of beheading of senile trees, the season of beheading and season of grafting decides the success of top working in cashew. Under Odisha conditions, beheading at 0.5m height in the month of May or June and grafting in the month of August resulted in the highest success rate of 81.80 per cent (Lenka *et al*.,1991). Under coastal Tamil Nadu conditions, the grafting success was highest between June to September (Pugalendhi *et al*., 1992). For Western ghat zone of Maharashtra, beheading in the first week of May followed by grafting in July resulted in highest success rate (85.70%) (Patil *et al*., 2004). One should exercise utmost precaution in beheaded trees as the cut trees are amenable for gummosis disease (Cardoso and Freire, 1998) or attack by cashew stem and root borer where mortality rate varies from 2.5 per cent to 100 per cent (Swamy,1995).

CONCLUSION

Canopy management is an 'art' of fruit growing - it is much more than cutting off a few branches. In fact, removing wood/branch from a tree is one of the last things growers want to do. To optimize fruit production and productivity, thoughtful canopy management is one of the most important subjects to sustain the yield and quality of fruits. To establish an ideal plantation, the young grafts are to be trained from the first year of planting itself which helps in facilitating easy and effective intercultural operations like base cleaning, trench making, fertilizer application, irrigation, pesticide spray against TMB, swabbing against stem and root borer, harvesting and picking nuts. In old and unthrifty plantations, the development of deadwood, water shoots crisscross branches, intermingling branches with the neighbouring trees and the branches crawling on the ground should be pruned to enhance nut yield. The leader shoot pruning should also be attended at least once in 2-3 years along with the removal of the above-mentioned unwanted growth which will be of help in boosting the nut yield. Meanwhile dwarfing rootstocks, planting density, use of growth retardants and selection of varieties also play an important role in successful management of cashew canopy.

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Original Research Paper

Phenotypic variability for horticultural and fruit quality attributes in plastic house grown tomato

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ABSTRACT

In Sudan agro-ecological zone, tomato production is constrained by dearth of high fruit yielding and quality (*Solanum lycopersicum* [L.]) varieties for cultivation in polyhouse. Exotic and indeterminate tomato genotypes with high fruit yield and quality were evaluated to gain information on variation for fruit yield, quality, shape, and interdependence between traits in Sudan agroecology. Seed were sown during 2018 and 2019. Fruit yield, quality and phenomic traits were measured. Development, °Brix, and fruit yield responded to microclimate factors in the polyhouse over years. 'Bruno' was the best for fruit size and 'Tofi' for fruit number. Vine length at flowering, fruits/cluster, days to 50% flowering and days to first flowering and fruit brix are heritable. The genotype responses suggest the need for stable and to develop high yielding and quality tomato varieties for protected cultivation in the Sudan agro-ecological zone. Testing stable genotypes in locations could enhance breeding efficiency with respect to genotypic stability. The yield data gained under tropical conditions identified traits of superior genotypes for multiple environment study and to encourage tomato growers to consider protected cultivation in the tropics.

Keywords: Character correlation, Fruit quality, Fruit shape, Fruit yield variability, Genotype by environment, Polyhouse and *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* [L.]) diploid (2n=24) is the second most commonly cultivated fruit vegetable after potato throughout the world (FAOSTAT, 2018). It is an annual herb, erect to prostrate stems, dicotyledonous, and grow as a series of branching stem with a terminal bud, determinate or indeterminate growth habit. Anthesis, fruit formation, and retention are temperature sensitive (Mohanty, 2002), and cloudy conditions reduces ripening and fruit yield (Nakia *et al.*, 2005). In West Africa, tomato production takes place in different agro-ecological zones under rain fed conditions, with a single cycle of tomato production annually. As an alternative, greenhouse production could likely allow 3 growth cycles annually. Tomato is a reliable source of nutrients (Arab and Steck, 2000; Ayandeji *et al.*, 2011). Total soluble solids are a measure of several chemicals and a proxy for sugar content. Higher TSS positively influences likeability and reduces cost associated with processing tomato fruit (Beckles, 2012). Consumers' choice for fresh tomato fruit is driven by fruit size, color, shape, and texture. Tomato production in the greenhouse is influenced by temperature (high and low), humidity (high or low), day length, and cloud cover which affect physiological and reproductive processes, and attack by insects and pathological organisms (Singh and Ashey, 2005; Tadele, 2016). Beefsteak and cluster tomatoes types are grown in greenhouses throughout the world; limited trials have occurred in sub-Saharan Africa, where greenhouse cultivation of tomato is limited. Local cultivars have low fruit yield, poor fruit quality traits, susceptible to diseases and insect attack,

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and unsuitable for cultivation in plastic house. Growers rely on seeds (hybrids or open pollinated) shipped from Europe and Asia for planting in greenhouse. A drawback in attaining a sustainable supply of tomato fruit is absence of quality seeds of promising genotypes and unfavourable climatic conditions (within and between years) and climate shocks.

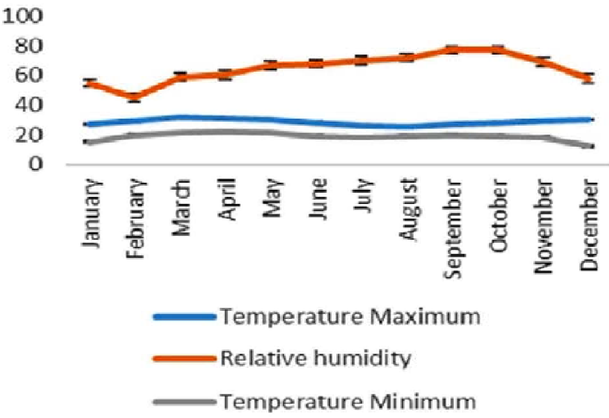
Under open field cultivation, high temperature and humidity are serious problems for crop production under tropical conditions. Tomato fruit set is very sensitive to low or high temperatures that affect pollen development and anther dehiscence (Gebisa *et al*., 2017). The cultivation of tomato under polyethylene house in the Sudan agro-ecological zone is limited due to inadequate knowledge of greenhouse production and absence of high yielding, early maturing and disease resistance with extended shelf-life and improved fruit quality traits. High temperature due to climate shocks have increased the incidence of heat stress in crops (Bitta and Gerrats, 2013), and in tomato grown under protected cultivation in Sudan agroecology. Exposure to temperature above 25ÚC during anthesis causes flower abortion, poor style development and pollen germination (Berry *et al.,* 1988; Peet *et al*., 1988), reduced fruit set and yield (Li *et al*., 2011; Zin *et al*., 2010; Giri *et al*., 2011). The genotypic response to both optimal and heat stressed conditions in the plastic house is important for fruit yield stability.

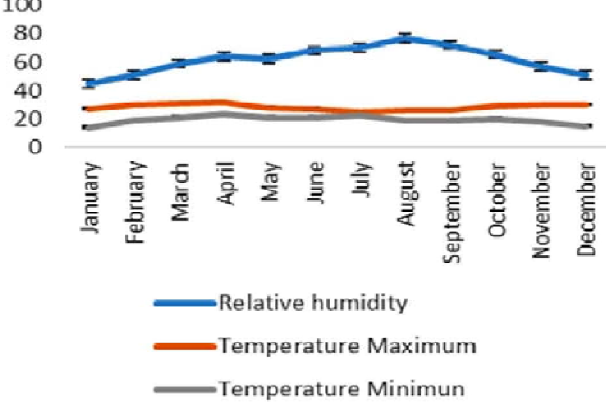
Tropical conditions encompass a wide array of environmental conditions and regions. Enhancing production in the tropics requires taking into consideration the diversity of climates and production systems that affect tomato production. Genotype x environment interaction results in variable performance of a genotype over time and space such that in many cases GXE interactions are treated as undesirable and confounding effects (Yan and Tinker, 2006), although they can provide breeding opportunities. The objectives of the research were to: a) evaluate variation for growth and development, fruit yield and fruit quality attributes, b) determine the magnitude of phenomic of fruit shape variability, c) estimate components of genetic variation, interdependence among developmental, fruit yield and fruit quality traits and heritability, and d) identify promising genotypes for fruit yield and fruit quality traits under Sudan agro-ecological zone.

MATERIALS AND METHODS

Location and nursery management

Two cycles of experiments were carried out at Greenhouse of Taraba Vegetable, Ardo Kola Local Government Area, Taraba state (latitude 08°46'N, Long 11°22'E), at 222 m above sea level. The experiments were begun in 2 July (rainy season) 2018 and 2019. Humus soil and perlite (Jubaili Nigeria, Ltd., Jalingo, Nigeria) was mixed in the ratio 3:1 (w:w). Fifty-six extruded plastics nursery multicell seedling trays were filled with the mix. Seed of the indeterminate, beefsteak, tomato genotypes viz, Bruno 29402, Dominique 539, Tomato 29206, IND 27812, Tomato 20209 (hybrids), and 'Tofi' (open pollinated), developed by Hazera Seed (Telaviv, Isreal) and Jubaili Seed (Jalingo, Nigeria) companies, respectively, were sown in cells in trays; each planting tray accommodated 260 seedlings.





The greenhouse was 102.72 × 57 m (~5,855 m^2) and 18 m high of which 99.37 × 54.72 m (~5,438 m^2) was cultivated. The slightly acidic (pH 5.67) sandy loam soil was ploughed, harrowed, and flat ridges constructed with tractor mounted implements. Each ridge contained double rows, 0.5 m apart with a 1.1 m pathway between double rows. Sixty flat ridges were established in the polyethylene house. The temperature and relative humidity in the plastic house were recorded using a CR200X Data Logger (Campbell Scientific Inc., Australia). Tomato seedlings were hand transplanted (18 April 2018, 20 August 2019 for first and second trials, respectively) in ridges with an inter- and within-row spacing of 0.5 × 0.6 m. Each ridge accommodated 140 plants (70 plants/row). A total of 8,400 plants were established in the polyethylene house. The experiment was arranged in a completely randomized design, each genotype was assigned to a double ridge plot 43 m long and replicated 4 times. Fertigation was begun 2 weeks after transplanting, 25 kg of N18:P18:K18 was dissolved in 100 L of water and applied through the drip irrigation system to plants, each plant received 10 mL of fertilizer. At 4 weeks after transplanting, N17:P9:K27 was dissolved in 100 L of water and applied through the drip irrigation system, each plant received 10 mL of fertilizer. At 6 weeks after transplanting, K61 soluble fertilizer was dissolved in 100 L of water and applied through the drip irrigation system to plants, with each plant receiving 10 mL. Weeding was by hand. Abamectin® (EC) (50 mL; Control Solution Inc., Genea-Red Bluff, Pasadena, CA), 40 mL of Imidacloprid® (EC; Hebei Xintian Biological Technology Co., Ltd Shijiazhuang, Hebe, China), and Mancozeb® (WP; Sigma-Aldrich Chemie, Taufkirchen, Germany) powder (100 g) was dissolved in 30 L of water and applied at 3 weeks after transplanting to control insect pests and insect-transmitted diseases. A T-shaped rod was inserted at both ends of the plot; tomato vines were trained on twine connected to overhang rods to support plant growth upward. Each tomato plant received 0.59 L of water 4 times a day (2.38 L of water per day) via drip irrigation.

Trait measurement and data analysis

The number of days to first flower (d), days to 50% flowering (d), vine length at first flowering and maturity (m), vine length at 50% flowering (cm), days to first fruit (d), days to first ripe fruit (d), interval between first fruit and fruit maturity (d), individual fruit weight (g), fruit weight/plot (kg), fruit length (cm) and fruit width (cm) were measured. A net plot of 1.1 × 3 m was used for determination of fruit number, fruit number/plot and fruit yield (kg). Twenty randomly picked tomato fruit (5 fruit per replicate) were blended for determination of fruit pH (MP 220; Mettler Toledo, Barcelona, Spain), and soluble solids using hand-held refractometer (model ATC-1, Atago, Bellevue, WA). At maturity, 12 tomato fruits were randomly chosen to measurement of fruit phenomic metric traits. A longitudinal cut was made on each fruit and digitalized (Scanjet G4010 scanner, Hewlett-Packard, Palo Alto, CA) at a resolution of 300 dpi. Scanned fruit images were subjected to morphometric analysis using Tomato Analyzer ver. 3 software (Rodriguez *et al.* 2010; Ohio State University laboratory website, http://www.oardc.ohiostate.edu/vanderknaap/). Fifteen fruit descriptors viz. fruit area, fruit perimeter, fruit width mid-height, fruit maximum width, fruit maximum height, fruit mid-width height, fruit maximum width, internal fruit shape index, fruit shape index eccentricity I, fruit shape index eccentricity II, proximal eccentricity, distal eccentricity, obovoid and fruit curved shape and fruit lobes defined by the manufacturer, were automatically received from Tomato Analyzer software (Rodriguez *et al*., 2010).

Quantitative traits were summarized, all data were subjected to analysis of variance using PROC GLM of SAS (ver. 9.4, SAS Institute, Cary, NC). If the interaction was significant it was used to explain results. Pearson correlation was performed for each year. The formula of Syukur *et al*. (2012) was used to calculate variance due to genotype, coefficient of variation due to genotypic effect (GCV), and phenotype effect (PCV). Heritability in broad sense for each trait was computed following the method of Allard (1960). Broad-sense heritability values >82% = very high, 60-79% = moderately high, 40-59% = moderately low, and <40% = low.

RESULTS AND DISCUSSION

A sustainable supply of fresh and high-quality tomato fruits to markets from polyethylene house requires development and deployment of high fruit yielding, early and medium maturity tomato varieties. This goal may be reached through the knowledge of phenotypic variability, association between traits and heritability. The combined analysis of variance showed statistically

significant (Pd”0.05) mean squares among the genotypes for development traits (vine length at flowering and vine length at maturity), earliness (days to first flowering and days to 50% flowering) and fruiting cycle (appearance of first fruit, appearance of first mature fruit and interval (days) between appearance of first fruit and first mature fruit) (Table 1a). These traits are important to ensure 2 or 3 production cycles annually in polyethylene house. The variability for earliness, vegetative growth and fruit growth cycle (early, medium or late maturity groups) among the genotypes have implications for harvest, shipment, shelf-life and delivery of fresh tomato fruits to the markets.

Table 1a. Combined analysis of variance and estimates of Genotypic variation (σ^2G), Phenotypic variation (σ^2P), Genotype by Year variation (σ^2GY), genotypic coefûcient of variation (GCV), phenotypic coefûcient of variation (PCV) and Heritability for developmental, earliness and fruiting cycle in tomato genotypes.

Source of variation	df	Days to first flower	Days to 50% flowering	Vine length at appearance of first flower	Vine length at 50% flowering	Days to first fruit	Days to 1st ripe fruit	Interval(days) between first fruit & maturity
Genotype (G)	5	48.90**	21.01**	774.12**	613.65***	19.28***	21.97***	30.14***
Year (Y)	1	1.22	14.40**	93.64**	18.15	38.03***	4.22**	22.58**
G × Y	5	7.78	2.58*	63.43**	76.40	7.52***	13.28***	13.00**
Error	36	5.27	0.75	11.37	38.08	1.06	0.91	1.68
CV (%)		3.88	1.30	5.43	7.73	1.05	1.37	4.58
Mean		61.64	66.41	62.98	79.87	97.74	69.47	28.45
σ^2P		7.00	2.82	103.37	163.90	4.69	2.91	5.18
σ^2G		6.03	2.53	95.44	134.98	2.63	1.47	3.55
σ^2GY		0.68	0.40	13.02	9.58	3.09	1.62	2.83
PCV		4.29	2.53	16.14	16.02	2.22	2.46	7.99
GCV		3.98	2.39	15.51	14.55	1.65	1.75	6.62
Hb (%)		85	89	92	82	56	51	69

*, **, *** significant at 5, 1, or 0.01%, level of probability, respectively, ANOVA.

Highly significant (Pd” 0.01) mean squares differences were recorded among the genotypes for individual fruit weight, number of fruits/plant, fruit weight/plot, number of fruits/plot, fruits/cluster, fruit length, fruit width, number of loculi/fruit, fruit pH and fruit brix (Table 1b). The foregoing may be associated with genetic factors and accumulation of photosynthates in the sink, in addition, the influence of microclimatic factors. Several authors (Dar and Sharma. 2011; Sharma and Singh (2015); Dhyani *et al*. 2017; Jindal *et al*. 2018) have reported significant genotypic effects for fruit yield and yield related traits among tomato varieties grown in polyethylene house condition.

The year (Y) effect significantly (Pd” 0.01) influenced days to 50% flowering, vine length at flowering, days to first fruit, days to first ripe fruit, interval between fruit appearance and maturity (Table 1a), and fruits/plant, fruit weight/plot and fruit brix (Table 1b). Findings are in accordance with reports by Dar and Sharma (2011) and Dhyani *et al*. (2017) in tomato varieties grown in polyethylene house and open field respectively. These traits could have been responsive to temperature, humidity and precipitation with low predictability. Therefore, the need for continuous evaluation over years for reliable inferences. On the other hand, vine length at 50% flowering, fruit/plot, fruit/cluster, fruit width, loculi/fruit and fruit acidity were not affected by environmental factors during the

Table 1b. Combined analysis and estimates of Genotypic variation (σ^2G), Phenotypic variation (σ^2P), Genotype × Year variation (σ^2GY), genotypic coefûcient of variation (GCV), phenotypic coefûcient of variation (PCV) and Heritability (H_b) for fruit yield, yield contributing traits and fruit quality attributes among genotypes.

Source of variation	df	Individual fruit weight	Number fruit/plant	Fruit weight/plot	Number fruit/plot	Number fruit/cluster	Fruit length	Fruit width	Number loculi/fruit	Fruit pH	Fruit Brix
Genotype (G)	5	353.31**	46.63**	7105.08**	496431.88***	7.14**	0.67**	3.34**	10.95**	0.27**	3.48**
Year (Y)	1	133.33	96.10**	2788.9**	60062.50	0.03	0.001	0.87	0.40	0.05	0.75*
G × Y	5	155.18	34.41**	3215.18**	221164.93	0.08	1.16**	0.80	0.40	0.06	0.91*
Error	36	77.63	11.55	363.79	113708.08	0.05	0.05	0.28	0.19	0.05	0.26
CV (%)		6.49	7.29	4.25	11.22	4.09	4.35	11.77	7.82	4.65	1.06
Mean		135.75	46.58	446.37	3004.63	5.42	5.14	4.64	5.62	4.85	4.64
σ^2P		53.85	5.82	2207.63	1245.14	178.53	0.31	0.48	2.68	0.11	0.46
σ^2G		34.46	1.52	882.84	842.75	178.48	0.19	0.38	2.63	0.03	0.32
σ^2GS		19.39	5.72	1628.4	712.84	0.008	0.01	0.13	0.05	0.04	0.16
PCV		5.41	5.19	10.52	1.17	247	10.83	14.93	29.13	6.84	14.62
GCV		4.32	2.64	7.27	6.50	246	8.48	13.28	28.85	3.57	12.19
Hb (%)		64	26	40	68	99	61	79	98	27	70

*, **,*** significant at 5, 1, or 0.01%, level of probability, respectively, ANOVA.

Table 2. Mean squares for fruit metric traits among tomato genotypes grown in a greenhouse.

Source of variation	df	Perimeter	Area	Maximum width	Maximum length	Curve height	Ellipsoidal	Circular	Lobeness	Distal ec-centricity	Eccentricity area index	Pericarp thickness	Proximal eccentricity	Obovoid
Genotype (G)	5	96.44**	261.66**	7.69**	6.34**	0.65	0.02**	0.01	14.72**	0.05**	0.13**	0.006	0.003	0.87**
Year (Y)	1	0.01	0.04	0.11	0.01	0.01	0.0001	0.0002	9.00	0.008	0.0008	0.0002	0.008	0.01
G × Y	5	0.05	0.10	0.06	0.12	0.04	0.003	0.0003	8.24	0.003	0.0009	0.0001	0.008	0.01
Error	24	12.59	59.01	0.65	0.63	0.54	0.001	0.007	8.66	0.009	0.0069	0.0069	0.006	0.009
CV (%)		14.6	26.00	14.84	15.94	12.97	0.13	0.13	3.12	0.82	0.42	0.24	0.87	0.31
Mean		24.21	29.24	5.46	4.99	5.68								

*, **, *** significant at 5, 1, or 0.01%, level of probability, respectively, ANOVA.

years of evaluation, due to non-significant (Pe” 0.05) mean squares (Table 1b). The high impact of the microclimatic factors on earliness and fruit yield and fruit quality traits may be linked to the polygenic nature of these traits and influence of microclimatic factors. The genotype effect accounted for a large proportion of the total variation compared to the year effect and genotype by year interaction (GYI).

The performance of the tomato genotypes for days to 50% flowering, vine length at flowering, number of days to first fruit, number of days to first ripe fruit, interval between fruit appearance and maturity, number of fruits/plot and fruit length and fruit brix were inconsistent with little or no predictability due to highly significant (Pd” 0.01) genotype by year interaction (GYI) mean squares. There are a number of previous studies (Carli *et al*., 2011; Cebolla-Cornejo *et al.,* 2011) among tomato varieties cultivated in the open field with significant GYI for traits considered in this study. The magnitude of GYI variation for fruit brix (total soluble solids) was attributed to temperature, reduced air flow and light intensity within the polyethylene house (Causse *et al.,* 2003). The sugar accumulation in tomato fruits depend upon the translocation of photo-assimilates from the leaves during fruit ripening (Cebolla-Cornejo *et al.,* 2011). The prospects of genetic improvement for these traits may not be achieved in the short run. The magnitude of genotype by year interaction for traits is useful to select optimal genotypes for earliness, fruit yield and quality traits. The GYI for some traits was responsible for the cross over performance of some genotypes (Table 4). Therefore, selection and recommendation of the genotypes for earliness and fruit yield will be complex. However, insignificant GYI mean squares for fruit pH is in conformity with findings of Causse *et al.* (2003).

A popular morphological feature distinguishing tomato varieties from undomesticated accessions is fruit shape (elongated). The mean squares for genotypes were significant (Pe” 0.01) for fruit perimeter, fruit area, fruit maximum width, fruit maximum height, fruit distal eccentricity, eccentricity area index and obovoid (Table 3). Also, ellipsoidal, lobeness, distal eccentricity, eccentricity area index and obovoid had significant (Pe” 0.01) mean squares due to genotypes (Table 6b). The mean squares due to the genotype × year interaction on fruit metric and phenomic traits were

Table 3. Mean values for fruit yield, yield contributing traits and fruit quality attributes among tomato genotypes.

Genotype	Days to first flower	Vine length at 50% flowering	Individual fruit weight	Fruit width	Number fruit/ cluster	Number loculi/ fruit	Fruit pH	Number of fruit/plot
Burno	61ab[a]	80.09cd	141.79a	4.79b	5b	6b	4.73b	3074.9ab
Dominique	59b	84.29bc	132.09ab	5.05a	5b	5b	4.93ab	2996.3ab
IND 27812	65a	81.08b	137.78a	5.23a	5b	5b	4.65b	2703.8b
Tom 29206	63a	93.98a	141.2a	5.21a	5b	5b	5.17a	2765.ob
Tofi	58b	50c	137.7ab	3.83b	8a	8a	4.83b	3480.8a
Tom 20209	62a	88.94b	124.01b	3.71b	5b	5b	4.85ab	3011ab

not significant (Pe” 0.05) for all traits (Table 6a and 6b). The differences for fruit size and shape among tomato genotypes is similar to report of Berwer *et al.* (2007), they indicated that tomato fruit can be small to large, round, with many loci contributing to fruit shape and size.

As shown in Table 3a, days to first flower appearance was early 58 d (‘Tofi’ and ‘Dominique’) and late 65 d (‘IND 27812’). The interval (days) between appearance of first flower and 50% flowering was 1 d in ‘IND 27812’ and 10 d in ‘Tofi’. In contrast, between 38 and 49 d from transplanting to flowering was recorded in tomato genotypes under rain fed (Mesecret *et al.* 2012). ‘Dominique’ was early for appearance of immature and ripe fruit. The interval (days) between seeding and appearance of first fruit was 67 d in ‘Dominique’, and 72 d in ‘Tomato 29206’. Tomato vines peaked (93.98 cm) in ‘Tomato 29209’, followed by ‘Tomato 29206’ with 88.94 cm. A vine length up to 154 cm occurred for determinate and indeterminate tomato genotypes grown in a

Table 3b. Genotype and year interaction[a] effects on fruit yield and quality traits.

Genotype ×	Year	Days to: 50% flowering	Days to: first fruit appearance	Days to: first ripe fruit	Fruit: Brix	Fruit: Height (cm)	Fruit: Weight per plot	Interval (days) from fruit first to maturity	Fruit number per plant (kg)
Bruno	1	63d	67e	98b	4.97d	5.73a	380.5e	31a	50.53d
	2	65c	70b	101a	3.50e	5.33c	467.0b	31a	75.00a
Dominique	1	65c	67e	96d	5.25a	5.33c	465.6b	29c	59.13c
	2	65c	67e	96d	5.25a	5.38b	465.5b	29c	59.18c
IND 27812	1	65c	68d	97c	3.65e	4.45h	439.8d	25e	58.53c
	2	67b	72a	100a	3.66e	5.73a	451.3c	25e	56.03b
Tofi	1	67b	72a	97c	5.25a	5.31d	425.3c	28d	58.44c
	2	67b	72a	97c	5.25a	5.15e	425.3c	28d	58.44c
Tom 209206	1	67b	71a	97c	5.05c	5.15e	428.0c	29a	55.50c
	2	69a	68b	99a	5.20b	4.45h	413.8c	30b	55.67c
Tom 29209	1	69a	69c	97c	3.50g	4.80f	509.3a	26e	68.84b
	2	69a	70b	100a	5.25a	4.50g	509.3a	32a	68.94b

[a] data in the interaction analyzed with Least Squares Means and means separated with Least Significant Difference.

[b] values in columns followed by the same letter are not significantly different, P<0.05

greenhouse (Kallo *et al*., 2012). Length of tomato vines is associated with adaptation and physiology.

The numbers of fruit harvested per plot was highest in 'Tofi' (Table 3a), this is a common trait of cluster tomato). Medium to high fruit per plant is consistent with effective pollination, fruit set and retention, and small sized fruit. Fruits of 'Bruno', 'Dominique' and 'IND 27812' are large (fruit length and width). Tomato fruits are sold by weight, 'Bruno', 'Tom 29206' and 'Tofi' appear to hold promise for individual fruit weight (Table 3a), and fruit weight/plot (Table 3b). The mean values for individual fruit weight in this study are larger than those reported by Cheema *et al*. (2013) for indeterminate tomatoes grown in a greenhouse. This may be linked to hereditary factors, high fruit set, large fruit size and efficient accumulation of photosynthate. The number of fruits/cluster is an index for fruit weight, 'Tofi' recorded the highest fruits/cluster (Table 3a). High fruits/cluster may be attributed to long fruits than wide. The total soluble solids (°Brix) were low ('Tom 20209') moderate ('Dominique' and 'IND 27812'). The mean values recorded for fruit brix are closer to those reported by Purkayastha and Mahanta, (2011). 'Bruno' and 'Domonique' were best for fruit size (fruit length and fruit width).

The mean values for fruit perimeter was on par with 'Dominique' and 'Bruno' and greater than mean values for 'IND 27218' and 'Tofi'. 'Bruno', 'Tomato 29206' and 'Dominique' had the best fruit area, fruit maximum width, and fruit height which agrees with mean values reported for fruit height and fruit diameter (Table 4). The proportion of fruit area outside the ellipse to total fruit area is important for fruit size. 'Bruno' performed best, followed by 'Tom 20209' and 'Tom 20906'. A morphological feature influencing preference for tomato cultivar is fruit shape. 'Burno' are obovoid, indicating the greater proportion of the fruit is below the mid-fruit height. 'Bruno', 'Tom 20206' and 'Tom 20209' are circular and ellipsoidal compared to 'Dominique' and 'IND 27812'. Fruit height measured along a curved line through the fruit was long in 'Dominique', but short in 'IND 27812'. 'Bruno' performed best for distal eccentricity and eccentricity area index. The spherical fruit shape was observed in the genotypes with fruit shape index (0.86 – 0.99). Variation in fruit size (fruit length and diameter) is associated with genetic makeup and moderated by cell size and intercellular space of the flesh, as was observed by Regassa *et al*. (2012) and Jindal *et al*. (2015).

Table 4. Mean values for some fruit phenomic traits among tomato genotypes grown in a greenhouse.

Genotype	Perimeter (cm)	Area(cm³)	Maximum width (cm)	Maximum length(cm)	Ellipsoidal	Lobeness	Eccentricity area index	Distal eccentricity	Obovoid
Dominique	28.79[a]	19.52d	5.59ab	4.38ab	0.20a	6.97b	0.21d	0.21d	0.31b
Bruno	27.03a	30.9b	6.06a	6.06a	0.10c	5.74d	0.56a	0.56a	0.97a
Tom 29309	24.54b	37.6a	6.41a	5.60ab	0.08d	6.17c	0.49b	0.50b	0.09b
Tom 29206	22.27b	30.9b	5.73ab	5.39b	0.09a	6.34bc	0.49b	0.50b	0.06b
1ND 27812	18.54c	27.23c	3.52c	3.51b	0.17ab	9.77a	0.32c	0.33c	0.09b
Tofi	18.33c	27.00c	2.33d	2.71c	0.08a	6.22c	0.22d	0.31c	0.08bc

[a] values in columns followed by the same letter are not significantly different, P<0.05 level, Tukey's test.

Table 5. Pearson's correlation coefficient between agronomic, fruit metric and fruit quality attributes in tomato genotypes.

	D.50FL[a]	FAMP	Frpp	Frl	Frw	FrPl	FrW/Pl	Fr Cl	Lo Fr	Fr pH
FAMP	-0.36									
FrPP	0.78**	-0.78**								
Frl	0.35	0.32	0.28							
Frw	-0.77**	0.57	-0.38	-0.11						
FrPl	0.40	-0.29	0.77**	0.77**	-0.77**					
FrW/P	0.77**	-0.16	0.59	0.67	-0.21	0.59				
Fr Cl	0.78**	-0.15	0.48	0.79*	-0.66	0.86**	0.66			
Lo Fr	0.87**	-0.26	0.59	0.76**	-0.57	0.78**	0.83**	0.91**		
Fr pH	-0.43	0.77**	-0.63	-0.08	0.84**	-0.63	-0.13	-0.61	-0.43	
Brix	-0.31	-0.51	-0.05	-0.97**	0.03	-0.62	-0.58	-0.76**	-0.63	-0.06

*, ** = significant at 1 and 5 % level of probability.

[a] D.50FL = Days to 50% Flowering, FAMP = Days between first and mature fruit, FrPP = Fruit/plant, Frl = Fruit Length, Frw = Fruit width, FrPl = Fruit/Plot, FrW/Pl = Fruit weight/plot, Fr Cl = Fruit/Cluster, Lo Fr = Loculi/Fruit, Fr pH = Fruit acidity, Brix = Total soluble solids.

The number of days to first flowering, days to first ripe fruit, fruit brix, fruit weight per plot were better during 2018 compared to 2019. Differences in solar radiation, temperature and humidity received in the polyethylene house over years influenced truss appearance and fruit yield. Pék and Helyes (2004) had noted differences in earliness and fruit yield in tomato varieties due to climatic factors. In contrast, fruit height, interval between fruit appearance and fruit maturity performed better during 2019 evaluation. Considering fruit weight per plot, 'Tom 29206' had higher fruit weight during 2018, while 'Bruno' and 'IND 27812' performed best during 2019. Trend of results for fruit yield and fruit quality traits in Sudan agro ecology may be due largely to inherent genetic factors and positive response by tomato genotypes to microclimate, which influences accumulation of photosynthate, growth and transpiration.

Genetic variability and Heritability

The amount of phenotypic variability in a crop is predicated on inherent genetic variation, the phenotypic expression is essential for selection. For all traits, the magnitude of phenotypic variance is greater than their corresponding genotypic variance, environmental variance, and variance due to genotype by year interaction. (Table 1a and 1b). Also, the genotypic variance had larger, or smaller magnitude than variance due to genotype by year interaction depending on trait. This is associated with the influence of microclimatic factors in the expression of these traits. As shown in tables 1 and 2, the mean values for phenotypic variance were farther apart for vine length at first flowering and 50% flowering, individual fruit weight, fruit weight/ plot and fruit/plot). The estimates for phenotypic coefficient of variation were larger in magnitude

than their corresponding genotypic coefficient of variation. In another study, Syukur and Rosidah (2014) reported large magnitude for PCV compared to GCV in pepper (*Capsicum annuum* L.). This suggest some influence of micro climatic factors. A little difference between PCV and GCV estimates indicates less environmental sensitivity. Therefore, selection based on phenotype will be worthwhile for improvement. Broad sense heritability estimates provides information about a trait and its interaction with the environment. It comprised additive and non-additive gene effects. Broad-sense heritability is classified as very high (e" 82%), moderately high (60-79%), moderately low (40-59%), and low (d" 40%). A high (e" 82%) broad sense heritability estimates were found for days to first flowering, days to 50% flowering, vine length at first flower, vine length at 50% flower, number of fruits per cluster, and number of loculi per fruit. This is indicative of high contribution of additive and non-additive gene effects compared to low contribution of microclimatic factors in phenotypic expression of these traits. These traits were least sensitive traits. In addition, fruit width and fruit brix had moderately high broad sense heritability estimates. This suggest a greater level of environmental sensitivity. A low (d" 40%) broad sense heritability indicates preponderance of environmental factors (precipitation and temperature) in the expression of these traits. However, it is possible to achieve improvements on a short run-in traits with high broad-sense heritability and with high phenotypic coefficient variance slightly larger than their genetic coefficient variance. In contrast, it would take more time to improve traits with low heritability, because of their low genetic variance component, and genetic coefficient of variation and genotype by year interaction.

The number of days to 50% first flowering had positive and significant correlation coefficient with fruit/plant (r= 0.78** P< 0.01, fruit weight/plot (r= 0.77** P< 0.01), fruit/cluster (r= 0.78** P< 0.01) and loculi per fruit (r= 0.87** P< 0.01). This suggest that early to medium flowering genotypes will account for higher fruits/plant and fruit yield. In addition, the desire to have 3 cycles of tomato production annually may be feasible. Similar findings were reported by Islam *et al*., 2010 and Tembe *et al*., 2017). The number of days between fruit appearance and mature fruit had significant negative correlation coefficient with fruits/plant (r= -0.78** P< 0.01) and significantly positive correlation coefficient with fruit pH (r= 0.78** P< 0.01). This suggest that genotypes with few days between fruit appearance and maturity will have low of fruit/plant and vice versa. 'IND 27812' had 25 d between fruit appearance and maturity with lowest fruit/plant. Results in this study are similar with those reported by Wali and Kabura, 2014 and Tembe *et al*., 2017). The correlation coefficient between number of fruit/plot and fruit/plant was positive and significant (r= 0.78** P< 0.01). Fruit length recorded positive and significant association with fruit/plant (r= 0.77** P< 0.01), fruit/cluster (r= 0.79** P< 0.01), loculi/fruit (r= 0.76** P< 0.01). This indicates that tomato fruits are oblong in shape and improvement in fruit length will account for more fruits/cluster. On the other hand, a significantly negative correlation coefficient was recorded in the association between fruit length and fruit brix (r= 0.97** P< 0.01). The number of fruits/plant correlated positively with fruit length (r= 0.84** P< 0.01) and number of loculi/fruits (r= 0.78** P< 0.01). The association between fruit weight/plant and number of loculi/fruit showed statistically significant (r= 0.83** P< 0.01).

Fruit development and size was dependent on micro climate, the 2019 evaluation was best for fruit yield. Moderate to high temperature, humidity, hot air and day length influenced physiological processes for high fruit yield and fruit quality, and earliness for 3 cycles of production annually. Tomato genotypes were responsive to microclimatic variables, inconsistent in fruit appearance, fruit development, fruit number and fruit brix, and fruit yield across years. Genotype × Year Interactions (GYI) are important to consider when developing stable varieties for a specific environment. For optimal performance, manipulation of micro-climate and breeding works are essential.

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Original Research Paper

Development and evaluation of novel gladiolus hybrid selections IIHRG-7 (IC620379) and IIHRG-11 (IC620380) for flower quality and *Fusarium* wilt resistance

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ABSTRACT

The present study was carried out to evaluate the performance of two novel gladiolus hybrid selections IIHRG-7 and IIHRG-11 along with commercial checks, for flower quality traits and *Fusarium* wilt resistance in completely randomized block design, during 2012-13 to 2014-15. Three years data were pooled and analyzed statistically. The hybrid selections IIHRG-7 and IIHRG-11 had been developed through hybridization by crossing Arka Meera x Picardy and Gold Medal 412 x Arka Poonam, respectively followed by selection. IIHRG-7 has novel flower colour (as per RHS Colour Chart) *i.e.,* Red-Purple (65.B) having Red-Purple (62.A) streaks with Red-Purple (67.B) splash and spike with variegated florets, while, IIHRG-11 has novel floret colour as Red (41.C) having Red (41.A) margin. Blotch Red (46.B) with yellow (13.C) border and resistance to *Fusarium* wilt disease. These hybrid selections are suitable for cut-flower and flower arrangement purposes. Further, these hybrid selections will be useful for developing new gladiolus hybrid selections with novel traits and resistance to *Fusarium* wilt disease.

Key words: Evaluation, Flowering, Fusarium wilt and Gladiolus Vase life

INTRODUCTION

Gladiolus is one of the most important bulbous flowering plants commercially grown for cut flowers, garden display and floral arrangement. It belongs to the family Iridaceae and sub-family Ixioideae. It ranks second in area (20.53 thousand ha) and production (132.58 thousand tons) among the cut flowers grown in India (Anon., 2016). The main emphasis in gladiolus improvement has to be given on development of varieties having attractive novel colour and more number of well-spaced large sized florets mainly for cut flower, long spikes and good corm multiplication ability (Swaroop *et al.,* 2018). Fusarium wilt is the most devastating disease in gladiolus which is caused by the fungus *Fusarium oxysporum* f. sp. *gladioli* (Massey) W.C. Snyder & H.N. Hansen (Massey, 1926 and Nelson *et al.,* 1981). It is a major bottleneck in gladiolus cultivation causing 60-80% crop damage and huge economic loss to flower growers (Lakshman *et al.,* 2012 and Kakade *et al.,* 2016).

As conventional management practices for Fusarium wilt disease include corm treatment with fungicides and soil fumigation are time consuming, labour intensive and increase the cost of cultivation, developing Fusarium wilt disease resistance gladiolus genotypes is an economically viable option in managing this disease. Identification of genetic resources for resistance to Fusarium wilt is crucial for harnessing resistance from these plants which can be deployed in development of resistant varieties. Therefore, the present study was carried out to evaluate two novel gladiolus hybrid selections IIHRG-7 and IIHRG-11 for their flower quality and Fusarium wilt disease resistance.

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MATERIAL AND METHODS

Hybridization followed by selection was employed to develop novel gladiolus hybrid selections IIHRG-7 and IIHRG-11 involving crosses Arka Meera x Picardy and Gold Medal 412 x Arka Poonam during 1986 and 1988, respectively. From hybrid seeds, cormels were produced. After the period of dormancy, cormels were planted and corms were harvested. Promising novel hybrid selections *viz.,* IIHRG-7 and IIHRG-11 were selected and multiplied vegetatively. Further, these hybrid selections with commercial checks Pink Friendship and *Psittacinus* hybrid, were evaluated for flower quality traits and resistance to Fusarium wilt disease, in replicated trial in completely randomized block design for three consecutive years i.e., from 2012-13 to 2014-15. The data on various biometrical parameters recorded were subjected to statistical analysis (Panse and Sukhatme, 1967).

Screening for resistance was undertaken in pot culture inside polyhouse in replicated trial in completely randomized block design. Uniform sized corms (5.5 cm to 6 cm) of IIHRG-11 and Pink Friendship (check) were planted in plastic pots containing 2 kg sterilized growing media @ 2:1:1 v/v (soil: sand: FYM). Sorghum based *Fusarium oxysporum* f. sp. *gladioli* inoculum was mixed in the soil at 4 g per 100 g of soil one day before planting and watering was done. The response of genotypes to *Fusarium* inoculation was evaluated at 90 days after planting (Elewa *et al.,* 2001). The disease incidence in per cent was recorded according to Riaz *et al.* (2010) and categorization of gladiolus genotypes based on disease incidence percentage was carried out as reported by Shanmugam *et al.* (2009) as follows: 0-10% = Highly resistant (HR); 10-25% = Resistant (R); 25-50% = Moderately susceptible (MS); 50-75% = Susceptible (S); 75-100% = Highly susceptible (HS). The results have been presented and discussed at the probability level of one per cent. The data regarding disease incidence and mortality were recorded using following formulae:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{No. of plants died due to disease}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

Perusal of data presented in Table 1 indicated significant differences between hybrid selection IIHRG-7 and Pink Friendship (check) for most of the vegetative and floral characters, however, plant height, floret diameter, number of marketable spikes per corm and vase life were found non-significant. The genotype Pink Friendship (check) recorded significantly early spike emergence (53.00 days) and flowering (62.67 days) in comparison to IIHRG-7 (63.95 days and 72.25 days, respectively). IIHRG-7 recorded significantly higher spike length (123.11 cm) than the check Pink Friendship (113.70 cm), however, longest rachis was recorded in Pink Friendship (57.41 cm). The spike length is one of the major criteria in selection of superior hybrid selection in gladiolus. The Pink Friendship (check) recorded more number of florets per spike (17.10) and florets remain open at a time (6.12) than IIHRG-7 (12.66 and 5.55, respectively). However, IIHRG-7 recorded significantly maximum number of total spikes per corm (1.66) than the Pink Friendship (1.40). The more number of spikes per corm are directly related to the higher productivity per unit area. The Pink Friendship recorded higher flowering duration (12.03 days) than the IIHRG-7

Table 1. Vegetative and floral traits of Gladiolus hybrid selection IIHRG-7 with check Pink Friendship (pooled data of three years)

Genotype	Days to spike emergence	Days to flower	Plant height (cm)	Spike length (cm)	Rachis length (cm)	Floret diameter (cm)	No. of florets per spike	Florets remain open at a time	No. of spikes/ corm	No. of marketable spikes/corm	Flowering duration (days)	Vase life (days)
IIHRG-7	63.95	72.25	142.06	123.11	46.77	10.66	12.66	5.55	1.66	1.43	9.61	9.33
Pink Friendship	53.00	62.67	144.88	113.70	57.41	10.79	17.10	6.12	1.40	1.40	12.03	9.00
C.D. at 5%	3.01	9.67	NS	6.23	2.97	NS	0.97	0.41	0.28	NS	1.11	NS

(9.61 days) owing to presence of more number of florets per spike in Pink Friendship which opened in acropetal successions for longer period. Sankari *et al.* (2012) reported variation in flowering traits in 42 gladiolus genotypes and recommended genotypes Pusa Swarnima, Pusa Shagun, Thumbolina, Priscilla and Candyman for cut flower production under Eastern Ghats of Tamil Nadu. Safeena and Thangam (2019) also evaluated ten cultivars of gladiolus for flowering traits and recommended Arka Amar and Darshan for cut flower purpose under Goa conditions.

Data presented in Table 2 indicate significant differences between hybrid selection IIHRG-7 and Pink Friendship (check) for corm and cormel characters. IIHRG-7 recorded significantly higher number of corms (1.46) than Pink Friendship (1.03); however, Pink Friendship recorded more number of cormels per corm (52.11) than IIHRG-7 (26.63). Significantly higher diameter of corm (6.75 cm), cormel (1.17 cm), weight of corm (72.44 g) and cormel (0.57 g) was recorded in IIHRG-7. Corm diameter and corm weight are important traits for producing quality spikes, with higher number of florets with bigger size. Sankari *et al.* (2012) and Safeena and Thangam (2019) reported that genotypes Thumbolina, Priscilla, Candyman, Arka Amar and Darshan were found superior for corm number, corm weight and corm diameter.

Table 2. Corm and cormels traits of Gladiolus hybrid selection IIHRG-7 with check Pink Friendship (pooled data of three years)

Genotype	Corm per corm (Nos.)	Cormel per corm (Nos.)	Diameter of corm (cm)	Diameter of cormel (cm)	Weight of corm (g)	Weight of cormel (g)
IIHRG-7	1.46	26.63	6.75	1.17	72.44	0.57
Pink Friendship	1.03	52.11	5.65	1.02	61.33	0.44
C.D. at 5%	0.23	4.07	0.29	0.04	9.58	0.08

The qualitative traits of IIHRG-7 and Pink Friendship are given in Table 3. The IIHRG-7 has novel flower colour (RHS colour chart) as Red-Purple (65.B) having Red-Purple (62.A) streaks with Red-Purple (67.B) splash with variegated spikes.

Table 3. Qualitative traits of Gladiolus hybrid selection IIHRG-7 with check Pink Friendship

Sl. No.	Trait	IIHRG -7	Pink Friendship
1.	Floret Type	Open-faced	Open-faced
2.	Floret texture	Medium	Medium
3.	Floret structure	Wavy	Wavy
4.	Floret placement	Good	Good
5.	Floret colour	Red-Purple (65.B) having Red-Purple (62.A) streaks with Red-Purple (67.B) splash	Red (50.D) having Red (51.C) margin and White (155.D) lines with Yellow (2.D) blotch

On the perusal of the data presented in Table 4, significant differences were observed between hybrid selection IIHRG-11 and *Psittacinus* hybrid (check) for most of the vegetative and floral characters, however, flowering duration and vase life were found non-significant. The hybrid selection IIHRG-11 recorded significantly early spike emergence (66.66 days) and flowering (76.65 days) in comparison to *Psittacinus* hybrid (check) (78.24 days and 89.245 days, respectively). Shaukat *et al.,* (2013) also reported early spike emergence in Applause and Peter Pears and early flowering in Priscilla and Peter Pears.

Psittacinus hybrid recorded significantly maximum plant height (150.38 cm), spike length (120.43 cm) and rachis length (60.07 cm) than IIHRG-11 (120.72 cm, 95.18 cm and 48.81 cm, respectively). However, IIHRG-11 recorded significantly maximum floret diameter (9.46 cm), number of florets per spike (17.54) and florets remain open at a time (6.86) than *Psittacinus* hybrid (8.25 cm, 16.68 and 4.75, respectively), while, maximum total number of spikes per corm (3.92) and marketable spikes per corm (2.43) were recorded in *Psittacinus* hybrid than IIHRG-11 (1.92 and 1.70, respectively). The genotypes

Table 4. Vegetative and floral traits of Gladiolus hybrid selection IIHRG-11 with check *Psittacinus* hybrid (pooled data of three years)

Genotype	Days to spike emergence	Days to flower	Plant height (cm)	Spike length (cm)	Rachis length (cm)	Floret diameter (cm)	No. of florets per spike	Florets remain open at a time	No. of spikes/ corm	No. of marketable spikes/corm	Flowering duration (days)	Vase life (days)
IIHRG-11	66.66	76.65	120.72	95.18	48.81	9.46	17.54	6.86	1.92	1.70	11.70	7.12
Psittacinus hybrid	78.24	89.24	150.38	120.43	60.07	8.25	16.68	4.75	3.92	2.43	11.96	7.00
C.D. at 5%	1.96	2.14	3.96	2.75	2.47	0.21	0.64	0.25	0.46	0.40	NS	NS

with more number of florets remain open at a time on the spike are more suited for exhibition purpose. The more number of spikes per corm are directly related to the higher productivity per unit area. Swaroop *et al.* (2018) evaluated 27 gladiolus hybrids and reported that hybrids Suchitra x Melody and Green Pasture x Regency recorded maximum plant height, spike length and rachis length, while, hybrids Suchitra x Melody and Bindiya (mutant) recorded more number of florets per spike, whereas, hybrids Suchitra x Melody and Green Pasture x Regency recorded higher number of shoots per plant. Bhat *et al.* (2017) evaluated 60 genotypes of gladiolus for growth and flowering traits and recommended that genotypes Eurovision, Jester Gold, Priscilla, Vink's Glory, White Friendship *etc.* are best suited for cut flower under temperate conditions of Kashmir.

Data presented in Table 5 indicate significant differences between hybrid selection IIHRG-11 and Psittacinus hybrid (check) for most of the corm and cormel traits except number of cormels per corm. Psittacinus hybrid recorded significantly higher number of corms per plant (3.64), diameter of cormel (1.78 cm) and weight of cormel (3.10 g) than IIHRG-11 (1.91, 1.53 cm and 1.08 g, respectively), whereas, IIHRG-11 recorded significantly higher corm diameter (6.64 cm) and corm weight (64.44 g) than Psittacinus hybrid (5.22 cm and 44.33 g, respectively). Bhat *et al.* (2017) evaluated 60 genotypes of gladiolus for corm and cormels traits and recommended genotypes Buff Beauty, Mayur, Priscilla, Pusa Suhagin, Regency etc. are best suited for corm production under temperate conditions of Kashmir.

Table 5. Corm and cormel traits of Gladiolus hybrid selection IIHRG-11 with check *Psittacinus* hybrid (pooled data of three years)

Genotype	Corm per corm (Nos.)	Cormel per corm (Nos.)	Diameter of corm (cm)	Diameter of cormel (cm)	Weight of corm (g)	Weight of cormel (g)
IIHRG-11	1.91	10.14	6.64	1.53	64.44	1.08
Psittacinus hybrid	3.64	10.90	5.22	1.78	44.33	3.10
C.D. at 5%	0.28	NS	0.18	0.06	4.47	0.12

The qualitative traits of IIHRG-11 and *Psittacinus* are given in Table 6. The IIHRG-11 has novel flower colour (RHS colour chart) as Red (41.C)

Table 6. Qualitative traits of Gladiolus hybrid selection IIHRG-11 with check *Psittacinus* hybrid

Sl. No.	Trait	IIHRG -11	Psittacinus hybrid
1.	Floret Type	Open-faced	Hooded
2.	Floret texture	Thick	Medium
3.	Floret structure	Slightly ruffled	Plain
4.	Floret placement	Double row	Fair
5.	Floret colour	Red (41.C) having Red (41.A) margin. Blotch Red (46.B) with Yellow (13.C) border	Red (39.A) with orange-Red (34.A) margin. Blotch Yellow (8.B)

Table 7. Disease incidence (%) and mortality (%) in IIHRG-11 with Pink Friendship (check) as influenced by *Fusarium* inoculum

Genotype	Disease incidence (%)	Mortality (%)
IIHRG-11	18.52 (18.47)	0.00 (2.87)
Pink Friendship	33.33 (30.95)	18.52 (18.47)
SEm±	3.46	3.14
CD (P=0.01)	20.63	18.70

Note: Values within parenthesis are *arc sign* transformed values

having Red (41.A) margin. Blotch Red (46.B) with Yellow (13.C) border and have resistance to Fusarium wilt disease.

Data presented in Table 7 indicate that the hybrid selection IIHRG-11 recorded 18.52% disease incidence with zero per cent mortality which comes under resistant category, while, check Pink Friendship recorded 33.33% disease incidence with 18.52 per cent mortality which comes under moderately susceptible category as categorized by Shanmugam *et al.* (2009).

On the basis of three years of evaluation, gladiolus hybrid selections IIHRG-7 was found promising for novel flower colour and variegated spike, and IIHRG-11 for novel flower colour and resistant to *Fusarium* wilt disease. These hybrid selections will be useful in developing new gladiolus hybrid selections with novel flower traits and resistant to Fusarium wilt disease.

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Original Research Paper

Evaluation of potassium salt of phosphonic acid in Nagpur mandarin with special reference to *Phytophthora* management

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ABSTRACT

***Phytopthora parasitica* var. *nicotianae* is a major fungal pathogen that causes foot rot, root rot, crown rot, gummosis, leaf fall and brown rot diseases in Nagpur mandarin in the entire Vidarbha region of Maharashtra. For the efficient management of root rot and gummosis due to *Phytophthora*, potassium salt of phosphonic acid (PSPA) was evaluated under field and laboratory conditions. In field trials, infected plants were treated with different concentration of PSPA by foliar spray and soil drenching. The results revealed that foliar spray + soil drenching of PSPA at 3 ml/liter water was better with respect to the average reduction in no. of lesion with oozing (28.39%), minimum in feeder root index (2.17), increase in canopy volume (11.15%) and higher fruit yield (65.89 kg/ per tree). Effect of PSPA was assayed at three difefernt concentarions against *P. nicotianae* under *in vitro*. PSPA was found most effective in arresting growth of *P. nicotianae* as complete (100%) inhibition obsereved in tested doses.**

Keywords: Foot rot, Gummosis, Nagpur mandarin, *Phytophthora* and Potassium salt of phosphonic acid (PSPA)

INTRODUCTION

Mandarins (*Citrus reticulata* Blanco) occupy a place of prime significance among the major fruit crops of India positioning third after mango and banana. They are a good source of vitamin C, as well as several other vitamins, minerals, and antioxidants. As per third advance estimates 2019-20, total land under orange (Mandarin orange/kinnow) cultivation in is 4.79 lakh hectare with production of 63.97 lakh tonnes (Anon., 2020). Major states engaged in orange cultivation are Madhya Pradesh, Punjab, Maharashtra, Rajasthan and Haryana.

Phytophthora spp. infect citrus plants at all stages and may infect most parts of the plant, including roots, stem, branches, twigs, leaves and fruits. Root rot, foot rot (also known as "gummosis", "trunk gummosis" or "collar rot"), fruit brown rot, twig and leaf dieback (often indicated collectively as "canopy blight") and rot (better known as "damping off") of seedlings, all incited by *Phytophthora* spp., may be considered diverse faces of the alike disease (Naqvi, 2000). In citrus, gummosis and foot rot (*Phytopthora parasitica* var. *nicotianae*) is reported as major constraint to sustain optimum production and it reduces yield by 46 per cent annually (Menge, 1993). It is responsible for 10-30 per cent yield loss in citrus cultivation around the world (Timmer *et al.,* 2000). The disease is also reported to pose a serious problem in mandarin grown in on large scale in Vidarbha region of Maharashtra (Naqvi, 2003). The severity of the disease is higher during monsoon season. Integrated disease management package that incorporates fungicides, biocontrol agents and organic amendments is required. Number of workers (Thind *et al*., 2004; Gade *et al.,* 2005; Kaur *et al.,* 2009; Jagtap *et al.,* 2012; and Singh *et al.,* 2015) used contact and systemic fungicides and bioagents for management of root rot/gummosis disease due to *Phytophthora* in citrus crops. Use of conventional fungicides can moderate the problem up to some level but cannot eliminate it; moreover, there are chances of resistance risk in the pathogen due to the use of systemic chemicals.

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Potassium salt of phosphonic acid is chemically known as potassium phosphonate (H_3PO_3). The dynamic component of this chemical within plants is phosphonate (phosphate) or phosphonic acid which is the active constituent working against the plant pathogen (Fenn and Coffey, 1987; Dunhill, 1990 and Guest and Grant, 1991). PSPA possess significant symplastic ambimobility or movement in both xylem and phloem (acropetally and basipetally). Translocation in phloem allows the chemical to move from leaf tissues to the crowns and roots (Ouimette and Coffey, 1990). Confirmation from histological and biochemical studies prove that PSPA application increases level of host resistance to pathogen invasion (Jackson *et al.,* 2000 and Daniel and Guest, 2006). Previously in India, effectiveness of PSPA against foot rot of black pepper incited by *Phytophthora capsici* (Lokesh *et al*., 2012) and nut rot disease in areca nut (*Phytophthora arecaea*) have been evaluated (Hegde, 2015) and was found effective.

Table 1. Treatment details

Sl. No.	Treatments	Dose (ml/ L of water)
1	Foliar spray of PSPA	02
2	Foliar spray of PSPA	03
3	Foliar spray of PSPA	04
4	Soil drenching of PSPA	02
5	Soil drenching of PSPA	03
6	Soil drenching of PSPA	04
7	Foliar spray+Soil drenching of PSPA	03 + 03
8	Foliar spray of Fosetyl -Al	02 g
9	Absolute control	-

First foliar spray and soil drenching was given in September and succeeding second application at one-month interval (October)

However, there is lack of information on use of PSPA for management of the Phytophthora root rot and gummosis disease in Nagpur mandarin. Therefore, an effort was made to explore the efficacy of PSPA in managing Phytophthora root rot and gummosis disease of Nagpur mandarin in the endemic region of Vidarbha in Maharashtra.

MATERIALS AND METHODS

The research trial was conducted on Nagpur mandarin at Dr. PDKV, Akola in randomized block design with nine treatments and three replicates. Application of respective potassium salt of phosphonic acid (PSPA) (trade name Sanchar 40) was given in one-month interval during September and October 2018. Fosetyl - Al @ 0.2% was used as standard check and (Table 1). Individual dosages were applied at foliar and basin region of plants. The observations on number were lesions with oozing, canopy volume, feeder root rot rating, number of fruits per tree and phytotoxicity were recorded.

Number of oozing lesions was recorded on experimental plants (main stem/side branches) before the application of PSPA and after second application of PSPA. Reduction in oozing lesion measured by using following formula-

= (Initial no. of oozing lesion - Final no. oozing lesions / Initial no. of oozing lesion x 100

The feeder root rating was recorded before the application and after second application of PSPA. The feeder root rotting using scale (1-5) given by Grimm and Hutchinson (1973) and Gade *et al.* (2005) was followed Root scale (1-5): 1= No visible symptoms, 2= A few roots with symptoms (1-25%), 3= Majority of roots with symptoms (26-50%), 4= All roots infected, cortex sloughed from major roots (51-75% rotted), 5= Majority roots dead or missing (>76% rotted). The canopy volume was as per the formula suggested by Westwood (1993) and increase in plant volume calculated as per the formula given below-

Increase in canopy volume (%)= (Final canopy volume-Initial canopy volume)/ Initial canopy volume X 100.

Observations on phytotoxicity symptoms were recorded for all treatments visually as per the guidelines of Central Insecticide Board, Govt. of India on 0–10 scale, 1-1 to 10%, 2-11 to 20%, 3-21-30%, 4-31-40% 5- 41-50% 6-51 to 60%, 7-61 to 70%, 8-71-80%, 9-81-90% and 10-91-100%. Effect on crop health *viz.*, leaf yellowing, tip necrosis, scorching, epinasty and hyponasty etc., were recorded on 0, 1, 3, 5, 7 and 10 days after application of each spray using the following score and per cent effect was worked out as per the method proposed by Nishantha *et al.* (2009).

In vitro study was also conducted to know the inhibition of pathogen by poison food technique (Nene

and Thapliyal, 1993). PSPA was added to cornmeal agar at various concentrations A 6 mm diameter agar disk, taken from an actively growing fungal colony on agar without PSPA, was placed with pathogen side downward at the center of the plate (9 cm diameter). Plates were incubated in the incubator at 25 ±1 ^{0}C for ten days. Radial growth of pathogen recorded and per cent inhibition in each treatment was calculated by using formula of Vincent (1927).

Sample of fruits for residue analysis (harvested 3 months after second application) were sent to Pesticides Residue Analysis Laboratory, National Horticultural Research and Development Foundation, Nasik. Residue of PSPA in fruits samples were quantified by utilizing LCMS technique (Saindrenan *et al*., 1985).

The data collected from the experiments were subjected to analysis of variance for different treatments. Fisher's protected critical difference (CD) test was used to indicate the difference between the treatments at the probability level of $p < 0.01$ following the procedure described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The number of oozing lesions before the first application of PSPA and after second application of one-month interval and final oozing lesions were counted. There were significant differences in the in number of oozing lesions in plates treated with PSPA at different concentration of (Taldiz) and Fosetyl- Al. The results revealed that amongst the different treatments, foliar and soil drenching of PSPA @ 3ml/ liter at one-month interval significantly reduced the number of oozing lesions (8.75) on tree trunk with enhanced reduction in number of oozing lesions (28.39%). It was on par treatments (T3, T5, T6, T7). Maximum number of oozing lesions was recorded in absolute control (14.04). This clearly indicates that by the application of PSPA through soil or foliar and combinations will reduce no. with oozing lesions from tree trunk. There was high reduction with T7 compared to other treatments (Table 2).

Subsequent to final application (2nd foliar and drenching) feeder root rot index (infected and healthy effective roots) observed from the basin of treated plants and was found in the range of 2.17 to 2.97 (Table 2).

Table 2. Efficacy of potassium salt of phosphonic acid on oozing lesion and feeder root index

Sl. No.	Treatments	Dose Product (ml/L of water)	Number of Oozing lesion		Reduction in oozing lesion (%)	Feeder root Index	Feeder root index reduction over control (%)
			Initial	Final			
1	Foliar spray of PSPA	02 ml/L	12.18	9.78	19.68	2.33	21.55
2	Foliar spray of PSPA	03 ml/L	11.73	8.98	21.60	2.30	22.56
3	Foliar spray of PSPA	04 ml/L	11.69	8.87	23.27	2.27	23.57
4	Soil drenching of PSPA	02 m/L	12.25	9.90	19.86	2.23	24.91
5	Soil drenching of PSPA	03 ml/L	11.70	8.87	22.64	2.20	25.92
6	Soil drenching of PSPA	04 ml/L	11.67	8.79	23.93	2.20	25.92
7	Foliar spray + Soil drenching of PSPA	03 ml/L + 03 ml/L	12.25	8.75	28.39	2.17	26.93
8	Foliar spray of Fosetyl -Al @ 02 g/L	02 g/L	12.75	10.37	19.30	2.30	22.56
9	Absolute control	—	11.93	14.04	-	2.97	
	SE (m)±		-	0.43		0.12	
	(CD at 5%)		NS	1.29		0.36	

Maximum extreme feeder root rot (2.97) index was recorded in control treatment. Highest per cent reduction (26.93%) in feeder root rot index over control was observed in treatment T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L each) followed by T6 and T5 (25.92%) and T4 (24.91%). The results indicated that concentrations and application methods were able to reduce roots infections of pathogen.

Maximum canopy volume (10.33 m^3) with an increase by 11.15% was recorded in treatment T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L). Next best array of treatments was T6, T3, T5, and T2 that recorded higher canopy volume 10.17,9.83, 9.76 and 8.99 m^3 with increase in canopy volume 7.96, 6.46, 5.63 and 5.19 respectively (Table 3).

The maximum yield of (65.89 kg/tree) was registered in the plots sprayed with Foliar spray + Soil drenching of PSPA @ 3ml/L (T7), which was par with 62.11 kg/tree in the plots soil drenched with PSPA @ 4ml/L (T6), foliar spray of PSPA @ 4ml/L (T3) 60.78 kg/tree and plots drenched with PSPA @ 3ml/L (T5) 59.78 kg/tree (Table 3). Commenly used fungicides and standard check Fosetyl-Al recorded yield 59.33 kg/tree (T8) which was notably lower in compared to PSPA application. The lower yield of 51.11 kg/tree was recorded in the untreated plots (T9) i.e., absolute control.

Table 3. Efficacy of potassium salt of phosphonic acid on canopy volume and fruit yield

Sl. No.	Treatments	Dose Product (ml/L of water)	Canopy volume (m^3)		Increase in canopy volume (%)	Fruit yield (kg/tree)
			Initial	Final		
1	Foliar spray of PSPA	02 ml/L	8.50	8.91	4.82	57.78
2	Foliar spray of PSPA	03 ml/L	8.55	8.99	5.19	59.56
3	Foliar spray of PSPA	04 ml/L	9.23	9.83	6.46	60.78
4	Soil drenching of PSPA	02 m/L	8.68	9.12	5.03	58.22
5	Soil drenching of PSPA	03 ml/L	9.24	9.76	5.63	59.78
6	Soil drenching of PSPA	04 ml/L	9.42	10.17	7.96	62.11
7	Foliar spray + Soil drenching of PSPA	03 ml/L+ 03 ml/L	9.29	10.33	11.15	65.89
8	Foliar spray of Fosetyl -Al @ 02 g/L	02 g/L	9.35	9.90	5.92	59.33
9	Absolute control	-	8.51	8.56	0.59	51.11
	SE (m)±		-	0.37		2.25
	(CD at 5%)		NS	1.11		6.38

During the course of investigation phytotoxicity symptoms not observed in any of the treatments at respective days of observation.

Efficacy of PSPA at respective concentration was tested *in-vitro* by following poison food technique for mycelial growth of *Phytophthora nicotianae.* After 10 days of inoculation, PSPA was found most effective in arresting complete growth of *P. nicotianae* as complete (100%) inhibition obsereved in tested doses i.e., 2, 3, and 4 ml/L. Radial growth of 20.32 mm was recorded in control plate on 10th day (Table 4).

Residue examination of treatment T3 (PSPA foliar shower @ 4ml/L) and T7 (Foliar spray + Soil drenching of PSPA @ 3ml/L) was done (Table 5). Treatment T3 recorded 0.329mg/kg residue content in harvested fruits however in treatment T7 recorded 0.666 mg/kg residue PSPA was observed that were below the recomond of 70g/kg level of MRL (according to EU).

Table 5. Residue analysis of PSPA

Sl. No.	Treatments doses	Equipment used	LOQ (mg/kg)	Residue content (mg/kg)	Fruits parts used
1	PSPA foliar spray @ 4ml/L	LCMS	0.010	0.329	Fruits with Peel
2	PSPA foliar spray @ 3 ml/L + Soil drenching @ 3 ml/L	LCMS	0.010	0.666	Fruits with Peel

Results demonstrated that application of PSPA significantly reduced the number of oozing lesions compared to chemical fungicide (Fosetyl -Al) at different concentrations. The results were also consistent with feeder root index, increase in canopy volume and fruit yield. Among the different concentrations and application methods of PSPA, two applications of PSPA @ 3ml/L foliar + soil drenching (one moths interval) significantly reduced the gummosis symptoms (28.39%) i.e., reductions in number of oozing lesions, reduced feeder root not index (Phytophthora root rot symptoms) (26.93%), increase in canopy volume (11.15%), and higher yield (65.89 kg/tree).

zone Use of PSPA at foliar plant and root challenge the infection adequately and reduce the gummosis and root rot. This had been observed by Hegde and Mesta (2014) who reported that in cocoa, spraying with PSAP @ 6 ml/L and soil drench @ 4 ml/L had reduced the incidence of pod rot caused by (*Phytophthora theobromae)*. Lokesh *et al*. (2012) also reported that application of potassium phosphonate @ 0.3 % as spraying and drenching with soil application of *T. harzianum*, @ 50 g/vine along with neem cake (1 kg/vine) to the black pepper vines against Phytophthora foot rot served as best treatment when compared to the farmers practice with use of 1 % Bordeaux mixture as spray. Moreover, current results are in agreement with the report of Hegde (2015) where potassium phosphonate effectively protected areca nut plants against nut rot disease incited by (*P. arecae)*. Compared to Fosetyl -Al, potassium phosphonate applied as a foliar spray or soil drench reduced stem infection of *Persea indica* seedlings by *Phytophthora citricola* (Fenn and Coffey, 1987). Numerous reports confirm that PSPA is readily absorbed by leaves and roots (Groussol *et al*., 1986; Schroetter *et al*., 2006 and Graham, 2011). After application of PSPA on the plant, the chemical gets translocated upwards in the xylem and downwards in the phloem (Guest and Grant, 1991). Its translocated in the phloem and its distribution is then subjected to normal source sink relationship in the plants. The translocation of phosphonate to different parts of black pepper plant was demonstrated by using radioactive ^{32}P Kumar *et al.*, 2009). Graham (2011) also experimentally proved that potassium phosphonate is highly systemic rapidly taken up by leaves and to move to fruit and provide protection against citrus brown rot of fruit caused by *Phytophthora palmivora*. In adding together, PSPA treated plants appear to be capable to create an anti-microbial environment more effectively by disrupting pathogen metabolism and triggering their own defense mechanisms (Daniel and Guest, 2006). Niere *et al.* (1994) proposed that the toxicity of PSPA on oomycetes was due to an increased level of inorganic poly-phosphonate, which is known to inhibit key phosphorylation reactions in them. In addition, PSPA also found to alter the nucleotide pools and pentose phosphate metabolism in *Phytophthora citrophthora* (Barchietto *et al*., 1992).

In present experiment, phytotoxicity symptoms were not visually observed on treated plants in respective concentrations and on respective days of observations. These results are in line with Pilbeam *et al.* (2000) who did not observe any phytotoxicity symptoms in *Eucalyptus* spp. treated with different concentrations of phosphonate. Guest and Grant (1991) observed that PSPA caused minimal toxicity when used at acute concentrations. Foliar sprays of phosphonate can cause phytotoxicity to citrus leaves and rapidly growing fruit later in the season if applied at high rates, at high temperatures, or if the tree is under drought stress (Le Roux, 2000). During the experimentation, congenial conditions prevailed and no phytotoxicity symptoms was observed.

PSPA effectively inhibited P. *nicotianae.* Anti-fungal chemical effects of the PSPA against *Phytophthora* spp. were reported by Cohen and Coffey (1986); Fenn and Coffey (1984); Bompeix et *al*., 1989), Grant *et al.,* (1990) and Truong *et al.* (2012). Wong (2004)

documented in his *in vitro* studies that phosphonate suppressed the growth rate of *Phytophthora capsici* by hyphal lysis.

Fruits samples with peel subjected for LCMS/MS for determination of PSPA residue and results confirmed that phosphonic acid present in treated plants. PSPA remarkably persistent in plants. Concentrations of PSPA in the fruits can be well below those MRL level (MRL level is 70g/kg of phosphonic acid as per EU).

Present results indicated that foliar sprays and soil drench of PSPA to Nagpur mandarin could be a practicable method of application for Phytophthora root rot and gummosis management.

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Original Research Paper

Genetic Analysis in mango (*Mangifera indica* L.) based on fruit characteristics of 400 genotypes

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ABSTRACT

The analysis of variance for 6 quantitative traits and 30 qualitative traits showed significant differences among the 400 genotypes of mango which indicates the existence of high heterozygosity. Among the 18 clusters formed, the highest fruit weight of 1404.27 g was recorded in cluster 10 followed by cluster 15 with 1280.67g whereas the lowest fruit weight was recorded in cluster 16 (30.94g). The highest fruit length (22.03 cm) was recorded in cluster 10 followed by 17.80 cm in cluster 14. Similarly, the fruit diameter was highest (12.18 cm) in cluster 10 followed by 12.03 cluster 4. The fruit thickness was highest (10.60 cm) in cluster 15 followed by cluster 4 with 9.96 cm. The pulp recovery was maximum (87.16%) in cluster-14 followed by followed by cluster 4 and 18 with 79.28 and 78.41 %, respectively. The clusters 15 had the varieties meant for pickle making and possessed the less TSS whereas the TSS of above 19°B was recorded in cluster 2. The maximum inter cluster (D^2) value was obtained between cluster 10 and cluster 11. These clusters may be used for hybridization programme due to wide variability and possibility of transgressive sergeants. Estimates of phenotypic variance and genotypic variance had only a narrow difference for all six characters studied indicating that these characters are not much influenced by environmental factors and highly heritable which can be exploited by adopting clonal selection or selection of chance seedlings and selection as parents for breeding purpose.

Key words: Biplot analysis, GCV, Genetic analysis, Heritability, Mango and PCV,

INTRODUCTION

Mango (*Mangifera indica* L.*)* is originated from the Indo-Burma region and genus *Mangifera* has more than 60 species world-wide, the highest diversity being found in the Malayan Peninsula, Borneo and Sumatra (Bompard,1993). Mukherjee (1953) opines that mango has been under cultivation for at least 4000 years with over 1000 varieties in cultivation. Almost all these are selections made from open-pollinated seedlings and selection by man from seedlings of unknown parentage has played the most significant role in the development of new mango cultivars (Singh, 1963). Mango is a premier fruit crop in India as well as some other countries in the tropical world with respect to being its eminent place in nutritional security and employment and income generation. The present scenario and expected future need of mangoes necessitate bringing improvement in mango with respect to the productivity not only per tree but also per unit area of land. A need has therefore, arisen to develop high yielding varieties of dwarf plant type, high fruit quality and resistant to biotic and abiotic stresses. Studies have been made for understanding diversity in the genus *Mangifera* and the possibility of its use in improvement of mango through introduction and selection of promising varieties for commercial cultivation and making further improvement in the existing varieties through inter-specific and inter-varietal hybridization and induction of useful mutations.

Mukherjee (1948) has described 72 mango varieties from Bengal, Bihar and Uttar Pradesh.

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Simultaneously, Naik and Gangolly (1950) have described 335 varieties of South India. Apart from the fruit characters, they have also laid great stress on the vegetative characters. The list of 1595 cultivars of mango in world (Pandey, 1998) was revised with the names of 1663 cultivars by Pandey and Dinesh (2010). The updated list now contains the names of 1682 cultivars. There are seven centres of diversity exists in India which includes (i) humid subtropical region (Manipur, Tripura, Mizoram and south Assam), (ii) Chhota Nagpur Plateau (trijunction of Madhya Pradesh, Odisha and Bihar), (iii) Santhal Paragana, (iv) Southern Madhya Pradesh (tribal area) adjoining Odisha and Andhra Pradesh, (v) Dhar Plateau of Madhya Pradesh adjoining South Rajasthan and Gujarat, (vi) humid tropical southern peninsular India and (vii) Andaman & Nicobar group of islands (Yadav and Rajan, 1993). A list of the names of cultivars available in the world as probable gene sources for dwarf-ness, fruit size, red peel colour, high pulp content, high content of total soluble solids, long shelf-life of fruit, regularity in fruit bearing, earliness and lateness in fruit maturity and good processing quality have been mentioned by Pandey and Dinesh (2010). Dinesh and Vasugi (2002) catalogued 151 cultivars of mango including *M. zeylanica*. Another lot of 223 varieties of mango were catalogued by Dinesh *et al.,* (2012) using Bioversity International Descriptors and they developed barcodes for these varieties through molecular characterization. There is a great variation in fruit weight in mango. Pandey and Dinesh (2010) categorized mango on the basis of fruit weight as very small (99g and below), small (100-149g), medium large (150-299g), large (300-500g) and very large (more than 500g) fruited varieties. Out of 61 varieties of mango registered in U.S.A., 19 varieties have been reported to bear large to very large fruits (Brooks and Olmo, 1972). The evaluation of genetic variability with in a cultivated crop has important consequences in plant breeding and germplasm management. The yield and its contributing traits improvement in this crop can be achieved through selection of superior genotypes with desirable traits existing in nature. Mahalnobis (D^2) statistics which is based on the multivariate analysis of quantitative trait is powerful tool for measuring genetic divergence among the population. Therefore, an attempt has been made to study the variability of fruit characteristics among 400 mango germplasm.

MATERIALS AND METHODS

The experimental materials consists of 400 mango genotypes belonging to different geographical regions of the world were evaluated for over the three years (2014-17) at ICAR-Indian Institute of Horticultural Research, Bengaluru. The experimental material comprised of 400 mango germplasm belonging to different geographical regions and evaluated in the year 2015, 2016 and 2017. The fruit characteristics such as fruit weight (g), fruit length (cm), fruit diameter (cm), fruit thickness (cm), pulp recovery (%) and TSS (ÚBrix) have been recorded by using the standard procedure. Thirty qualitative characteristics have been used to catalogue 400 varieties as per the standard descriptors given by the Bioversity International (IPGRI, 1997). At the first instance, statistical tools such as ANOVA and F-test were used to evaluate the significant difference ($p<0.05$) among the varieties/hybrid individually for all the traits. Least Significant Difference (LSD) was computed as a Post-hoc test (Cochran and Cox, 1957). SAS GLM was used to develop suitable codes for the statistical analysis (SAS V 9.3 2012).

Biometrical Analysis

With a view to understand the extent of diversity to which the observed variation were due to genetic factors, the phenotypic variance (PV), genotypic variance (GV), phenotypic coefficient of variation (GCV), genotypic coefficient of variation (GCV), broad sense heritability (h2), genetic advance (GA) and genetic advance as per cent over mean (GAM) were computed (Falconer,1985; Venugopalan, 2015).

Estimation of genetic parameters

Genotypic variance (σ_g^2) = $\frac{\text{Treatment MSS} + \text{Error MSS}}{\text{r}}$

Environmental variance (σ_e^2) = Error mean sum of squares

Phenotypic variance (σ_p^2) = $\sigma_g^2 + \sigma_e^2$

Coefficient of variation

The coefficient of variation (CV) being a standardized form of variance is useful for comparing the extent of variation between different characters with different scales. Genotypic and phenotypic coefficients of variation were estimated according to

Burton and Dewane (1953) based on estimate of genotypic and phenotypic variance.

$$\text{Genotypic coefficient of variation} = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

$$\text{Phenotypic coefficient of variation} = \frac{\sqrt{\sigma_p^2}}{\bar{x}} \times 100$$

Where $\bar{x}$ = General mean of the character

σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

Heritability (h2)

Heritability in broad sense was calculated as the ratio of genotypic variance to the phenotypic variance and expressed in percentage (Falconer, 1985).

Heritability (h2) =

Where σ_g^2 = Genotypic variance

σ_p^2 = Phenotypic variance

Expected genetic advance

Expected genetic advance (EGA) was calculated using the formula given by Robinson *et al.,* (1949).

EGA = I x h2 x áp

Where i = Selection of differential (2.06) at five per cent selection intensity

h2 = Heritability in broad sense

áp = Phenotypic standard deviation

Genetic advance over mean

Genetic advance as per cent over mean was worked out as suggested by Johnson *et al.,* (1955).

GAM =

Where GA = Genetic advance

= General mean of the character

Correlation

Genotypic (rg) and phenotypic (rp) coefficients of correlation were estimated as suggested by Al-Jibourie *et al.,* (1958).

$$\text{Genotypic correlation} = \frac{C_oV_{xy}(G)}{\sqrt{V_x(G) \times V_y(G)}}$$

$$\text{Phenotypic correlation} = \frac{C_oV_{xy}(P)}{\sqrt{V_x(P) \times V_y(P)}}$$

Where

C0Vxy (G) = Genotypic covariance between x and y

C0Vxy (P) = Phenotypic covariance between x and y

Vx (G) = Genotypic variance of character x

Vx (P) = Phenotypic variance of character x

Vy (G) = Genotypic variance of character y

Vy (P) = Phenotypic variance of character y

Test of significance of correlation was tested by comparing the 'r' value with obtained value.

Estimated heritability (broad sense) was classified as low (< 30 %), medium (30 – 60 %) and high (> 60 %) and the range of genetic advance as a percentage of mean was classified as low (< 10 %), moderate (10 – 20 %) and high (> 20 %) as suggested by Johnson et al. (1955). SAS package was used to develop suitable codes for the statistical analysis (SAS V 9.3 (2012)).

Using quantitative traits, the genetic distance among the populations is calculated using D2 statistics (Rencher, 1995) on the basis of multiple characters. The clustering of genetic groups is done by a method suggested by Tocher (Rao, 1974). The means of all the characters were subjected to Squared Euclidian Cluster analysis and a dendrogram was derived using Ward's method (Rencher, 1995).

RESULTS

The analysis of variance for 6 quantitative traits showed significant differences among the 400 genotypes of mango which indicates the existence of genetic diversity. Means for different qualitative characters of 400 accessions of mango are given as Supplementary Table S1 (Available online). The maximum fruit weight of 1404.27g in variety Sora followed by 1280.67g in variety Amini where as the

minimum fruit weight was of 29,51g in variety Kana Appe followed by 39.31g in variety Halasage. The fruit length was maximum (22.03 cm) in var. Sora followed 16.77cm in hybrid 7/15 where as the minimum fruit length of 4.33cm was recorded in var. Pacharasi. The maximum fruit diameter 13.50 cm was recorded in var. Dorgani Kavi followed by 11.33 cm in var. Maharaja Pasand where as minimum fruit diameter of 3.33 cm was recorded in var. Dodderi Jeerige. The maximum fruit thickness of 11.40cm was recorded in var. Dorgani Kavi followed by 9.87 in var. Amini whereas the minimum thickness was recorded (2,80cm) in Haldotta Appe. The highest pulp recovery (89.67%) was recorded in var. Manoranjan followed by 88.20 % in var. Lahara whereas the lowest pulp recovery was recorded in var. Halasage. The highest TSS (ÚBrix) of 31.00 was recorded in var. Dattatreya local followed by 30.67 in var.K-0-7 where as the lowest was recorded in var. Halasage (5.5%)

The cluster mean analysis (Table 1) reveals that there is a huge variation among the clusters. Highest fruit weight of 1404.27 g was recorded in cluster 10 followed by cluster 15 with 1280.67g. Whereas the lowest fruit weight was recorded in cluster 16 (30.94g). The highest fruit length (22.03 cm) was recorded in cluster 10 followed by 17.80 cm in cluster 14. Similarly, the fruit diameter was highest (12.18 cm) in cluster 10 followed by 12.03 cluster 4. The fruit thickness was highest (10.60 cm) in cluster 15 followed by cluster 4 with 9.96 cm. The pulp recovery was maximum (87.16%) in cluster-14 followed by cluster 4 and 18 with 79.28 and 78.41 %, respectively.

Table 1. Cluster mean for quantitative traits of 400 mango genotypes

Clusters	Fruit w eight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit thickness (cm)	Pulp (%)	TSS (°Brix)
Cluster 1	376.51	10.81	8.19	7.25	74.48	18.95
Cluster 2	246.93	9.27	7.16	6.43	70.35	19.36
Cluster 3	144.69	8.04	5.94	5.27	63.28	18.31
Cluster 4	1188.69	15.30	12.03	9.96	79.28	14.97
Cluster 5	197.24	8.78	6.62	5.94	68.42	19.23
Cluster 6	527.53	12.60	9.09	7.91	78.60	19.26
Cluster 7	1015.20	16.77	11.07	9.60	79.26	17.47
Cluster 8	452.59	11.20	8.73	7.72	73.79	18.64
Cluster 9	835.55	13.70	10.75	9.26	79.25	17.02
Cluster 10	1404.27	22.03	12.18	9.50	78.78	13.53
Cluster 11	86.84	5.97	5.24	4.62	60.68	17.50
Cluster 12	965.09	15.32	11.08	9.14	79.49	16.27
Cluster 13	305.87	10.09	7.66	6.85	72.30	19.18
Cluster 14	1077.39	17.80	11.00	9.13	87.16	13.60
Cluster 15	1280.67	15.93	11.67	10.60	74.23	11.23
Cluster 16	38.94	6.70	3.40	3.12	27.54	13.98
Cluster 17	625.93	13.25	9.46	8.70	78.37	17.14
Cluster 18	714.91	14.11	9.70	8.41	78.41	17.79
Average	340.525	10.076	7.59	6.7289	70.7	18.581

The maximum inter cluster (D^2) value was obtained between cluster 10 and cluster 11. These clusters may be used for hybridization programme due to wide variability and possibility of transgressive sergeants. The minimum cluster distance was obtained in cluster 2 and cluster 5 which indicates that the accessions belonging to such clusters are relatively close. The selection of parents from genetically close clusters may be due to narrow genetic base and inbreeding depression.

The pattern of distribution of accessions in different clusters indicating the existence genetic diversity which is related to geographical distribution. These 400 genotypes were grouped in to 18 clusters as presented in Table 2 which is apparent that cluster 1 (28 accessions), cluster 2(63 accessions), cluster 3(50 accessions), cluster 4 (3 accessions), cluster 5 (56 accessions), cluster 6 (20 accessions), cluster 7 (1 accessions), cluster 8 (34 accessions), cluster 9 (7 accessions), cluster 10 (1 accession), cluster11 (24 accessions), cluster 12 (6 accessions), cluster 13 (53 accessions) cluster14 (1 accession), cluster15 (1 accession), cluster16 (3 accessions), cluster17 (12 accessions) and cluster 18 (10 accessions).The clusters such as 7, 10, 14 and 15 had 1 accession in each with hybrid 7/15, Sora, Tella Gulabi and Amini, respectively. All these accessions possessed the fruit weight ranged from 1015.20g to 1280.67g which are meant for pickle making.

Table 2. Distribution mango accessions in various clusters

Clusters	Varieties/Hybrids
Cluster 1 (48accessions)	Ananas, Arka Puneet, Ashrafi, Bangalore Sindhura, Bennet Alphonso, Bombay Green, Chambeliwala, Chinnarasam, Cipia, Dalbia, Dilpasand, Dori,Fazrizafrani,H-165,H-85, Himsagar, Kacha Meetha, Kadikai, Kari Ishad, Karkanchavadi Rumani, Keitt, Khazri, Kitchner, Kurd, Lord, Madan Rao Pasand, Mahmood Bahar, Maya, Motichoor, Muffarai, Mulgoa, Mumbaigaro, Murshidabad, Navneet, Nom- Dok-Moi, Nr.25, Pattar, Peddarasam, Potte, Prior, Rangoon Goa, Rumani, Salem, Sanakalu, Santi, Mulgoa, Tatamidi, Tenkasi Banganpalli, Thogarapalli.
Cluster 2 (63accessions)	Almas, Alphonso black, Ambika, Ameer gola, Andamans local, Apple Rumani, Ashruf-Us-Samar, Asiquot, Badami modal, Bhopdya, Borsha, Botlimavu, Brindabani, Chimut, CISH M-2, Devrakhio, Dofasala, Gidagana Mavu, Gopal Bhog, Guruvam, Hindustan Ball,Hy-87, Hyder Sahib, IRS (Long fruit), Jawahar, Kasturi Mamidi (R), Kohinoor, Kottur Konum, Kove Sara, Lal Sundari, Lat Sundari, Mahamoozda, Malai Misri, Malgesh, Mandor Katta, *Mangifera zeylanica*, Manibhatta Appe, Manipur, Manoranjan (Sreddy), Moreh, Muvandan, Nagin, Neelgoa, Olour, Panchavarnam, Papayakhas, Prabhashankar, Puttu, Raja Pasand, Ramphalya, Raspuri, Rosa, Safeda Malihabad, Santhura Collection, Sardar, Sensation, Sepia, Sindhu, Sundar Langra, Sundarshan, Tenkasi Rumani, Tofanchan and Vellaikulamban
Cluster 3 (50accession)	Adderi jeerige, Amrapali, Anfas, Balekoppa appe, Bappakkai, Barbalia, Bombay darsha, Chengavarikai, Coorg Collection, Dattatreya local, Dashehari Clone-51, Ec 95862, Gomavu, H-12 (Arka udaya), H-151, Hamsa Mamidi, Hilario, Hittalahalli Appe, Jeerige, Kalapadi, Kalkuni, Karigal Appe, Kerala Kalapadi, Khas-Ul-Khas, Kintalavenipeta, K-o-22, Kobbe, Kurukkan, Kutumba Appe, La resorce -1, La Resource-2, Lalmuni, Lazzat baksh, Licthi, *Mangifera griffithi*, Narayanasheni, Narela (SR), Nekkare, Paiyur–1, Sabre, Sadamidi, Safeda Lucknow, Siddapura Alavalli, Siroli, Terpentine, Thali, Thumbebeedu, Vattam, Vinayaka Hegde, Willard
Cluster 4 (3 accessions)	DorganiKayi, Maharaja Pasand(L), Safed Mulgoa
Cluster 5 (56 accessions)	Achar Pasand, Agarabathi, Akhadya, Alfazli, All season, Ambalavi, Ananthabhatta appe, AtiMadhuram, Bandariya, Bhutto Bombay, Bobbalipunasa, Bombayno.1, Carabao(g), Carabao(s), Chandanum, Chitanga, Fernandin, Furtad, H-14, IRS (Small fruit), Isagoor Appe, Janardhan Pasand, Java, Kalapara, Kalgundi Koppa Appe, ,Karanjio, Khuddus, Kishen Bhog, Zardalu, KM P7, *Mangifera odorata*, Miranda, Mohammada Vikarabad, Mohandas, Mylupilian, Naati, Nalla Mamidi, Neeleshwari, Neelphonso, Neeluddin, Pacharisi (TN), Panakalu, Peach, PKM-2, Ratnagiri Alphonso, Ratul, Ropeday, Royal Special, Samarbehisht Chausa, Shandariya, Sharbathi Bagri, Shidadakke Appe, Surankundi, Virudhanagar, Yakutti and Zardalu
Cluster 6 (20 accessions)	Arya Samaj, Azam-Us –Samar, Badaaam, Bandar bandal, Chausa, Fakir, Fakirwala, Hansraj, Jalal, Kerala Dwarf, Lahara, Lal Khatra, Manoranjan, Ostin, Padiri, Sai Sugandh, Samparpatti Totapuri, Swathantram, Thatnur and Vanraj.
Cluster 7 (1 accession)	Hybrid 7/15

Cluster 8 (34 accessions)	Abbas, Allampurbaneshan, Aryavarthana Rasalu, Black ceri, Bombay alphonso, Bombay natasala, Bombay peda, Chandrama, Chettalli, Dannalli appe, Danti Mamidi, Fazli, Goa Kodur, H-56, Harsha, Himam Pasand, Hyb-11/14, Intimax (P3), Jehangir, Kalami Hindustani, Kensington, K-o-11, Ku-8, Mage Mavu, Manjeera, Mombasa, Mundappa Black, Navaneetham, Nazukbadan, Neeleshan, Pkm 1, Ruswani, Thorappadi, Warate Gidaga
Cluster 9 (7 accessions)	Arka Aruna, Begum Pasand, Black Andrews, Hamlet, Katta Gola, Rajapuri, Thambva
Cluster 10 (1 accession)	Sora
Cluster 11 (24 accessions)	Appemidi, Chandrakaran, Chanshi, Creeping, DodderiJeerige, Elaichi, Gurumurthy Appe, Hajeera, Heera Chowki, Huli Appekai, Kalakai, Kempikundi, Lalpasand, Malange, Muregeer, Musoore, Mylapuri, Nuha (M), Pacharasi, Rasool, Rubi, Starch, Tenkasi Neelum, Vhout
Cluster 12 (6 accessions)	Himayat Pasand, Kerali Goa, Kothapalli Kobbari, Mutwar Pasand, Shahjahan, Tenneru
Cluster 13 (55 accessions)	Alphonso, Anda, Arka anmol, Arka Neelkiran, Asif Pasand, Au Rumani, Badagulab, Chauthi, Cherukurasam, Chitha, Colaso, Dashehari, Dwarf Rumani, Faluda, Gaddahall Appe, Goa Bunder, Goa Mankurd, Gola, Goran Appe, Hur (SR), Jamedar, K-0–7, Kadari, Kadri, Kalakand, Kalwa Gudda, Kesar, Kirsapati, Kolanka Goa, Krishna, Laddu, Langra, Latif, Lemon, Maharaja of Mysore, Mallika, Mandamane, Nagalapalli Rasalu, Neelum,Nr-34 Local, Panchadara Kalasa, Papayaraju Goa, Peter, Pulihara, Ratna, Rehman, Pasand, Salem Bangalora, Shakkar Gola, Sushan Bhog, Suvarna Rekha, Swarna Jehangir, Taimur Pasand, Tephala, Tokio, Xavier
Cluster 14 (1 accession)	Tella Gulabi
Cluster 15 (1 accessions)	Amini
Cluster 16 (3 accessions)	Halasage, Haldotta Appe, Kana Appe-1
Cluster 17 (17 accessions)	Balakondapari, Balakrishnan, Banganapalli, Cowasji Patel, Ebatti Mavu, Eldon, Elephant Head, K -0-32, Kasturi Mamidi (l), Khudadath, K-0-15, Lily, Maharaja Pasand(rd), Shendriyo, Tommy Atkins, Totapuri, Whiteceri
Cluster 18 (10 accessions)	Anardana,Gaddemar,Kmh-1, Makaram, Mohan Rao Pasand, Mulgoa Black, Nymath, Pahilwan, Papaya (SR), Rebello

Estimates of phenotypic variance and genotypic variance had only a narrow difference for all six characters studied indicating that these characters are not much influenced by environmental factors (Table 3). This also suggests the presence of sufficient genetic variability which can be exploited by adopting clonal selection or selection of chance seedlings. The maximum PCV was recorded for fruit weight followed by fruit length and fruit diameter. This indicates the better scope for phenotypic selection of these traits for improvement.

Heritability and genetic advance for fruit characters varied considerably. The high heritability (0.84 to 0.94) and high estimate of genetic advance recorded for fruit weight, fruit length, fruit diameter, fruit thickness, pulp recovery and TSS. High heritability indicates the effectiveness of selection through phenotypic performance but it does not mean a high genetic gain. However, high heritability associated with high genetic advance proves more useful for efficient improvement of a character through simple selection. In the present study, high heritability estimates with high genetic advance as per cent over mean was observed for the all the fruit traits studied indicating the possible role of additive gene action, whereas moderate heritability with low genetic advance as per cent over mean indicating the non-additive gene action. In the present investigation, the estimates of genotypic correlations in general were higher than phenotypic correlations, indicating the presence of inherent association between various characters.

A total of 30 characters have been used to group the 400 genotypes as per standard descriptor of the Bioversity International (Table 4) which is evident that there are gradations of variations due to the heterozygosity nature of the crop.

Table 3. Genetic parameters for fruit characteristics of 400 mango accessions

Characters	Mean	Range	CV	GCV	PCV	h^2	Genetic advancement as % of mean
Fruit weight (g)	336.61	29.10-1404.2	15.141	63.946	65.714	0.946	128.37
Fruit length (cm)	10.050	4.33-22.03	6.228	25.317	26.072	0.942	50.72
Fruit diameter (cm)	7.57	3.23-13.50	5.589	20.529	21.276	0.930	40.86
Fruit thickness(cm)	6.71	2.80-11.40	8.287	19.346	21.046	0.844	36.68
Pulp recovery (%)	70.76	18.93 -89.67	4.762	13.234	14.065	0.885	25.69
TSS (°Brix)	18.61	5.50-31.00	8.124	20.501	22.052	0.864	39.32

Table 4. Grouping of 400 germplasm of mango based on qualitative traits

Character	Percentage (%)	Character	Percentage (%)	Character	Percentag e (%)
1.Fruit shape		**11.Fruit beak type**		1. Slightly Juicy	50.00
1. Oblong	67.5	1.Absent	79.75	2. Juicy	41.75
2. Elliptic	1.50	2. Pointed	14.75	3. Very Juicy	8.25
3. Roundish	30.5	3. prominent	3.75	**21.Pulp aroma**	
4. Ovoid	0.25	4. Mammiform	1.75	1 Mild	55.50
5. Obovoid	0.25	**12.Fruit sinus type**		2. Intermediate	28.75
2.Shape of fruit apex		0. Absent	53.50	3. Strong	15.75
1. Acute	48.75	1. Shallow	38.25	**22.Presence of turpentine flavour**	
2. Obtuse	38.75	2. Deep	8.25	0. Absent	61.00
3. Round	12.50	**13.Fruit skin waxiness**		1. Mild	22.00
3.Fruit attractiveness		1. Waxy	95.50	2. Intermediate	9.50
1. Poor	15.75	2. Non-waxy	4.50	3. Strong	7.50
2. Average	35.25	**14.Pulp colour of ripe fruit**		**23.Veins on stone**	
3. Good	39.00	1. Light yellow	1.00	1. Level with surface	46.75
4. Excellent	10.00	2. Golden yellow	-	2. Depressed	14.50
4.Fruit skin surface texture		3. Yellow orange	66.00	3. Elevated	38.75
1.Smooth	84.75	4. Orange	14.50	**24.Pattern of stone venation**	
2. Rough	15.25	5. Greenish yellow	0.25	1. Parallel	46.00
5.Density of lenticels on fruit skin		6. Yellow	17.50	2. Forked	54.00
3. Spare	21.25	7. Light orange	0.25	**25 Quantity of fiber on stone**	
5. Medium	23.00	8. Dark orange	0.50	3. Low	57.50
7. Dense	55.75	**15.Pulp texture of ripe fruit**		5. Intermediate	27.50
6.Fruit stalks insertion		3.Soft	25.50	7. High	15.00
1.Vertical	71.25	5. Intermediate	40.00	**26.Adherence of fiber to stone**	

2. Oblique	28.75	7. Firm	34.50	3. Weak	12.25
7.Depth of fruit stalk cavity		**16.Adherence of fruit skin to pulp**		5. Intermediate	29.75
0. Absent	41.25	0. Absent (free)	17.75	7. Strong	58.00
1. Shallow	43.75	3. Weak	38.00	**27Space occupied by seed inside the stone (%)**	
2. Medium	6.50	5. Intermediate	27.00	1. < 25	1.75
3. Deep	7.75	7. Strong	17.25	2. 26 - 50	2.25
4. Very deep	0.75	**17.Quantity of fiber in pulp**		3. 51 - 75	19.25
8. Fruit stalks attachment		0. Absent	3.75	4. 76- 100	76.75
3. Weak	6.50	3. Low	48.75	**28.Seed shape**	
5. Intermediate	55.25	5. Medium	32.25	1. Ellipsoid	2.75
7. Strong	38.25	7. High	15.25	2. Oblong	13.75
9.Fruit neck prominence		**18.Adherence of fiber to fruit skin**		3. Reniform	83.50
0. Absent	80.75	3. Low	49.75	**29.Type of embryony**	
1. Slightly prominent	12.75	5. Intermediate	39.25	1. Monoembryony	91.50
2. Prominent	5.50	7. High	11.00	2. Polyembryony	8.50
3. Very prominent	1.00	**19. Fiber length in the pulp.**		**30.Eating quality**	
10.Slope of fruit ventral shoulder		3. Short (0.58 -2)	44.25	3. Poor	16.75
1. Slopping abruptly	9.00	5. Medium (2.1-5)	6.50	5. Good	63.50
2. Ending in a long curve	44.75	7. Long (5.1 & above)	49.25	7. Very good	15.50
3. Rising and then rounded	46.25	**20.Pulp juiciness**		9. Excellent	4.25

DISCUSSION

The analysis of variance for 6 quantitative traits exhibited the significant differences among the 400 genotypes of mango which indicates the existence of genetic diversity within the *Mangifera indica*. The maximum fruit weight of 1404.27g in var. Sora followed by 1280.67g in var. Amini whereas the minimum fruit weight was of 29,51g in var. Kana Appe followed by 39.31g in var. Halasage. The results are similar as reported by Pandey and Dinesh (2010). While selecting the parents for hybridization programme, the quantitative characters such as fruit weight, fruit length, fruit thickness, fruit diameter, and pulp recovery should be considered as all of them have high heritability. Transgressive segregation was observed for fruit size in the progeny (Iyer and Subramanyam, 1987) and it appeared to be governed by additive genes (Sharma and Majumder, 1988a). Detailed study conducted by Prabhuram (1998) based on heritability, expected genetic advance and total genetic variance gave useful information on inheritance of different fruit characters. Fruit weight was influenced considerably by the environmental conditions. Equal proportion of the additive and non-additive components of the total genetic variance influenced its genotype. Rajan *et al.,* (2009) found high degree of broad sense heritability in mango varieties for the length and weight of fruit, peel weight and length and weight of stone. The fruit length was maximum (22.03 cm) in var. Sora followed 16.77cm in hybrid 7/15 where as the minimum fruit length of 4.33cm was recorded in var. Pacharasi. Fruit length shows transgressive segregation in either side of the parental limits and therefore, suggested a polygenic control of this character. Also, this character was influenced by environment to a considerable extent (Pandey, 2012). The maximum fruit thickness of 11.40cm was recorded in var. Dorgani Kavi followed by 9.87 in var. Amini whereas the minimum thickness

was recorded (2,80cm) in Haldotta Appe. Fruit thickness showed polygenic control and was highly influenced by environmental factors. For breadth of fruit the genotypic contribution appeared to be comparatively lesser than in most of the traits. The peel colour and attractiveness are considered as important qualitative traits in mango, only 10% of the germplasm possess excellent attractiveness out of 400 germplasm screened. A high frequency of hybrids with red peel or burgundy blush can be recovered from crosses where one of the parents has an intense red blush (Brittell *et al.,* 2004). The highest pulp recovery (89.67%) was recorded in var. Manoranjan followed by 88.20 % in var. Lahara whereas the lowest pulp recovery was recorded in var. Halasage. The highest TSS (ÚBrix) of 31.00 was recorded in var. Dattatreya local followed by 30.67 in var.K-0-7 where as the lowest was recorded in var. Halasage (5.5%). Sharma and Majumder (1988) stated that total Beta carotenoid pigments and T.S.S. content in these two hybrids exceeded the better parent Dashehari suggesting the gene action showing transgressive segregation for this trait. On the other hand, light yellow colour of pulp appeared dominant over orange yellow in the progenies of Alphonso x Neelum cross (Iyer, 1991). According to Prabhuram (1998), total soluble solids (T.S.S.) content in some hybrids transgressed either of the parents Amrapali and Sensation, which suggested a polygenic control for this trait and that it was influenced considerably by environmental factors.

Bretell *et al*., (2004) observed that many important fruit quality aspects such as fruit weight, fruit shape, ground skin colour, fruit width and pulp depth have high heritability, and can therefore be readily selected in a breeding programme. For non-ordered traits scored in discrete categories (blush colour, bloom, lenticel colour, embryo type and flavour), an estimate was made of data consistency from multiple scores for individual hybrids at different times and locations. Relatively high consistency value was recorded for fruit flavour, and in combinations involving Kensington Pride. The analysis of blush colour and fruit flavour in twelve families of hybrids has confirmed that these characters have a strong genetic component, the high frequency of hybrids with red or burgundy blush can be recovered from crosses where one parent has an intense red blush colour. Singh *et al*., (2004) observed wide magnitude of phenotypic coefficient of variation with high genetic advance for yield per plant and fruit weight in 31 chance seedlings of mango.

Biometrical studies

The analysis of variance for 6 quantitative traits showed significant difference among 400 germplasm of mango indicating the existence of diversity. These germplasms have been grouped in to 18 clusters and distribution of germplasm among the clusters varied in numbers which indicates that genetic divergence was related to geographical differentiation. The clustering of genotypes from different eco-geographical locations in to one cluster can be attributed to possibility of free exchange of germplasm. Similar observation was recorded by Singh and Gupta. However, unidirectional selection practiced for a particular trait or a group of linked traits in several places may produce similar phenotype which can be aggregated in to one cluster irrespective of their geographical origin as reported by Singh and Gupta (1968). The maximum inter cluster (D^2) value was obtained between cluster 10 and cluster 11. These clusters may be used for hybridization programme due to wide variability and possibility of transgressive segregants (Singh, 1991; Singh *et al.,* 1991). The minimum cluster distance was obtained in cluster 2 and cluster 5 which indicates that the accessions belonging to such clusters are relatively close. The selection of parents from genetically close clusters may be due to narrow genetic base and inbreeding depression (Singh and Gupta, 1968).

Qualitative characters

Out of 30 qualitative characters studied in 400 germplasm, the fruit shape distribution was observed to be oblong (67.5%), .elliptic (1.50%), roundish (30.5%), ovoid (0.25%), and obovoid (0.25%), fruit attractiveness, fiber free, pulp recovery (>70%) , pulp aroma (mild 55.50 (%), intermediate (28.75%) and strong (15.75%)); TSS and eating quality (poor (16.75%), good (63.50%), very good (15.50%) & excellent (4.25%) are very important for commercial point of view.

Sharma (1987) opined that flesh colour is controlled by additive genes. However, Iyer (1991) observed that light yellow colour is dominant over orange-yellow in the progenies of Alphonso x Neelum cross. Dinesh (2003), carried out using half-sib analysis and found that fruit characters like fruit weight, TSS and

pulp percentage are controlled by non additive factors and heritability is less. Lavi *et al.* (1998) reported that parents should not be chosen on the basis of phenotype since offspring performance is quite unpredictable. With regard to skin colour, it was found that when red-coloured varieties were crossed with green-coloured varieties, gradation of colour in the progenies indicated that it is controlled by a number of loci (Sharma, 1987; Iyer and Subramanyam, 1987). The presence of beak on the fruit seems to be dominant as the entire progenies had beak on their fruits when 'Totapuri' was used as one of the parents (Iyer and Subramanyam, 1979). Bunch bearing was found to be dominant over single fruiting (Sharma *et al*., 1972). Inheritance of red peel colour in the cross Amrapali x Sensation suggested no clear out dominance, as the cross between these parents with yellow and red peel respectively yielded hybrids with yellow, green yellow, fully green and red colouration on peel in various intensities. A high heritability with a high expected genetic advance clearly suggested that the inheritance of this trait was governed more by additive genes (Prabhuram, 1998). Sharma and Majumder (1988) in the crosses involving Totapuri Red Small and Sensation (both red peeled ones) and yellow-peeled varieties Dashehari and Amrapali revealed that the red peel colour was dominant and governed by duplicate genes, thereby showing various gradations of pink blush on the fruits. They reported that a few hybrids bore fruits with green colour which suggested that the red colour is in heterozygous condition. Fruit peel colour was found to be governed by a number of loci (Iyer and Subramanyam, 1987). Red peel colour is dominant over yellow and green and gradation of red peel suggested role of duplicate gene. Fruit quality varied in different hybrids developed at IARI, New Delhi. Fruit pulp colour is governed more by additive genes and that the environmental influence is very low (Prabhuram, 1998). Based on two hybrids, *viz*., Amrapali and Mallika, Fibre in pulp showed high heritability and high expected genetic advance, which suggested the genetic variance to be additive in nature. Aroma in pulp showed high heritability with a moderately high expected genetic advance. This suggested equal contributions of additive and non-additive genetic variance. On the other hand, genetic variance of fruit taste might have been governed mostly by non-additive genes.

Most of the commercially important varieties have been evolved as open pollinated progenies; still there are lot scopes to explore the OP varieties through exploitation of variability by selections. However, the varieties such as Alphonso, Amrapali, Arka Anmol, Arka Puneet, Bobbali punasa, Bombay no.1, Creeping, Danti Mamidi, Dashehari, Goa Kodur, Maharaja of Mysore, Mohammada Vikarabad, Mohan Rao Pasand, Neelgoa, Prabha shankar, Prior and Sardar, possess an excellent eating quality which can be utilized the breeding programme as well as commercial cultivation for table purpose.

CONCLUSION

The analysis of variance for 6 quantitative traits showed significant differences among the 400 genotypes of mango which indicates the existence of genetic diversity. The maximum inter cluster (D^2) value was obtained between cluster 10 and cluster 11. These clusters may be used for hybridization programme due to wide variability and possibility of transgressive sergeants. Estimates of phenotypic variance and genotypic variance had only a narrow difference for all six characters studied indicating that these characters are not much influenced by environmental factors. This also suggests the presence of sufficient genetic variability which can be exploited by adopting clonal selection or selection of chance seedlings.

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Original Research Paper

Standardization of Nitrogen Application for Potted *Chrysanthemum morifolium cv. Kikiobiory*

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ABSTRACT

The present investigation was carried out to standardize nitrogen application for standard potted *Chrysanthemum morifolium* cv. Kikiobiory at Department of Floriculture and Landscaping, PAU, Ludhiana during the year 2015-16. Six treatments of nitrogen *viz.* 0, 100, 200, 300, 400 and 500 mg/pot were applied twice in the last week of September and October. The results of the study revealed that nitrogen application had significant effect on all the vegetative and floral parameters. The largest flower size (17.69 cm) was obtained with the nitrogen application of 400 mg/pot which was at par with 500 mg/pot (17.67 cm). Application of nitrogen at 500 mg/pot recorded the highest plant height (75.47 cm), number of leaves per plant 75 days after planting (30.92), number of root suckers per plant (11.47) and delayed flower bud appearance (93.78 days), color break stage (122.59 days) and also shown flower quality deterioration by reducing the flowering duration (5.84 days) as compared to the other treatments. Therefore, it was concluded that 300 mg of nitrogen per pot applied twice was the standard dose for quality flower pot production in *Chrysanthemum morifolium* cv. Kikiobiory.

Key words: Chrysanthemum, Kikiobiory, Nitrogen, Standardize and Potted plants

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat), belongs to the family Asteraceae, commonly known as Queen of East or Autumn Queen, is a popular cut flower of commercial importance and native of Europe and Asia (Koley and Sarkar, 2013). Nitrogen applied as fertilizer is the main sources used to meet the N requirements of plant growth (Konnerup and Brix, 2010). Nutrient status of the plants can be indicator to the response of plant to the fertilization and internal content of the nutrient determine the fertilizer requirements (Polara *et al.*, 2014). Chrysanthemum is a heavy feeder of nitrogen and phosphorus with high requirement for N during first seven weeks of their growth period. William *et al.* (2013) reported that chrysanthemum accumulates applied N in the form of NO^{3-} during its active growth period which is later remobilized from vegetative tissues and directed to the developing bud during the bud emergence stage. Excessive nutrient concentrations caused an imbalance in other essential nutrients and reduced flower yield (Chawla *et al.*, 2007). The plant height, number of branches, flower per plant and flower size increased with increase in nitrogen dose in annual chrysanthemum (Baboo and Sharma, 1997). Though, there is lot of literature available pertaining to amount of nutrient requirement in field grown chrysanthemum and however, there is a need to work in particular for potted plants used as cultivars. Keeping this in view, the study was undertaken to standardize the nitrogen dose for standard potted *C. morifolium* cv. Kikiobiory.

MATERIAL AND METHODS

The present study was conducted at Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana during 2015-16. The terminal cuttings were taken from the mother stock plants pinched in end of May to encourage more number of axillary shoots of pot standard *C. morifolium* cultivar Kikiobiory. The terminal cuttings (5-7 cm) were

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treated with IBA 400mg/L and rooted in burnt rice husk in June-July. The rooted cuttings were then transplanted during August in the earthen pots (8") containing mixture of soil and FYM (2:1) along with diammonium phosphate DAP incorporated as a basal dose @ 1 kg/100 cubic feet. The application of nitrogen in the form of urea (N 46%) was done twice in the last week of September and October as per the treatments *viz*. control, 100 mg/pot, 200 mg/pot, 300 mg/pot, 400 mg/pot and 500 mg/pot. The liquid fertigation of potassium @ 200 ppm (muriate of potash) was given after transplanting the cuttings at 15 days interval till mid-October through watering can. The effect of different doses of nitrogen on vegetative growth (at 15 days interval) and floral parameters were recorded and statistically analyzed by SAS software using Duncan multiple range test (DMRT) at 5 per cent level of significance (Duncan, 1955).

RESULT AND DISCUSSION

The results indicated that vegetative parameters like plant height, number of leaves and root suckers per plant increased significantly ($p<0.05$) with increase in nitrogen application from 100 mg/pot to 500 mg/pot (Table 1). The plant height at 45 days after planting (DAP) varied significantly in all the treatments and was highest (25.80 cm) with the nitrogen application of 500 mg/pot whereas, it was shorter (22.29 cm) in the control. The plant height at 60 and 75 DAP were significantly high in the treatment of nitrogen 500 mg/pot (55.67 and 75.47 cm) and 400 mg/pot (54.30 and 72.85 cm). However, all the other treatments were at par among themselves. The shorter plant height at 60 and 75 DAP was observed in the control (46.44 and 60.30 cm) which was at par with 100 mg/pot (46.66 and 60.59 cm) and 200 mg/pot (47.60 and 63.31 cm). The numbers of leaves per plant 45, 60 and 75 DAP, were significantly more in 500 mg/pot (18.14, 25.92 and 30.92, respectively) followed by 400 mg/pot (17.41, 24.32 and 29.92, respectively), whereas minimum were observed in the control (14.62, 20.17 and 23.50, respectively). The maximum number of root suckers per plant was obtained in 500 mg/pot (11.47) which is significantly better than the other doses, whereas minimum was recorded in 100 mg/pot (8.78) which was at par with the control (8.61).

Table 1. Effect of nitrogen application on plant growth of chrysanthemum cv. Kikiobiory

Treatments (mg urea/ pot)	Plant height (cm)				No. of leaves per plant				No. of root suckers per plant
	30 DAP	45 DAP	60 DAP	75 DAP	30 DAP	45 DAP	60 DAP	75 DAP	
0	13.90 a	22.29 d	46.44 c	60.30 c	10.18 a	14.62 c	20.17 c	23.50 e	8.61 c
100	13.75 a	22.56 cd	46.66 c	60.59 c	11.17 a	15.57 bc	20.86 c	25.40 de	8.78 c
200	13.40 a	23.63 bcd	47.60 c	63.31 c	10.07 a	15.63 bc	21.85 c	27.16 cd	9.41 bc
300	13.62 a	24.17 abc	50.31 b	66.46 b	9.95 a	15.62 bc	22.22 bc	28.19 bc	10.82 ab
400	13.05 a	25.02 ab	54.30 a	72.85 a	9.86 a	17.41 ab	24.32 ab	29.92 ab	11.21 ab
500	13.68 a	25.80 a	55.67 a	75.47 a	10.95 a	18.14 a	25.92 a	30.92 a	11.47 a
F- test	ns	*	*	*	ns	*	*	*	*

Values followed by common alphabets do not differ significantly

The increased plant height, number of leaves and root suckers per plant obtained were due to the effect of nitrogen which increased the number of cells, cell size and an overall leaf production (Joshi *et al.,* 2013). The plants in control produced less vegetative growth due to non-availability of nitrogen and its involvement in photosynthesis. The increase in plant height in chrysanthemum at the higher dose of nitrogen might be due to the increase in transportation of metabolites and rate of photosynthesis in the plant which enabled the plant to have quick and better upward vegetative growth (Lodhi and Tiwari, 1993; Belgaonkar *et al.,* 1996). These results are in accordance with Joshi *et al.* (2013) and Dorajeerao *et al.* (2012) reported on chrysanthemum.

The flowering parameters *viz.,* days to flower bud appearance, colour break stage, full bloom, duration of flowering, flower diameter and nitrogen content in

plants were significantly ($p<0.05$) affected with increase in nitrogen application from 100 mg/pot to 500 mg/pot. The flower bud appearance, colour break stage and full bloom were delayed with the application of nitrogen at the rate of 500 mg/pot. The flower diameter, nitrogen content in plant are increased and duration of flowering decreased with increased nitrogen dose from 100 mg/pot to 500 mg/pot with increase in nitrogen application. The earliest flower bud appearance (84.50 days) was obtained in the control which is at par with 100 mg/pot (84.89 days) and the maximum days were observed in 500 mg/pot (93.78 days). The earliest color break stage was recorded in the control (111.84 days) and the maximum days were obtained with the application of nitrogen 500 mg/pot (122.59 days). The earliest full bloom was obtained in the control (138.31 days) and the maximum days were observed with the nitrogen dose of 500 mg/pot (144.87 days). The flower duration was significantly better in control (11.00 days) followed by nitrogen 100 mg/pot (10.20 days) whereas, shortest flower duration was recorded in the nitrogen dose 500 mg/pot (5.84 days) followed by 400 mg/pot (6.08 days). The largest size of flower (17.69 cm) was obtained in 400 mg/pot of nitrogen, it was at par with 500 mg/pot (17.67 cm) whereas, smallest flower size in control (15.18 cm). The nitrogen content increased significantly with increased nitrogen dose, maximum at 500 mg/pot (1.24 %) followed by 400, 300 and 200 mg/pot (1.12, 1.02 and 0.95, respectively) and minimum at control (0.81%).

The delayed flowering with higher dose of nitrogen in chrysanthemum has been reported earlier by Ingle *et al.* (1993) and Sharma *et al.* (2006). This delay in blooming is better as flowering of chrysanthemum is confined only to limited period from October to December thus, the monitoring of nitrogen dose application provides growers with an efficient crop schedule according to demand of flowers in the market. Joshi *et al.* (2013) also reported decreased vase life in chrysanthemum with increased nitrogen application of 300kgha^{-1}. These results are in conformity with the findings of De and Barman (1997) and John and Paul (1999) in chrysanthemum.

Table 2. Effect of nitrogen application on flowering of chrysanthemum cv. Kikiobiory

Treatments (mg urea/ pot)	Days to bud appearance	Days to color break stage	Days to full bloom	Duration of flowering (days)	Flower diameter (cm)	N content in plants (%)
0	83.79 c	112.02 d	138.31 c	11.00 a	14.56 b	0.81 f
100	84.89 c	116.08 c	140.39 bc	10.20 a	16.29 ab	0.86 e
200	88.53 b	117.89 c	141.64 ab	8.15 b	16.03 ab	0.95 d
300	89.38 b	118.83 bc	142.48 ab	7.29 bc	17.01 ab	1.02 c
400	90.40 b	120.82 ab	142.91 ab	6.08 c	17.69 a	1.12 b
500	93.78 a	122.59 a	144.87 a	5.84 c	17.67 a	1.24 a
F- test	*	*	*	*	*	*

Values followed by common alphabets do not differ significantly

The increased flower size with higher nitrogen application was due to the accelerated photosynthetic activities due to increase in number of leaves for providing facility to develop more flowers and increased flower size (Kumar *et al.,* 2002). The increased number of flowers per plant with increased nitrogen application is in conformity with the earlier findings reported in chrysanthemum (De and Barman, 1997; John and Paul, 1999). The increased nitrogen dose increases photosynthesis and enhances food accumulation and diversion of photosynthates towards sink resulting in better growth and subsequently higher number of flowers per plant and higher flower yield per hectare (Verma *et al.,* 2011).

The higher dose of nitrogen application of 500 mg urea/pot produced maximum vegetative growth with increased flower size and delayed flowering, but deteriorated the flower quality by shortening the duration of flowering. Therefore, it was concluded

that 300 mg urea/ pot applied twice was standardized nitrogen dose for quality flower pot production with better plant growth in *C. morifolium* cv. Kikiobiory.

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Original Research Paper

Influence of inorganic nutrients on growth, flowering and quality of *Dendrobium* cv. Singapore white

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ABSTRACT

***Dendrobium* orchid cv. Singapore White is cultivated commercially for cut flower purpose. The performance of this orchid in response to the different nutrient concentrations was evaluated under the agro-climatic conditions of Kodagu during 2017-2020. Twelve nutrient doses were applied as foliar sprays at weekly intervals to study their effect on vegetative growth, flower production and quality parameters of the cut flowers. Plant height, number of leaves plant^{-1}, leaf area, number of pseudobulbs plant^{-1}, number of spikes plant^{-1} year^{-1}, number of flowers spike^{-1} and spike length varied significantly with the nutrient doses. Foliar spray of 30:20:20 NPK 0.1% at weekly intervals recorded the maximum plant height of 53.21cm, number of spikes plant^{-1}year^{-1} (10.01) with spike length of 44.43 cm and 16.20 flower spike^{-1}.**

Key words: Cut flower production, *Dendrobium,* Nutrients, Orchid and Quality

INTRODUCTION

Dendrobium orchid cv. Singapore White is an epiphyte belonging to the family Orchidaceae. It produces long spikes bearing attractive white flowers and is commercially cultivated for cut flower purpose. Globally orchids are traded as cut flowers and potted plants and are estimated to comprise of about 10% of the total cut flower trade (De *et al*., 2014). Indian orchid trade registered an increased trend of import value of orchids as compared export value during 2013-2014 to 2018-2019. According to De (2020) the highest import of orchids was recorded in 2013-2014 (Rs. 3425.76 lakh) followed by 2015-2016 (Rs. 2985.19 lacs) and 2018-2019 (Rs. 2321.84 lakh). Commercial cultivation of orchids in India has lot of scope to meet the demand for orchid cut flowers thereby reducing our dependence on imported flowers.

In India, states such as Kerala, Tamil Nadu, Karnataka, and Maharashtra are commercially cultivating *Dendrobium* orchids for cut flower production. This species comprises of 90% of the orchids in commercial cultivation (Rajeevan and Shobhana,1993). Similarly, in Karnataka, *Dendrobium* cultivation has been taken up in a cluster mode under Kanflora Society (Hegde, 2017). Kodagu, in Karnataka harbours about 75 species of native orchids and has conducive climatic conditions for commercial cultivation of orchids (Rao,1998). Among the exotic orchids, *Phalaenopsis, Dendrobium* and *Oncidium* are grown by orchid enthusiasts in this region. There is a lot of potential for commercial cultivation of orchids in this region.

Orchid plants receive water and nutrition from rainfall, air, breakdown of humus accumulated in the crevices of tree trunks and bird droppings in its natural habitats (Naik *et al*., 2009). For commercial cultivation orchid plants have to be provided with inorganic nutrients through foliar sprays for proper growth and development. Frequent application of diluted fertilisers to cater to the nutrient absorption and storage capacity is the general norm. Considering these facts, the present study was conducted to identify the

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optimum nutrient regime for *Dendrobium* orchids to realise higher cut flower yield of superior quality under Kodagu conditions.

MATERIALS AND METHODS

The present investigations to study the effect of inorganic nutrients on growth and flowering of orchids, *Dendrobium* cv. Singapore White was conducted during 2017-2020 at the Central Horticultural Experiment Station of ICAR- Indian Institute of Horticultural Research, located in Chetalli in Kodagu district of Karnataka. Chetalli is located between 12^0 37' North latitude and 75^0 83' East longitude at an elevation of 1050 m above MSL, with temperature ranging from 19^0C to 32^0C and relative humidity from 55- 90%. Hardened tissue cultured plants were planted in 8" plastic pots in a medium comprising of broken tile bits, chopped coconut husk and charcoal in 1:1:1 ratio (v/v) and was housed in a naturally ventilated polyhouse. The experiment comprised of twelve different ratios of NPK *viz*., T_1: 10:10:10 NPK @ 0.1% ;T_2: 10:20:10 NPK @ 0.1% ; T_3:10:10:20 NPK @ 0.1%; T_4:10:20:20 NPK @ 0.1% ; T_5:20:10:10 NPK @ 0.1% ; T_6: 20:20:10 NPK @ 0.1% ; T_7:20:10:20 NPK @ 0.1% ; T_8:20:20:20 NPK @ 0.1% ; T_9: 30:10:10 NPK @ 0.1% ;T_{10}:30:20:10 NPK @ 0.1% ; T_{11}:30:10:20 NPK @ 0.1% and T_{12}: 30:20:20 NPK @ 0.1%. The nutrients were applied at weekly intervals as foliar sprays. The experiment was laid out in completely randomized design (CRD) with three replications and ten plants per replication. The observations were recorded for three consecutive years on plant height, number of leaves plant^{-1}, leaf area, number of pseudobulbs plant^{-1}, internodal length, girth of pseudobulbs number of spikes plant^{-1} year^{-1}, number of flowers spike^{-1}, spike length, flower size, pedicel length and vase life of cut flowers. Data recorded for the vegetative and floral parameters over the three-year period was pooled and analysed using the OPSTAT statistical package (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

Among the vegetative parameters presented in Table 1, plant height, number of leaves plant^{-1}, leaf area and number of pseudobulbs plant^{-1} varied significantly among the treatments. Foliar spray of 30:20:20 NPK @ 0.1% (T_{12}) at weekly intervals recorded the maximum plant height of 53.21 cm and was at par with treatments T_2:10:20:10 NPK@0.1% (53.11 cm); T_3:10:10:20 NPK @ 0.1%; (52.41 cm), T_9: 30:10:10 NPK @ 0.1% (50.71 cm) and T_{11}:30:10:20 NPK @ 0.1% (49.43 cm). The results are in accordance with the findings of Bichsel *et al*. (2008) who found that an increase in nitrogen was found to have a favourable effect on plant height in *Dendrobium* hybrids. This is also corroborated by the findings of Anitha and Kannan (2015a) in *Dendrobium* orchid cv. Earsakul. The minimum plant height (43.80 cm) was recorded in treatment T_8:20:20:20 NPK @ 0.1%. Weekly foliar application of T_{10}:30:20:10 NPK @ 0.1% recorded the maximum number of leaves plant^{-1} (47.82) and was at par with treatments T_4:10:20:20 NPK @ 0.1% (46.44); T_{11}:30:10:20 NPK @ 0.1% (46.02) and T_{12}:30:20:20 NPK @ 0.1% (44.72). Application of higher levels of nitrogen was found to significantly increase the total chlorophyll content in *Dendrobium* orchid cv. Earsakul according to Anitha and Kannan (2015b). The number of leaves affects the photosynthetic efficiency of the plant and this is in accordance with the findings of Wang (1996) in *Phalaenopsis*. The minimum number of leaves plant^{-1} (25.05) was recorded in treatment T_7:20:10:20 NPK @ 0.1%. Foliar spray of T_2:10:20:10 NPK @ 0.1% recorded the maximum leaf area (48.40 cm^2) whereas, minimum leaf area (30.75 cm^2) was recorded in treatment T_5: 20:10:10 NPK @ 0.1%. The treatment T_4:10:20:20 NPK @ 0.1% recorded the maximum number of pseudobulbs plant^{-1} (9.10) and was at par with T_{12}:30:20:20 NPK @ 0.1% (8.89), whereas application of T_3:10:10:20 NPK @ 0.1% resulted in the minimum number of pseudobulbs plant^{-1} (6.63). Improved vegetative growth of the plants may be attributed to the increase in the photosynthetic capacity of the plants. This is in corroboration with the findings of Sailo, *et al.* (2014) that though the leaves are the source of photosynthates for inflorescence development, the pseudobulb is responsible for the redistribution of assimilates from the leaves. There is substantial mobilisation of carbohydrate to the inflorescence through the pseudobulb. The internodal length and girth of pseudobulbs did not vary significantly with the treatments.

Table 1. Effect of inorganic nutrients on vegetative characters of *Dendrobium* cv. Singapore White

Treatments	Plant height (cm)	No. of leaves per plant	Leaf area (cm^2)	No. of pseudo-bulbs per plant	Internodal length (cm)	Girth of pseudobulbs (mm)
T_1	48.63	30.67	37.29	7.15	3.35	12.56
T_2	53.11	43.42	48.40	8.44	3.12	12.76
T_3	52.41	37.38	38.11	6.63	3.19	12.31
T_4	46.78	46.44	31.68	9.10	3.44	12.03
T_5	46.82	36.65	30.75	7.33	3.57	11.79
T_6	48.89	36.76	35.67	8.07	3.57	12.06
T_7	46.79	25.05	32.07	8.34	3.22	11.96
T_8	43.80	43.16	34.57	8.18	3.28	12.05
T_9	50.71	29.79	36.34	6.98	3.07	12.67
T_{10}	47.76	47.82	37.17	7.86	3.18	12.08
T_{11}	49.43	46.02	36.83	7.99	3.26	12.55
T_{12}	53.21	44.72	35.19	8.89	3.37	13.01
CD (P=0.05)	3.78	4.02	3.50	0.63	NS	NS

Treatment details :

T_1: 10:10:10 NPK @ 0.1%	T_2: 10:20:10 NPK @ 0.1%	T_3: 10:10:20 NPK @ 0.1%	T_4: 10:20:20 NPK @ 0.1%
T_5: 20:10:10 NPK @ 0.1%	T_6: 20:20:10 NPK @ 0.1%	T_7: 20:10:20 NPK @ 0.1%	T_8: 20:20:20 NPK @ 0.1%
T_9: 30:10:10 NPK @ 0.1%	T_{10}: 30:20:10 NPK @ 0.1%	T_{11}: 30:10:20 NPK @ 0.1%	T_{12}: 30:20:20 NPK @ 0.1%

The floral traits and the vase life of cut flowers were recorded and have been presented in Table 2. The number of spikes $plant^{-1}year^{-1}$, number of flowers $spike^{-1}$ and spike length varied significantly among the treatments. Maximum number of spikes $plant^{-1}year^{-1}$ (10.01) was recorded with foliar spray of T_{12}: 30:20:20 NPK @ 0.1% and the minimum number of spikes $plant^{-1}year^{-1}$ (7.07) were recorded with application of T_7:20:10:20 NPK 0.1%. Maximum number of flowers $spike^{-1}$ (16.69) was recorded in treatment T_4: 10:20:20 NPK @ 0.1% and was at par with foliar application of T_{12}:30:20:20 NPK @ 0.1% (16.20) and T_3:10:10:20 NPK @ 0.1% (16.00) whereas the minimum number of flowers $spike^{-1}$ was recorded in the treatment T_8:20:20:20 NPK @ 0.1% (10.68). Maximum spike length was recorded in the treatment T_2:10:20:10 NPK @ 0.1% (49.06 cm) and was at par with foliar application of T_5:20:10:10 NPK @ 0.1% (46.69 cm), whereas T_7:20:10:20 NPK @ 0.1% had the minimum spike length (41.28 cm). The main factors that could increase profitability of orchid cultivation is the improvement of flowering characteristics such as number of spikes produced and number of flowers per spike. This is in accordance with the findings of Wang (2004). Increase in levels of NPK in the foliar spray has played a significant role in increasing the spike length and the number of flowers per spike even while the production of flower spikes per plant has substantially increased, which is desirable for profitable commercial production. At higher levels of nutrient application, there was significant increase in spike length which might be due to

higher nutrient absorption in plants (Sudeep *et al*., 2018). Other parameters like flower size, pedicel length and vase life did not vary significantly with the treatments. This might be due to the fact that these are predominantly governed by the genetic traits. This contradicts the findings of Higaki and Imamura (1987) that increasing the level of N generally increases the flower size of Vanda 'Miss Joaquim' and addition of P and K further increases Vanda flower size.

Table 2. Effect of inorganic nutrients on floral characters and vase life of *Dendrobium* cv. Singapore White

Treatments	No. of spikes $plant^{-1}$ $year^{-1}$	No. of flowers / spike	Spike length (cm)	Flower size (Length-cm)	Flower size (Breadth - cm)	Pedicel length (cm)	Vase life (days)
T_1	8.26	15.23	45.52	6.78	6.65	5.60	14.38
T_2	8.70	14.43	49.06	6.90	6.73	5.78	13.11
T_3	8.80	16.00	43.80	7.00	6.92	5.75	12.13
T_4	8.03	16.69	45.40	6.91	6.93	5.83	11.67
T_5	7.77	13.87	44.43	6.86	6.94	5.84	13.24
T_6	7.90	11.48	43.59	6.90	6.93	5.30	12.38
T_7	7.07	11.10	41.28	6.57	6.82	5.61	11.82
T_8	7.59	10.68	42.89	6.47	6.89	5.71	14.51
T_9	8.15	11.92	45.98	6.93	7.10	5.81	13.84
T_{10}	7.45	11.99	45.35	6.82	7.35	5.88	13.36
T_{11}	7.11	12.25	43.74	6.40	7.12	5.86	15.29
T_{12}	10.01	16.20	46.69	6.77	6.76	5.70	12.45
CD (P=0.05)	0.83	1.32	3.22	NS	NS	NS	NS

Table 3. Correlation of vegetative and floral traits with flower spike yield as influenced by inorganic nutrient doses

Character	No. of leaves/ plant	Leaf area (cm^2)	No. of Pseudo bulbs/plant	Inter -nodal length	Pseudo-bulb girth (mm)	No. of flowers / spike	Spike length (cm)	No. of spikes / plant
Plant Height (cm)	0.059	0.628*	-0.125	-0.336	0.791**	0.491	0.626*	0.731**
No. of Leaves / plant		0.212	0.480	0.073	0.120	0.239	0.371	0.162
Leaf area (cm^2)			-0.064	-0.550	0.586*	0.110	0.667*	0.317
No. of pseudo bulbs / plant			0.224	0.043	0.084	0.140	0.084	
Internodal length					-0.436	0.174	-0.224	0.002
Pseudobulb girth (mm)						0.353	0.658*	0.674*
No. of flowers / spike							0.513	0.707*
Spike length (cm)								0.624*

* Significant at 5% level ** Significant at 1% level

Estimates of the correlation coefficients among the various characters indicated that economic characters like spike length, number of flowers spike^{-1} and spike yield were positively correlated (Table 3). Among the vegetative characters, plant height recorded highly significant positive correlation (0.731) followed by girth of pseudo stem (0.674) which was significantly and positively correlated with spike yield. The results are in accordance with the findings of Zimmerman (1990). Pseudobulbs have ability to store water, mineral and carbohydrates. The active accumulation of mineral nutrients during the period of pseudobulb development constitutes an important source of reserve for the subsequent development of the inflorescence and new shoots as reported by Hew and Ng (1996). Hence vegetative growth in sympodial orchids has a direct effect on flowering and flower quality. Significant positive correlation with the number of spikes plant^{-1} was observed for number of flowers spike^{-1} (0.707) followed by spike length (0.624). Low positive correlation with spike yield was recorded for number of leaves plant^{-1}, leaf area, number of pseudobulbs plant^{-1} and internodal length. Significant positive correlation was recorded for spike length with leaf area (0.667) followed by pseudostem girth (0.658) and plant height (0.626). Girth of pseudobulb recorded a highly significant positive correlation with plant height (0.791) and was also positively correlated with leaf area (0.586). Leaf area recorded a significant positive correlation with plant height (0.628).

It may be concluded that foliar spray of NPK in the ratio of 30:20:20 at 0.1% at weekly intervals was found to be promising, resulting in higher cut flower yield and superior quality of spikes in *Dendrobium* cv. Singapore White under the agro climatic conditions of Kodagu.

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Original Research Paper

Palynological investigations in *Jasminum* spp.

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ABSTRACT

The present investigation was carried out at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2017-2019. The study involved nine jasmine genotypes, four falling under the commercially cultivated types and five belonging to underutilized species or 'lesser-known species'. The study was undertaken to investigate and document the palynological parameters of jasmines which could serve as a reliable reference for future jasmine breeding programmes. The palynological investigations were carried out using by Scanning Electron Microscopy (SEM), haemocytometry, acetocarmine test and i*n vitro* pollen germination. The pollen morphology analysis indicated wide variation among the species for shape of pollen grain, ranging from tricolpate to prolate; the exine ornamentation was reticulate in all the genotypes. Pollen output was the highest in *J. rigidum* (28,660 pollen/flower) and the lowest in (625 pollen/ flower) in *J. sambac* cv. Ramanathapuram Gundumalli. The maximum pollen germination rate and pollen tube length was recorded in *J. rigidum.*

Key words: *Jasminum* spp., Palynology, Pollen morphology and Pollen germination

INTRODUCTION

Jasmine (*Jasminum* sp.) belonging to family Oleaceae is one of the most important and popular traditional flowers of India. It is native to South and Southeast Asia. A large number of species of *Jasminum* are centered around the regions comprising India, China and Malaysia (Nirmala *et al.*, 2017). The area coverage under flower crops in India is 3,07,000 ha with a production of18,05,000 MT of loose flowers and 7,04,000 MT of cut flowers (2017-18) (www.indiastat.com). In India, Tamil Nadu is the leading producer of jasmine in the country with an annual production of 1,36,901 tonnes from an area of 13,246 ha with a productivity of 11.21 t/ha (Anonymous , 2019). The genus *Jasminum* consists of more than 200 species and is mostly tropical in distribution (Dickey, 1969).

Though there are a large number of species and varieties of jasmine, commercial cultivation is confined to only a very few species. Besides the three commercial jasmine species namely, *J. sambac, J. grandiflorum* and *J. auriculatum* which have attained importance in commercial cultivation (Rimando, 2003; Green and Miller, 2009) and the less exploited, *J. multiflorum* (Syn: *J. pubescens*) which is cultivated to some extent in Karnataka, three lesser known species namely, *J. nitidum*, *J. calophyllum* and *J. flexile* possess economic importance since they produce flowers which are suitable for use as loose flower, besides being ideal garden plants. These three species have the added merit of flowering throughout the year (Ganga *et al*., 2015), unlike the three popular commercial species namely, *J. sambac, J. grandiflorum* and *J. auriculatum* which undergo 'off-season' during the cooler months. The species *J. rigidum* also possesses year-round flowering potential, though the flowers have shorter shelf life.

Since the demand for jasmine flowers is growing day by day owing to its wide range of uses, there arises a pressing need for improving its production and productivity, besides exploring newer strategies to evolve improved genotypes. Flowers of the three commercially important jasmine species are

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unavailable in the market during the 'off-season' coinciding with the cooler months (November to March). The above mentioned lesser-known species possess year-round flowering potential but produce flowers with milder fragrance. Interspecific hybridization can enable introgression of desirable genes within the species. Interspecific hybridization in jasmine has been attempted earlier (Anon., 1974; Veluswamy, 1981) but was not successful, the predominant reason being failure of seed germination owing to hybrid unviability and a possible endosperm antagonism in operation (Veluswamy, 1981).

Palynological data are essential pre-requisites of any plant breeding programme. Domez and Isik (2008) and Hanif *et al.* (2013) emphasized the association of palynological aspects with the cytological status of plant species. The present study was undertaken to investigate and document the palynological parameters of commercial and lesser-known species of *Jasminum* which would serve as a reliable reference for future jasmine breeding programmes.

MATERIALS AND METHODS

(i) Plant materials

Plant materials were collected from the jasmine germplasm of the Department of Floriculture and Landscape Architecture, TNAU, Coimbatore located at 11°02' N latitude and 76°57' E longitude at an altitude of 426.72 m above MSL. The weather condition at Coimbatore during the study was moderately warm with hot early summer months during March-May. In open field conditions, the maximum temperature fluctuated between 25^{0}C and 35^{0}C with a mean of 30^{0}C. The minimum temperature ranged between 17^{0}C and 23.5^{0}C with a mean of 20^{0}C. The annual rainfall was 750 mm and relative humidity ranged between 60 and 90 per cent with a mean of 75 per cent.

The study involved nine genotypes of jasmine belonging to eight *Jasminum* species. The commercial species category included four genotypes namely, *J. sambac* cv. Ramanathapuram Gundumalli, *J. auriculatum* cv. CO.1 Mullai, *J. grandiflorum* cv. CO.1 Pitchi and *J. grandiflorum* (White). The lesser known (underutilized) species category included five genotypes namely, *J. calophyllum, J. flexile, J. multiflorum* (Pink flowered type), *J. nitidum* and *J. rigidum.*

(ii) Methods adopted

The methods adopted to study the palynological aspects of the above listed *Jasminum* species are briefly discussed below.

Pollen morphology

For assessing the morphological characteristics of pollen, flower buds at mature bud stage were involved. In the laboratory, anthers were isolated from the flower buds in petri-dishes and were maintained for 24-48 hours at room temperature (24°C) to facilitate release of pollen. Then the petri- dishes with pollen were transferred to a desiccator, where they were kept until analysis. Preparation of pollen for analysis was performed by mounting two-layer transparent tape on the object carrier on the microscope and applying pollen with a brush. The prepared samples were observed under a Scanning Electron Microscope (SEM) at a magnification of 2000X (whole grain) to15,000X (exine pattern). The pollen size was measured and the range was recorded for a sample of 30 pollen grains from each of the nine genotypes of jasmine.

Pollen output

Pollen production per flower was estimated using Haemocytometer as suggested by Sathiamoorthy (1973). Three samples of anthers from each *Jasminum* species were collected just prior to dehiscence. The anthers were crushed with a small glass rod in a vial containing 2.5 ml of distilled water and a drop of teepol for obtaining a good suspension of pollen grains in water. The contents were thoroughly shaken and two drops of it were pipetted out and placed on each of the two counting chambers of a Spencer bright line Haemocytometer. The number of pollen grains in each of the eight "corner squares" was recorded. This was repeated five times for each sample and was designated as sub samples. The average number of pollen grains per square multiplied by 2500 gave the quantity of pollens per anther.

Pollen viability

The pollen viability and fertility were assessed by the following two methods.

(a) Acetocarmine test

Freshly dehisced pollen grains were collected from each of the *Jasminum* species in sterilized petri-

dishes. The pollen grains were dusted on the cavity slide and a drop of Acetocarmine stain was placed on the pollen grains. Deeply stained, normal and plump pollen grains were considered viable while shriveled, deformed and weakly stained pollen grains were considered as sterile ones. Pollen viability was assessed for three days *viz*., first day, second day and third day of anther dehiscence and expressed in percentage.

(b) *In vitro* pollen germination

Pollen from freshly dehisced anthers were collected and tested for germination on the day of anther dehiscence. Pollen was dusted in cavity slide in which 10 per cent of sucrose and 10 ppm of boric acid solution were dropped over gently with a pointed dropper and pollen grains were mixed thoroughly in the solution with the help of a needle. The cavity slides were kept in petri dishes over small glass rods containing moist filter paper at the bottom and closed properly. These were then incubated at ambient room temperature (25°C) for optimum germination. The slides were examined under microscope after one hour, two hours and three hours. Germinated and non-germinated pollen grains were counted separately in several random fields containing a total of 100 pollen grains and the length of the pollen tube was measured in micrometer (µm).

RESULTS AND DISCUSSION

Pollen morphology

The details of pollen morphology of the nine genotypes of jasmine are furnished in Table 1 Scanning Electron Microscope (SEM) images of the *Jasminum* genotypes indicated wide variations in shape of pollen among the genotypes.

Pollen size

The pollen size ranged between 36.2 µm and 57.9 µm in *J. sambac* cv. Ramanathapuram Gundumalli, 33.0 µm and 42.9 µm in *J. auriculatum* cv. CO. 1 Mullai, 32.32 µm and 41.22 µm in *J. grandiflorum* cv. CO. 1 Pitchi, 33.0 µm and 46.2 µm in *J. grandiflorum* (White), 53.5 µm and 68.4 µm in *J. calophyllum,* 30.2 µm and 53.2 µm in *J. flexile*, 30.5 µm and 53.5 µm in *J. multiflorum* (Pink), 33.0 µm and 52.8 µm in *J. nitidum* and 39.6 µm and 49.5 µm in *J. rigidum* (Table 1).

Table 1. Pollen morphology, pollen size (µm) and pollen output in *Jasminum* species

Sl. No.	*Jasminum* genotype	Morphological characters: Pollen shape	Exine ornamentation	Aperture	Pollen size range (µm)	Pollen output
1.	*J. sambac* cv. Ramanathapuram Gundumalli	Tricolpate	Reticulate	Poorly defined	36.2-57.9	625
2.	*J. auriculatum* cv. CO.1 Mullai	Late obovatus	Reticulate	Sunken	33.0-42.9	14,175
3.	*J. grandiflorum* cv. CO.1 Pitchi	Prolate	Reticulate, granular	Prominent	32.32-41.22	17,920
4.	*J. grandiflorum* (White)	Spheroidal	Coarsely Reticulate	Poorly defined	33.0-46.2	23,816
5.	*J. calophyllum*	Obtuse-angular	Reticulate	Prominent	53.5-68.4	8,769
6.	*J. flexile*	Circular	Reticulate and smooth	Poorly defined	30.2-53.2	13,778
7.	*J. multiflorum*\ (Pink)	Prolate	Distinctly reticulate	Poorly defined	30.5-53.5	17,002
8.	*J. nitidum*	Circular	Coarse	Sunken	33.0-52.8	21,056
9.	*J. rigidum*	Prolate	Reticulate, conspicuous furrows	Prominent	39.6-49.5	28,660

Pollen output

Pollen output (average number of pollen produced/ flower) for the *Jasminum* species ranged between 625 and 28,660. The pollen output was 28,660 in *J. rigidum* which was the highest among the nine genotypes and 625 in *J. sambac* cv. Ramanathapuram Gundumalli which was the least among the genotypes. The pollen output was 14,175 in *J. auriculatum* cv. CO.1 Mullai, 17,920 in *J. grandiflorum* cv. CO.1 Pitchi, 23,816 *J. grandiflorum* cv. White, 8,769 in *J. calophyllum*, 13,778 in *J. flexile,* 17,002 in *J. multiflorum* (Pink) and 21,056 in *J. nitidum* and (Table 1).

Makde (1982) opined that in *Jasminum* species, a majority of pollen grains were characterized by large scale vacuolation and scanty cytoplasm resulting in degeneration of pollen grains prior to dehiscence, leading to low pollen output.

Pollen viability

Data on pollen viability are furnished in Tables 2 and 3 and pictorial details are furnished in Fig. 1 to 3. The percentage of fertile pollen in *J. sambac* cv. Ramanathapuram Gundumalli was 5.67 per cent on the day of dehiscence and it gradually decreased to 0 per cent on the third day. The percentage of fertile pollen in *J. auriculatum* cv. CO.1 Mullai was 94.87 per cent on the day of dehiscence and it gradually decreased to 43.33 per cent on the third day. The percentage of viable pollen in *J. grandiflorum* cv. CO.1 Pitchi was 77.17 per cent on the day of dehiscence and gradually decreased to 18.67 per cent on the third day. The percentage of viable pollen in *J. grandiflorum* cv. White was 95.50 per cent on the day of dehiscence and it gradually decreased to 18.67 per cent on the third day. (Table 2).

In *J. flexile, t*he percentage of viable pollen was 90.67 per cent on the day of dehiscence and it gradually decreased to 22.33 per cent on the third day. The percentage of viable pollen in *J. calophyllum* was 77.17 per cent on the day of dehiscence and it gradually decreased to 18.67 per

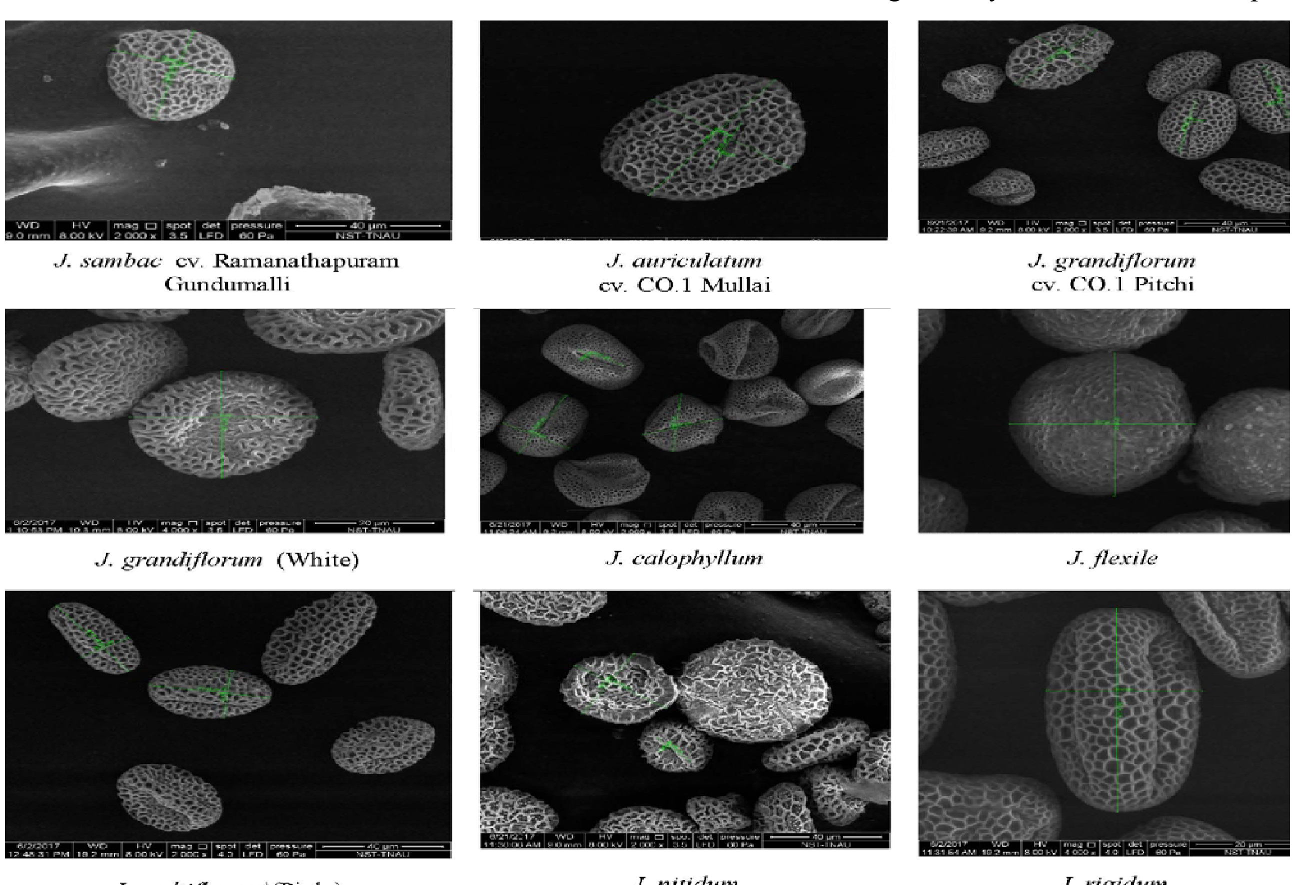


Fig. 1. Pollen morphology *Jasminum* species visualized under SEM

cent on the third day. The percentage of viable pollen in *J. multiflorum* (Pink) was 54.67 per cent on the day of dehiscence and gradually decreased to 17.33 per cent on the third day. The percentage of fertile pollen in *J. nitidum* was 53.33 per cent on the day of dehiscence and it gradually decreased to 18.33 per cent on the third day. The percentage of fertile pollen in *J. rigidum* was 95.33 per cent on the day of dehiscence and it gradually decreased to 27.67 per cent on the third day (Table 3).

Pollen germination rate

The mean germination percentage in *Jasminum* species ranged between 0 per cent and 94.34 per cent, with the highest in *J. rigidum.* No germination was observed in *J. sambac* cv. Ramanathapuram Gundumalli. The mean germination percentage was 42.56 per cent in *J. auriculatum* cv. CO.1 Mullai, 25.3 % in *J. grandiflorum* cv. CO. 1 Pitchi, 59.9 % in *J. grandiflorum* cv. White, 31.72 % in *J. flexile*, 24.0 % in *J. calophyllum*, 1.39 % in *J. multiflorum* (Pink) and 8.86 % in *J. nitidum* (Table 4).

Pollen tube growth

In the present study, pollen tube growth as measured in terms of the length of pollen tube in the *Jasminum* species ranged between 0 µm and 1917.10 µm. The maximum pollen tube length of 1917.10 µm was recorded in *J. rigidum.* The mean length of pollen tube was 447.81 µm in *J. auriculatum* cv. CO. 1 Mullai, 552.61 µm in *J. grandiflorum* cv. CO. 1 Pitchi, 449.82 µm in *J. grandiflorum* cv. White, 222.15 µm in *J. flexile*, 641.15 µm in *J. calophyllum,* 884.27 µm in *J. nitidum*. Pollen tube was not conspicuous in *J. multiflorum* (Pink).

Pollen viability is an important factor, since the probability of fertilization usually declines when pollen grains with low viability are deposited on the stigma (Wilcock and Neiland, 2002; Zhao *et al*., 2004; Teng *et al*., 2012). Lai (1995) and Deng *et al*. (2014) attributed low pollen fertility and Deng *et al*. (2016)

Table 2. Pollen of commercial jasmine genotypes

Age of pollen grains	No. of. pollen grains tested	*J. sambac* cv. Ramanthapuram Gundumalli		*J. auriculatum* cv. CO. 1 Mullai		*J. grandiflorum* cv. CO. 1 Pitchi		*J. grandiflorum* (White)	
		Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)
1st day of dehiscence	100	5.67	94.33	94.87	5.13	77.17	22.83	95.50	4.50
2ndday of dehiscence	100	3	97	78.33	21.67	56.00	44.00	62.67	37.33
3rd day of dehiscence	100	0	100	43.33	56.66	18.67	81.33	18.67	81.33

Table 3. Pollen viability of underutilized jasmine genotypes

Age of pollen grains	No. of. pollen grains tested	*J. nitidum*		*J. calophyllum*		*J. multiflorum* (Pink)		*J. flexile*		*J. rigidum*	
		Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)	Fertile pollen (%)	Sterile pollen (%)
1st day of dehiscence	100	53.33	46.67	83.33	16.67	54.67	45.33	90.67	9.33	95.33	4.67
2ndday of dehiscence	100	44.33	55.67	75.33	24.67	45.33	54.67	69.33	30.67	78.00	22.00
3rd day of dehiscence	100	18.33	81.67	25.67	74.33	17.33	82.67	22.33	77.67	27.67	72.33

Table 4: *In vitro* pollen germination in *Jasminum* species (after 3 hr incubation)

Species	Germination %	Length of pollen tube (µm)
J. sambac cv. Ramanathapuram Gundumalli	0.00	-
J. auriculatum cv. CO. 1 Mullai	42.56	447.81
J. grandiflorum cv. CO. 1 Pitchi	25.3	552.61
J. grandiflorum (White)	59.9	449.82
J. flexile	31.72	222.15
J. calophyllum	24.0	641.15
J. multiflorum (Pink)	1.39	-
J. nitidum	8.86	884.27
J. rigidum	94.34	1917.10

Fig. 2. Pollen stainability in *Jasminum* genotypes

J. sambac cv. Gundumalli

J. auriculatum cv. CO.1 Mullai

J. grandiflorum cv. CO. 1 Pitchi

J. grandiflorum (White)

J. calophyllum

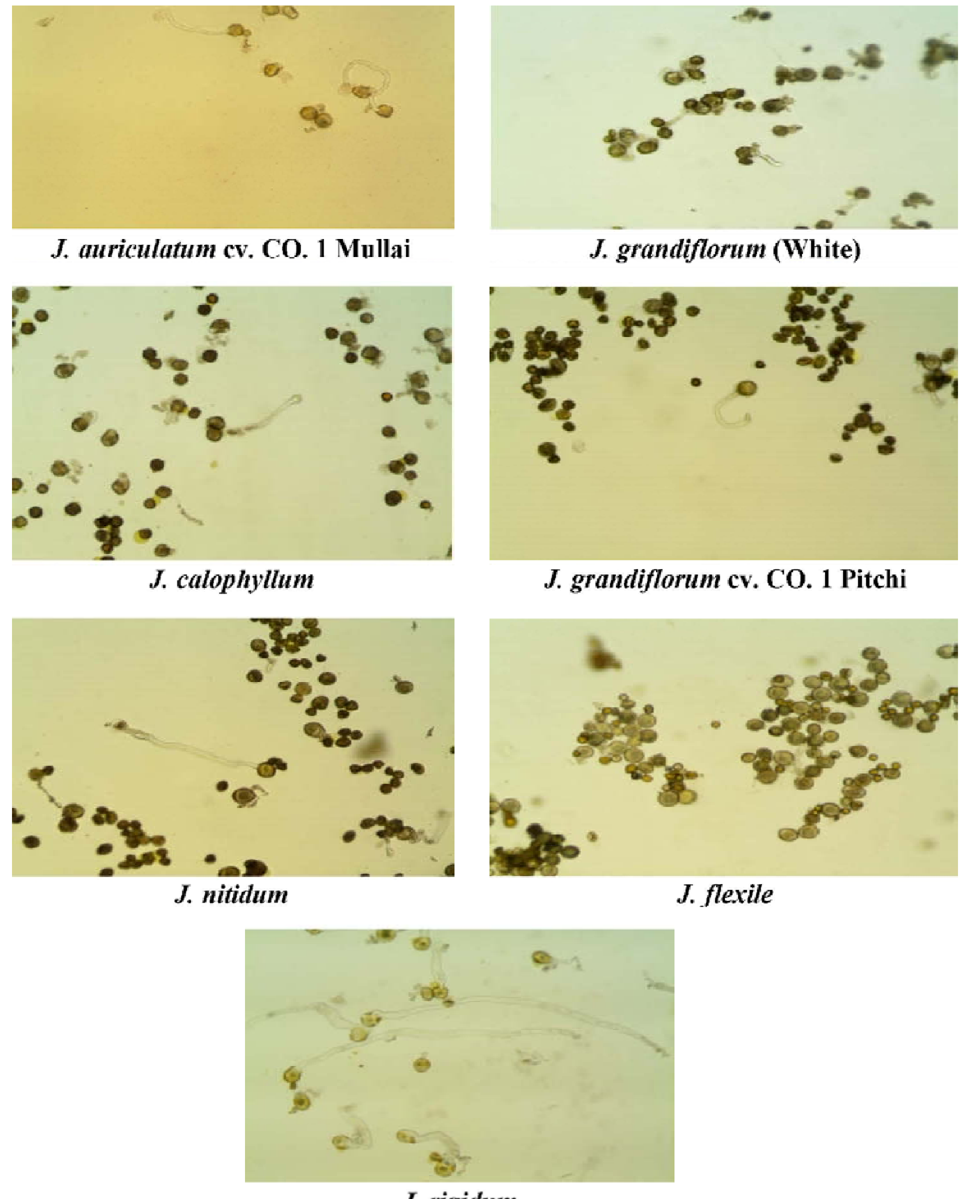
J. flexile

J. multiflorum (Pink)

J. nitidum

J. rigidum

Fig. 3. *In vitro* pollen germination in *Jasminum* genotypes



attributed low pollen viability for poor fertilization in jasmine. Deng *et al*. (2016) also reported that among 15% of anthers, tetrads formed pollen mass instead of free microspores, and only one tube grew normally from the tetrad pollen.

The differential pollen viability of the jasmine species recorded in the present study is attributable to the varied ploidy levels and chromosomal forms. In *J. sambac* cv. Ramanathapuram Gundumalli, higher pollen sterility is attributed to its triploid status which leads to meiotic abnormalities. These observations are supported by the earlier reports of Raman (1955) and Deng *et al*. (2017) in *Jasminum* species.

In the various jasmine species studied, pollen germination percentage was low to moderate (Plate 2). This might be associated with the irregular meiosis leading to defective pollen and egg cells, ultimately resulting in sterility. Datta *et al*. (1960) elucidated that structural changes lead to loss of genes as expressed in the suppression of the female reproductive development in *J. grandiflorum*.

CONCLUSION

The inferences from the present study are (i) there is wide variation among the nine genotypes with respect to shape of pollen grain while exine

ornamentation was reticulate in all the genotypes, (ii) *J. auriculatum* cv. CO.1 Mullai, *J. grandiflorum* cv. White, *J. flexile* and *J. rigidum* had high pollen viability. Pollen output (average number of pollen produced/flower) was the highest in *J. rigidum* (28,660) and the least (625) in *J. sambac* cv. Ramanathapuram Gundumalli; (iii) The highest rate of pollen germination (94.34%) and length of pollen tube (1917.10 µm) was recorded in *J. rigidum.*

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Original Research Paper

Effect of putrescine and benzyl adenine on growth, flowering and post-harvest keeping quality parameters in chrysanthemum (*Chrysanthemum morifolium* Ramat.)

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ABSTRACT

The experiment was conducted with the objective to study the effect of different anti-senescence compounds like putrescine and benzyl adenine (BA) on vegetative, floral and post-harvest keeping quality of chrysanthemum cv. Punjab Shyamli. The experiment was conducted in randomized block design (RBD) and replicated thrice. Putrescine (@ 50, 100 and 150 ppm) and benzyl-adenine (@ 100, 150 and 200 ppm) were sprayed twice on cv. Punjab Shyamli (spray type and pompon) at bud initiation stage and at fully developed flower buds. Control plants were sprayed with normal water. Floral parameters were delayed and vase life of cut stems was enhanced by benzyl adenine @ 200 ppm. The maximum delay of flower opening stage (116.33 days), number of sprays (5.00) and vase life (27.22 days) was obtained with benzyl-adenine @ 200 ppm treatment. Flower diameter was 4.53 cm with benzyl-adenine @ 200 ppm compared to 2.87 cm in control.

Key words: Anti-senescence compound, Chrysanthemum, Delayed flowering and Vase life

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) belonging to family Asteraceae is one of the most beautiful leading commercial flowering plants grown for its diverse uses including cut flower, loose flower for making garlands, floral ornaments, hair decoration as well as for pot culture and also in garden decoration as bedding plant (Joshi *et al.*, 2010). Chrysanthemum is gaining tremendous popularity among floriculture products in the recent times due to wide range of flower colours, diverse forms and their excellent keeping quality. The word chrysanthemum is derived from two Greek words '*chrysos'* referring to gold and '*anthemon'* or '*anthos*' referring to flower (Anderson, 1987). It is believed to be native to the Northern hemisphere chiefly Europe and Asia and it is believed to have originated in China (Bose *et al.*, 2002).

Chrysanthemum ranks 3rd in the international flower market next to roses and carnation in terms of both production volume and trading and it also ranks 5th amongst the potted flowering plants in the world flower market (Anonymous, 2017a). In India, the chrysanthemum is commercially cultivated in the states of Karnataka, West Bengal, Tamil Nadu, Madhya Pradesh and Himachal Pradesh on an area of 20.55 thousand ha with about 184.31 thousand MT production of loose flower and 14.64 thousand MT of cut flower (Anonymous, 2017b).

The use of plant growth regulators is becoming a very common practice in agricultural crops. Plant growth regulators act as either inhibiting or promoting agents to govern the plant growth depending upon the concentration of the dose and internal plant characteristics on which these are applied. The application of benzyl adenine and gibberellic acid has been reported to accelerate blooming and increase in number of flowers in lilium (Kioshi, 2003). Application of benzyl adenine resulted in increased length of blooming stem, flower diameter, number of flowers and accelerated blooming in narcissus flowers (Nakhaee *et al.*, 2009). Parameters like weight of a leaf, number of leaves, leaf surface and diameter of stem have also been improved with benzyl adenine in croton plant (Ibrahim *et al.*, 2010). Increased post-

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harvest keeping quality of anthurium flowers and miniature roses and increase in rate of water absorption have also been reported (Serek and Anderson 1993, Paull and Chantrachit, 2001). Exogenous application of putrescine in many plant species has also been reported that resulted in reduction in loss of chlorophyll and thus delay aging of leaves (Lee *et al.*, 1997). This effect may be related to the inhibition of peroxidase activity (Ma *et al.*,1996). The main objective of this study was to standardize optimum concentrations of putrescine and benzyl adenine (BA) in chrysanthemum for improvement of growth, flowering and post-harvest keeping quality parameters.

MATERIAL AND METHODS

The study was conducted at the Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana, during the year 2016-17 to study the effect of putrescine and benzyl adenine (BA) on vegetative, floral and post-harvest keeping quality parameters of chrysanthemum cv. 'Punjab Shyamli'. It is a pompon-type variety having purple flowers with deep purple, suitable for cut flower production. Healthy terminal stem cuttings (5-7 cm) free from any disease or insect pest symptoms were prepared in the month of July and planted in plug trays filled with burnt rice husk as rooting medium. New roots developed 15-20 days after planting and rooted cuttings were transplanted in the field in the first week of August. Cultural operations like weeding, irrigation and management of insect pest and diseases were performed as per recommended package of practices of Punjab Agricultural University, Ludhiana. Staking was done by using sticks to keep the plants erect and maintain the proper shape of plant and bloom. The experiment consisted of seven treatments *viz.* putrescine (@ 50, 100 and 150 ppm) and benzyl adenine (@ 100, 150 and 200 ppm) along with control. Foliar spray of growth regulator concentrations was done twice, first at bud initiation stage and the other when flower buds developed completely along with control plants that were sprayed with normal water. The observations on various growth and flowering parameters like plant height stem girth, internodal length, leaf chlorophyll content (SPAD), plant spread, days to bud appearance, days to colour showing stage, days to flower opening, flower diameter, duration of flowering, peduncle length, number of branches per plant, length of cut stem, vase life, final flower diameter in vase, days to initiation of flower senescence, days to complete senescence, days to initiation of leaf yellowing and total water absorbed by cut stem. The experiment was conducted in randomized block design (RBD) with seven treatments with three replications per treatment. Data was subjected to statistical analysis by using CPCS-1, software developed by Department of Mathematics and Statistics, Punjab Agricultural University (Ludhiana). The treatment comparisons were made at 5% level of significance.

RESULTS AND DISCUSSION

Foliar sprays with different concentrations of putrescine and benzyl adenine on chrysanthemum cv. Punjab Shyamli did not show any significant effect on various vegetative parameters (Table 1).

Table 1. Effect of different growth regulators on vegetative growth parameters of chrysanthemum cv. Punjab Shyamli

Treatments	Plant height (cm)	Stem girth (cm)	Internodal length (cm)	SPAD	Plant spread (cm)
Control	53.73	0.61	4.60	66.07	20.94
Putrescine @ 50 ppm	54.00	0.57	2.99	72.88	24.61
Putrescine @ 100 ppm	52.27	0.61	3.69	69.28	20.99
Putrescine @ 150 ppm	55.80	0.67	3.55	72.37	21.49
Benzyl adenine @ 100 ppm	59.40	0.63	4.21	67.01	22.66
Benzyl adenine @ 150ppm	54.60	0.57	4.43	72.98	24.61
Benzyl adenine @ 200 ppm	58.60	0.65	3.95	77.90	25.27
CD (P=0.05) SEm±	NS 0.99	NS 0.01	NS 0.21	NS 1.54	NS 0.70

The data on number of days taken to bud appearance, days to flower opening, flowering duration, number of sprays per plant , peduncle length, flower diameter and length of cut stem is presented in Table 2. Results revealed that spray of BA @ 150 ppm and 200 ppm significantly delayed the flower bud appearance as compared to control and other treatments. The minimum numbers of days (87.33 days) were taken by control plants for flower bud appearance. Hence, increase in concentration of putrescine and BA delayed the bud formation in chrysanthemum cv. 'Punjab Shyamli'. It has been reported that nutritional and climatic conditions during the growing period also play a major role to determine the flowering characteristics of plants (Boodley, 1975). Increase in number of days taken to bud appearance in Lilium cv. 'Tiger' by foliar spray of BA @ 50 ppm has also been reported earlier (Attiya *et al*., 2015).

Table 2. Effect of different growth regulators on flowering parameters of chrysanthemum cv. Punjab Shyamli

Treatments	Days to bud appearance	Days to colour showing stage	Days to flowe-ring	Flowe-ring duration (days)	Number of branches/ plant	Peduncle length (cm)	Flower diameter (cm)	Length of cut stem (cm)
Control	87.33	94.33	101.67	29.13	3.73	7.54	2.87	46.40
Putrescine @ 50 ppm	88.00	95.00	103.33	28.00	3.87	7.82	3.11	48.53
Putrescine @ 100 ppm	91.33	96.33	104.67	27.40	4.20	7.57	3.92	49.61
Putrescine @ 150 ppm	92.00	98.33	107.33	27.93	4.53	7.97	3.97	51.48
Benzyl adenine @ 100 ppm	91.33	99.67	109.67	28.93	4.13	8.95	4.10	53.12
Benzyl adenine @ 150 ppm	93.33	100.67	112.67	26.80	4.53	8.49	4.23	54.99
Benzyl adenine @ 200	97.67	105.67	116.33	24.53	5.00	8.24	4.53	56.99
CD (P=0.05)	4.24	1.89	1.87	1.21	0.34	NS	0.13	1.41
SEm±	1.94	0.86	0.87	0.55	0.16	0.31	0.06	0.65

The maximum number of days to colour showing stage (105.67 days) was observed in treatment with BA @ 200 ppm, followed by BA @ 150 ppm with 100.67 days for colour showing stage. The least number of days to colour showing stage (94.33 days) was observed control. Days taken to bud appearance and colour showing stage also determine the earliness or late flowering of any cultivar thus, both habits are helpful in regulating the availability of flowers for longer period in the flower market reported by Behera *et al*. (2002). The maximum delayed flowering for 116.33 days was recorded in treatment where plants were sprayed with BA @ 200 ppm, whereas the earliest flowering was recorded in control (101.67 days). Similar to our study, delayed flowering has been reported by the application of BA in salvia and lilium crops earlier (Carey *et al*., 2013).

Among different concentrations of putrescine and BA, the longest duration of flowering (29.13 days) was obtained in control plants followed by plants treated with BA @ 100 ppm with 28.93 days flowering duration. The minimum duration of flowering (24.53 days) was obtained in treatment comprising of BA @ 200 ppm. Blooming period of flower is an essential criteria for selection of flowering cultivars. Flowering duration is helpful to determine the availability of flowers for a longer time period. Early senescence of tulip flowers, when treated with higher concentrations of BA has also been reported by Kim and Miller (2008). It may be due to increased localization of the cytokinins within the gynoecium which results in early senescence of flower petals. Similar to our findings, results have also been reported in carnation by various workers (Woodson and Brandt, 1991). The maximum number of sprays per plant (5.00) was obtained with BA @ 200 ppm as compared to control (3.73). Besides this, putrescine @ 150 ppm and BA @ 150 ppm treatments were at par with each other having 4.53 sprays per plant in both the treatments. Similar to our research finding, effect of BA on increase in growth characters has also been reported by Asgari *et al*. (2014). The data showed that plant growth regulators did not affect

peduncle length of plants significantly. The maximum flower diameter (4.53 cm) was recorded with treatment BA @ 200 ppm followed by BA@150 ppm with 4.23 cm flower diameter and BA@100 ppm (4.10 cm). The lowest flower diameter (2.87 cm) was reported in control where plants were sprayed with water only. Similar to our findings, flower diameter was increased with BA @ 500 ppm as reported by Asgari *et al.* (2014) in narcissus and Al-Hasnawi (2011) in chrysanthemum. The longest stem length (56.99 cm) was obtained with BA @ 200 ppm BA as compared to control where stem length of 46.40 cm was obtained. There was significant increase in the cut stem length at all levels of putrescine and BA over control. Similarly, benzyl adenine (BA) increased stem length in salvia and tuberose as reported earlier (Kheiry, 2006; Carey *et al,* 2013).

The data on the effect of plant growth regulators on vase life of cut flower in distilled water at room temperature after harvest, water absorbed by cut stem, days to initiation of flower senescence, days to complete senescence and final flower diameter are presented in Table 3. It is clear from the results that different concentrations of putrescine and BA significantly improved the freshness of flower over control. BA @200 ppm significantly improved vase life of cut stems up to 27.22 days, followed by BA @150 ppm (26.45 days) and BA @ 100 ppm (24.44 days) . The minimum vase life (18.11 days) was recorded in control. The reason might be due to the increased protein content in petals or might be due to the ability of plant growth regulators to reduce and delay the production of endogenous ethylene hormone (Lukaszewska, 1994). Similarly, increased vase life with the application of BA @ 150 and 300 ppm has also been reported earlier in gerbera (Chavan *et al.,* 2012). The maximum water absorption (29.44 ml) was observed in cut flowers taken from the plots where plants were sprayed with BA @ 200 ppm followed by BA @ 150 ppm (25.78 ml). The least water absorption (11.89 ml) was observed in cut stems taken from control. In general, water absorption is closely related with persistency of flowers and any factor that improves water absorption rate would be effective. Similarly, results have also been recorded by Nagarja *et al.,* (1999) in tuberose. The cut stems showed sign of senescence in control after 15.89 days where no growth regulator was sprayed on plants at the time of growth phase. Cut flowers harvested from the plants treated with BA @ 200 ppm showed delay in flower senescence up to 29.78 days, followed by BA @ 150 ppm (28.56 days) and BA @100 ppm (26.89 days).The reason might be that cytokinins are responsible for reduction in ethylene sensitivity of plants that delayed flower senescence (Serek *et al.,* 1994). Leaf yellowing is an important factor triggering leaf senescence in plants, the oldest leaves at the bottom of a canopy enter senescence earlier than the upper leaves. In this study, no sign of yellowing of leaves in cut stems of chrysanthemum cv. Punjab

Table 3. Effect of different growth regulators on post-harvest keeping quality parameters of chrysanthemum cv. Punjab Shyamli

Treatments	Vase life (days)	Water absorbed (ml)	Days to initiation of flower senescence	Days to complete flower senescence	Final flower diameter (cm)
Control	18.11	11.89	15.89	21.00	3.63
Putrescine @ 50 ppm	20.56	14.11	17.89	22.33	4.00
Putrescine @ 100 ppm	21.89	16.11	19.00	23.89	4.35
Putrescine @ 150 ppm	22.11	19.55	19.33	24.67	4.72
Benzyl adenine @ 100 ppm	24.44	19.67	21.89	26.89	4.85
Benzyl adenine @ 150 ppm	26.45	25.78	23.55	28.56	5.25
Benzyl adenine @ 200 ppm	27.22	29.44	24.78	29.78	5.75
CD (P=0. 05)	1.51	2.52	1.59	1.01	0.35
SEm±	0.69	1.16	0.73	0.46	0.16

Shyamli was seen till the termination of their vase life. Hence, it can be inferred that putrescine and benzyl adenine (BA) delayed the leaf yellowing of cut stems as reported by (Singh and Bala, 2018) in addition to the important roles of the cytokinins and its derivatives in controlling and stimulating cell division, inhibition of leaf senescence.

The research findings of this study depicted that foliar spray of benzyl adenine (BA) @ 200 ppm (BA) twice i.e., at bud initiation stage and when flower buds were fully developed was found to be the effective treatment in improving the floral parameters and enhancing the vase life of the cut stems of chrysanthemum cv. Punjab Shyamli.

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Original Research Paper

Studies on bioavailability of iron from iron fortified commercial edible mushroom *Hypsizygus ulmarius* and standardization of delivery system for human nutrition

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ABSTRACT

Iron is one of the most important micronutrients for human health. Iron deficient diet and defective iron absorption are among the major reasons for iron malnutrition. Mushrooms are edible fungi, which are a very good source of iron and can be easily grown on agricultural residues at home scale, dehydrated and stored in the powder form, which can supplement the daily diet. Although mushrooms in general and oyster mushrooms in particular are a rich source of iron, yet, have not become a recommended diet by the nutritionists due to lack of data pertaining to its bioavailability from mushrooms. Data has been generated in the present study on the *in vitro* bioavailability of iron from non-fortified and iron fortified *Hypsizygus ulmarius,* which is a commercially grown species. This is the first report pertaining to the bioavailability of iron from iron fortified mushrooms. A delivery system for human nutrition was also standardized in the form of Arka Mushroom Fortified Rasam Powder using iron fortified and non-fortified mushrooms, which can be used to mitigate iron malnutrition. The data generated in this study will help in providing application techniques to use mushrooms as dietary source of iron in everyday diet to mitigate iron deficiency.

Key words: Arka Mushroom Rasam, Bioavailability, *Hypsizygus ulmarius* and Iron fortified

INTRODUCTION

Iron is one of the most important micronutrients for human health. It is required in the process of oxygen transport and storage (as a constituent of hemoglobin and myoglobin), for electron transfer (in cytochromes), desaturation of fatty acids, tyrosine iodination (in thyroid peroxidase), prostaglandin synthesis, formation of erythrocytes, maintenance of heat balance, as well as humoral and cellular resistance (Burke *et al.,* 2014; Nadadur and Mudipalli, 2008). Iron deficiency can also result in decreased cognitive health. Nutrition Monitoring Bureau Survey (NNMBS, 2006) showed that anemia prevalence in adolescent girls in India was 69.7% which caused 1.8% loss of GDP. Indian government has been working towards reducing the load of anaemia in girls and women by 50%. Numerous interventions like biofortification and fortification of diet are being undertaken to mitigate this problem. A very important intervention can be adoption and popularization of alternate foods rich in iron, which have not yet become a part of daily diet. Although mushrooms in general and oyster mushrooms in particular are a rich source of many minerals including iron, yet, have not become a recommended diet by the nutritionists due to lack of data pertaining to bioavailability. Any iron fortification method is incomplete until a proper delivery system is also standardized so that the benefit can reach the target population. The innovation for the process of production of iron fortified mushroom was taken to a logical and meaningful conclusion through the standardization of recipe for iron rich Arka Mushroom Rasam Powder which is available as licensed technology from ICAR-IIHR. The data

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generated in this study will fill this gap on bioavailability of iron from mushrooms and provide application techniques to use mushrooms as dietary source of iron in everyday diet.

MATERIALS AND METHODS

Hypsizygus ulmarius (Elm oyster) was cultivated on sterilized paddy straw-based substrate. Paddy straw was cut into small pieces (about 2.5-3.0 cm) using a motorized chaff cutter and soaked in clean water for 4 h. The soaked straw was drained for removal of excess water and spread on meshed tray under sun to reduce the moisture content to 65-70%. One kg wet straw was filled in polypropylene (PP) bags measuring 304 x 406 mm, plugged with non-absorbent cotton and sterilized in autoclave at 121°C, 15lb pressure for 25 min. The moisture content of the wet straw was determined by gravimetric method by drying 100 g of wet straw to a constant weight in oven at 70°C. The sterilized bags were cooled to room temperature and spawned (seeded) using grain spawn of *H. ulmarius* @ 5% (50g spawn per bag) in an aseptic bag inoculation chamber. Spawned bags were incubated at 26±2°C for spawn running (vegetative growth). After 25 days of completion of spawn running, the bags were shifted to cropping room where a temperature of 26±2°C and humidity of 80-85% was maintained throughout the cropping period of 30 days. Four to five holes were made in each bag to induce mushroom initiation. Iron fortified mushroom was developed through an innovative technique using iron salt (Patent application No -201841022601). Mature mushrooms of both the non-fortified *H. ulmarius* (NFHU) and iron fourtified *H. ulmarius* (IFHU) were harvested, hand shredded and dehydrated in a tray dryer covered with PP sheet at 48±2°C for 12-14 h. Dry mushrooms were powdered in wooden pestle mortar. Necessary precautions like using gloves while handling mushrooms, using plastic knife to cut mushrooms, were taken in handling of iron enriched mushrooms to avoid external contamination. NFHU powder and IFHU powder prepared was used for the *in vivo* iron bioavailability studies.

In vivo studies

The experiment was conducted in the Laboratory Animal House at Experimental Livestock Unit, National Institute of Animal Nutrition and Physiology (NIANP), Bangalore, India. The animal experiment protocol was as approved by the Institutional Animal Ethics Committee (IAEC) and carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments in Animals (CPCSEA), Ministry of Environment, Forests and Climate Change, Government of India.

Experimental design

Forty-eight numbers of healthy weaned (40 days old) Wistar strain (*Rattus norvegicus*) albino rats were randomly divided into four dietary groups, with four replicates of three rats in each group. All the rats were housed in polypropylene cages and maintained under similar managemental conditions. Temperature and humidity were maintained at 23±2ºC and 50 to 70% respectively in proper ventilated animal house. All rats were fed pelleted feed and offered purified drinking water *ad libitum*.

Preparation of experimental diets

Commercial pelleted rat feed was served as the control diet (A). The control diet pellets were finely powdered and mixed well separately with dried NFHU and FHU mushroom powder in 75:25 proportion to prepare experimental diet B and C respectively. The experimental diet D comprised of control feed supplemented with ferrous sulfate (Analytical grade, SD fine chemicals, Mumbai, India) equivalent to the iron content of fortified mushroom. Diets B, C and D were again pelleted in a manual pelletizer, air dried for 24 hours, later oven dried at 60ºC for 24 h and stored in respective air tight containers until fed to the experimental rats. Necessary precautions were taken at each step of diet preparation, pelletizing and storage to avoid contamination.

Sample collection

All the rats of different dietary groups were offered daily weighed amount of respective experimental diets in the morning at 9:30 h. They were provided with fresh and clean deionized water *ad libitum.* The feeding experiment was continued for 30 days and initial as well as final body weight of each rat was recorded. At the end of experiment, a digestibility trial of 5 days duration was conducted to record dry matter (DM) intake, DM digestibility and apparent Fe absorption in gut.

Analytical technique for iron bioavailability studies

The samples of diets offered, residues and faeces were analyzed for DM as per standard procedures of Association of Official Analytical Chemists

(AOAC 2000). The feeds and faeces were taken in pre-weighed silica crucibles and oven dried at 80°C for 24h, decarbonized and ashed at 550-600°C in a muffle furnace. The total ash was digested with 5N HCL over a hot plate for 15 min, cooled and filtered through Whatman filter paper (No. 41) into volumetric flasks of desired volume. Iron content in mushroom, diets and faeces was estimated using Optima 8000 inductively coupled plasma optical emission spectrophotometer (ICP-OES) (Perkin Elmer, Shelton CT#064840, USA).

Statistical analysis

The data were analyzed using the statistical Package for Social Sciences (SPSS, version 20.0 Chicago, USA) by one-way ANOVA and comparison of means was tested using Duncan's multiple range tests (Duncan 1995).

Standardization of delivery system for human nutrition

Standardization for a socially acceptable value-added product with iron fortified mushroom was undertaken. A local daily food accompaniment called 'Rasam or Soup' which is used in every Indian home of all strata was standardized using powder made by dehydration of both iron fortified and non-fortified mushrooms. The mushroom fortified rasam powder was made with traditional spices like coriander, jeera, pepper, fenugreek and tomato powder, chili powder, tur (*Cajanus cajan*) and urad dal (*Vigna mungo*), crushed garlic, salt and fresh coriander. This traditional mix was fortified with mushroom powder made from dehydrated non fortified and iron fortified Elm oyster mushroom. Ready to serve rasam or soup was made by boiling 10g of the rasam or soup powder in 350ml of water for 15 minutes and subjected to acceptance test by 501 people of rural and urban backgrounds. The rasam powder was also analyzed for its nutritive values. Taking a cue from the available literature on low availability of iron in human from legumes (Lynch et al 1984), the Arka rasam powder was also prepared with and without two legume ingredients (*C. cajan* and *V. mungo*). The five kinds of products *viz*. Rasam without legumes + iron fortified mushroom, Rasam with legumes + iron fortified mushroom, Rasam without legumes+ non fortified mushroom, Rasam with legumes+ non fortified mushroom and Rasam without mushroom (traditional recipe) were analyzed for iron content as per the standard AOAC method.

Proximate nutritive analysis of Arka Rasam

The total soluble carbohydrates of the dry powders of rasam, rasam powder with mushroom and rasam powder containing iron rich mushroom was determined by the phenol-sulfuric acid method as described by Dubois *et al.* (1956). Protein content was calculated by multiplying the total nitrogen value by the factor 6.25. Fat percentage was determined in the samples by the Soxhlet procedure using petroleum ether (60-80 ^{0}C) (AOAC, 1984). Dietary fiber percentage was estimated in dry, defatted samples by the Official AOAC Method No. 982.29 (Prosky *et al*., 1992). All the results were expressed as mg/100g dry weight or fresh weight basis. The extraction procedure of water-soluble vitamins from mushroom was followed as described earlier by Esteve *et al*. (2001). A composite sample of 2 g of freshly cultivated mushrooms was homogenized using a domestic blender with 5 ml of MilliQ water to which 10 mL of 0.1 N HCl was added and incubated in a water bath at 95–100°C for 30 min. The extract was then allowed to cool at room temperature and the pH was adjusted to 4.0–4.5 using 5 M sodium acetate solution. One ml of takadiastase enzyme solution, contain 200 mg/ml was added to this extract and incubated in a water bath at 45–50°C for 8 hrs. After the enzymatic hydrolysis, 1 mL of 50% trichloroacetic acid was added to the extract and incubated at 95–100°C in a water bath for 5 min. After cooling it was centrifuge at 10000 rpm for 10 min, supernatant was collected and made up the volume to 25ml with 0.1N HCl. To the 5 mL of the stock solution 300 µL of potassium ferricyanide (1% in 15% NaOH) was added and left to react for 10 min in the dark. The extract was then neutralized with 200µL of 15% ortho-phosphoric acid, filtered through a nylon membrane syringe disc filter with a pore size of 0.45 µm followed by 0.2 µm and 1µl was injected immediately into the UPLC-MS/MS.

Mineral analysis of Arka Rasam

Mushroom samples were dried in the oven at 70ºC to constant weight and analyzed for mineral nutrients as per the methods described by Piper (1966). Nitrogen content was determined by Kjeldahl method (Kjeltek Auto-Analyzer, Gerhardt, Germany),

phosphorus was estimated calorimetrically by vanado molybdophosphate method and potassium content by flame photometer. Calcium, magnesium, iron and zinc were determined using atomic absorption spectrophotometer (AAS) by Wet digest method with HNO_3 and $HClO_4$ in 10:4 ratio.

Water soluble vitamins analysis

Reagents

The water-soluble vitamin standards biotin (B7), niacin (B3), pyridoxine (B6), pantothenic acid (B5), folic acid (B9), cyanocobalamin (B12), thiamine (B1) and riboflavin (B2) were from Sigma Chemical Co., USA. The standard vitamin solutions were prepared in 0.01 N HCl. The enzyme takadiastase was purchased from Fluka, Switzerland. The organic solvents used for mobile phase in liquid chromatography were of chromatographic/MS grade and all other reagents were of analytical grade. Milli-Q water (Millipore system) was used to prepare the mobile phases. All mobile phases were filtered through 0.45 µm pore size membranes before use.

Equipment

Freshly harvested mushroom samples were used for the validation of the methodology. An Acquity UPLC-H class coupled with TQD-MS/MS from Waters, USA with ESI source was used for vitamin determinations; the system was equipped with a degasser, quaternary pump, automatic injection system (0–10 µL), with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths and MRM for individual masses. The overall system was controlled by the Mass lynx software, which also administered the data collection and treatment system.

MS-MS Methodology

The methodology for estimation of vitamins by UPLC-MS/MS system was validated for linearity, quantification and detection limits, and repeatability; recoveries of standards were 85-95%. The most sensitive, Multiple Reaction Monitoring (MRM) mode was employed for detection. The optimal operational parameters of the MS/MS system, to select the most abundant mass-to-charge ratio (m/z), was determined for each vitamin by direct sample infusion, using positive ionization mode (ES^+): the precursors ion and the product ions, the MS/MS capillary voltage, extractor voltage and RF lens values were set at 3.2 kV, 4 V and 0.1 V respectively, the gas flow for de-solvation and cone was set at 550 and 50 L/h. The cone voltage and the collision energy for individual vitamins were optimized (Table 1).

Table 1. MRM of water-soluble vitamins standards

Water soluble vitamins	Formula/Mass	Parent m/z $[M+H]^+$	Cone Voltage	Daughters	Collision energy (CE)	Ion Mode
Thiamine (B1)	264	265.03	20	122.06(Q)	16	ES+
		265.03	20	144.02	14	ES+
Riboflavin (B2)	376	376.97	40	243.05	24	ES+
Niacin (B3)	123	123.9	34	80.523(Q)	20	ES+
		123.9	34	77.47	18	ES+
Pantothenic acid (B5)	219.03	220.01	28	202.21	12	ES+
		220.01	28	124.16	20	ES+
Pyridoxine (B6)	169	169.97	24	152.09(Q)	12	ES+
		169.97	24	134.04	20	ES+
Biotin (B7)	244	245.03	26	227.14	14	ES+
Folic acid (B9)	441	442.1	24	295.16	16	ES+
Cyanocobalamin (B12)	677	678.29	38	147.18	68	ES+

* “Q” taken as quantified ion.

LC and MS/MS conditions

The mobile phase consisted of the aqueous phase 0.1% formic acid in water (A) and organic phase acetonitrile (B). The initial gradient of 60% A and 40% B was held for 0.5 min; at 6.0 min the gradient was changed to 5% A and 95% B, held for 0.5 min, and a linear gradient then followed, and later 30% A and 70% B in 8.0 min, held for 0.5 min. The system was then returned to initial conditions at 14 min and held for 1 min for equilibrating before the next injection. The flow rate was 0.3 ml/min. The analytical column 2.1x50 mm UPLC BEH-C18 column (Waters) with 1.7 µm particle size, protected by a Vanguard BEH-C18 with 1.7 µm. guard column (Waters). The column temperature was maintained at 25 °C and sample injection volume was 1.0 µl. The vitamins eluted were monitored using a PDA detector and the UPLC column effluent was pumped directly without any split into the TQD-MS/MS (Waters, USA) system, optimized for the vitamins analysis.

Extraction of Water-Soluble Vitamin

The extraction procedure of water-soluble vitamins from mushroom was followed as described earlier by Esteve *et al*. (2001). Two gram of composite sample of freshly harvested mushrooms was homogenized in a domestic blender with 5 ml of MilliQ water, added 10 mL of 0.1 N HCl and incubated in a water bath at 95–100 °C for 30 min. The extract was cooled to room temperature and pH was adjusted to 4.0–4.5 with 5 M sodium acetate solution. 1 ml of takadiastase enzyme solution (200 µg/ml) was added to this extract and incubated in a water bath at 45–50 °C for 8 hrs. After the enzymatic hydrolysis, 1 mL of 50% trichloroacetic acid was added to the extract and incubated at 95–100 °C in a water bath for 5 min. After cooling, the extract was centrifuged at 10000 rpm for 10 min; supernatant was made up to 25 ml with 0.1 N HCl. To 5 mL of this stock solution 300 µL of potassium ferricyanide (1% in 15% NaOH) was added and reacted in the dark for 10 min. The extract was then neutralized with 200 µL of 15% *ortho*-phosphoric acid, filtered through a nylon membrane syringe disc filter (0.45 µm pore size), followed by 0.2 µm filter, 1 µl was injected immediately into the UPLC-MS/MS.

Statistical analysis

The statistical analysis was done by t-test using the Excel (2019) software. The significant differences ($p<0.01$) were evaluated for the mineral and vitamin contents of each parameter and for each sample individually. The statistical analysis of iron content data of Arka Mushroom Rasam using iron fortified mushroom was analyzed using the software Agres.

RESULTS AND DISCUSSION

The data presented in Table 1 shows that the dry matter intake of the experimental animals reduced significantly with both NFHU (diet B) and IFHU mushroom diet (diet C) as compared to diet without mushroom (diets A & D) by 22.09, 21.40 and 10.46, 9.67% respectively. The reduction was more pronounced in NFHU diet (diet B) as compared to IFHU diet (diet C). Mushrooms are known to be low calorie, high fiber diets with high amounts of non-digestible polysaccharides as β glucans, which act as prebiotic. Such diets can generally reduce the dry matter intake and body weight gain as is revealed in the present study. Hence mushrooms have been recommended as a diet to fight obesity as well (Friedman, 2016). However, no significant reduction in the daily weight gain of the experimental animals was observed in the present investigation. The data in table 1 also reveals that the gut absorption of iron was significantly higher both for NFHU (Diet B) and IFHU (Diet C) as compared with diets A and D without mushrooms. The gut absorption of iron from NHFU diet was 36.30% higher as compared to non-mushroom diet A and 39.52% higher as compared to non-mushroom diet D. The iron absorption from FHU was 66.76% higher as compared to non-mushroom diet A and by 70.70% higher as compared to diet D. This observation is of immense importance as despite its abundance in nature, iron still remains one of the major factors of mineral deficiency leading to numerous health issues. An estimated 20% of maternal deaths are directly related to anemia and another 50% of maternal deaths are associated with it (Anand *et al.,* 2014). Regula *et al.* (2016) studied the effect of feeding iron deficient Wistar rats with cereal products enriched with 10 and 20% *Pleurotus ostreatus* powder. The authors reported a complete restitution of iron deficient rats in a short time. They also reported that iron bioavailability from cereal products enriched with mushroom powder was higher in comparison to standard reference Fe (II) gluconate. Such results further corroborate the

results obtained in the present investigation especially in reference to the higher bioavailability of iron from iron fortified mushrooms (IFHU) as compared to non- fortified (NFHU) mushrooms which can become an important strategy to mitigate iron deficiency.

Table 2. Performance of rats fed diets with and without iron enriched mushroom

Group	Dry matter intake (g/r/d)	Fecal dry matter outgo (g/r/d)	Dry matter digestibility (%)	Average daily gain (g/r/d)	Iron intake (mg/r/d)	Fecal iron outgo (mg/r/d)	Gut absorption of Iron (%)
A control	17.20 ±0.13 [a]	3.54±0.10	79.66±0.54 [a]	2.09 ±0.22	8.90 ±0.06 [a]	7.74 ±0.06 [a]	13.00± 0.92 [c]
B (NFHU) non fortified mushroom	13.40±0.98 [b]	3.70±0.34	72.38±1.82 [b]	1.66± 0.25	5.76 ±0.42 [c]	4.74 ±0.35 [b]	17.72 ±0.59 [b]
C (FHU) iron fortified mushroom	15.40±1.07 [ab]	4.23±0.41	72.62 ±1.48 [b]	1.53 ±0.07	6.80± 0.47 [b]	5.30 ±0.31 [b]	21.68 ±1.30 [a]
D equivalent of FeSo4	17.05±0.27 [a]	3.68±0.15	78.42 ±1.00 [a]	2.10± 0.04	9.38± 0.15 [a]	8.18 ±0.11 [a]	12.70 ±0.67 [c]
SEM+_	0.51	0.14	1.03	0.10	0.41	0.40	1.04
F value	5.709	1.12	8.510	2.813	27.646	50.033	21.661
P value	0.012	0.379	0.003	0.084	0.000	0.000	0.000

The delivery system developed in the form of Arka Mushroom fortified Rasam or Soup powder was found to be highly acceptable during the acceptance trials. As evident from Table 2, 91.21% of the respondents liked and accepted the product Arka Mushroom fortified Rasam or Soup while 68.39% liked it very much. None of the respondents disliked the product. This was an important observation as developing iron fortified value-added products acceptable to masses is very challenging due to the color and flavor changes imparted by iron and the protection of the fortified iron from iron inhibitors generally present in cereal-based foods (Hurrell 1997). Both these issues were resolved in the present delivery system through choosing of the right kind of traditional recipe. The traditional recipe of rasam or soup is originally reddish brown and the addition of iron fortified mushroom does not bring about any color changes and is well accepted. The recipe of rasam includes many traditional Indian spices with very strong aroma and flavor which masks any changes brought about in flavor due to the addition of iron fortified mushroom to the recipe.

Table 3. Acceptance score of Arka Mushroom Rasam or Soup by 501 panelists

	9-point Hedonic scale	Acceptance among panelists (Nos)	Acceptance score (%)
9	Like extremely	52	10.379
8	Like very much	291	58.08
7	Like moderately	114	22.75
6	Like slightly	32	6.38
5	Neither like nor dislike	7	1.39
4	Dislike slightly	5	0.998
3	Dislike moderately	NIL	-
2	Dislike very much	NIL	-
1	Dislike extremely	NIL	-
	Total	501	100

The traditional recipe has legumes like tur dal *(Cajanus cajan*) and urad dal (*Vigna mungo*), which contain phytates known to inhibit iron absorption. The recipe was suitably modified without the usage of the legumes to enhance iron bioavailability. The proximate and vitamin B composition of the standardized raw Arka Mushroom Rasam powder with and without mushrooms has been tabulated in table 3a & 3b. As per the data in table 3a, the protein content of the mushroom fortified rasam powder significantly increased by 3.4%, carbohydrate by 2.14% and fiber by 1.43%. The fat content of the sample with mushroom significantly decreased by 3.53%. There was appreciable and significant increase in vitamin B content in the rasam powder with mushroom vis-à-vis without mushroom. (Table 3b).

Table 3a. Comparative proximate composition of raw Rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' Stat value
Protein	20.85 g	17.45 g	9.89**
Carbohydrate	27.64 g	25.5 g	27.74**
Fat	94.93 g	13.02 g	86.90**
Fiber	10.63 g	92.08 g	5.77**

** Significant at 1%

Table 3b. Comparative vitamin B composition of raw Arka Mushroom rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' Stat value
Biotin	2.576	0.857	11.15**
Cyanocobalamin	0.003	0	59.00**
Folic acid	0.059	0.003	8476**
Niacin	161.00	39.82	33.61**
Pantothenic acid	270.433	15.09	468.56**
Pyridoxin	8.104	1.829	77.35**
Riboflavin	9.057	1.62	83.90**
Thiamine	2.576	0.396	113.43**

** Significant at 1%

Table 4a & 4b depicts the data obtained of the proximate and vitamin B content of the cooked and ready to use rasam. There was a significant increase in the protein content of cooked and ready to use rasam with mushroom by 7.34%, carbohydrate by 16.19% and fiber by 3.15%. The fat content in the product with mushroom significantly decreased by 7.3%. There was significant increase in the biotin, niacin, pantothenic acid, riboflavin and thiamin content in the cooked product with mushroom as compared to without mushroom.

Table 4. Comparative proximate composition of cooked Arka Mushroom rasam or Soup

Composition obtained after boiling 10g of the powder in 350ml of water	Cooked & ready to use Arka Mushroom Rasam with Mushroom mg/100ml	Cooked & ready to use Rasam without Mushroom mg/100ml	't' test value
Protein	3.19 g	2.46 g	64.90**
Carbohydrate	8.32 g	6.70 g	105.76**
Fat	0.1 g	1.42 g	29.135**
Fiber	4.31 g	3.99 g	40.93**

** Significant at 1%

Table 4b. Comparative vitamin B composition of cooked Arka Mushroom rasam or Soup

Vitamin B	Cooked & ready to use Arka Mushroom Rasam with Mushroom µg/100ml	Cooked & ready to use Rasam without Mushroom µg/100ml	't' test value
Biotin	2.555	0.316	7.88**
Cyanocobalamin	0	0	65535 NS
Folic acid	0	0.0035	65535 NS
Niacin	134.184	34.914	151.18**
Pantothenic acid	49.905	14.50	114.89**
Pyridoxin	4.628	0.677	27.80**
Riboflavin	3.404	0.354	272.92**
Thiamine	2.594	0.268	59.94**

** Significant at 1% NS – Non significant

Table 5 shows the macro and micro element composition of the Arka rasam powder with and without mushroom. As seen from table 5a, there was a significant increase in Phosphorus, potassium and calcium levels in the rasam powder with mushroom as compared to without mushroom. The levels of other minerals remained same in both treatments. As per table 5b, the level of micronutrients Manganese significantly increased in the product with mushroom as compared to without mushroom. The level of Zinc decreased significantly by the addition of mushroom. The levels of iron and copper remained statistically unchanged in both treatments.

Table 5a. Comparative macro and micro element composition of raw Arka Mushroom rasam or Soup powder

Composition	Raw Arka Mushroom Rasam powder with Mushroom mg/100g raw powder	Raw Rasam powder without mushroom mg/100g raw powder	't' test value
Macro-elements			
Phosphorus	516.66	341	10.106**
Potassium	2170	1943.33	6.552**
Calcium	348.33	451	19.479**
Magnesium	120	131.33	4.25 NS
Micro-elements			
Iron	9.23	13.52	4.066NS
Manganese	5.43	2.01	80.781**
Copper	1.11	1.05	1.889 NS
Zinc	1.20	3.20	22.863**

** Significant at 1% NS – Non significant

Lynch *et al*. (1984) reported a low bioavailability of 0.84-1.91% of iron from legumes in human beings. The data in table 6 shows the iron content of the Arka Rasam Powder made without the use of legumes.

Table. 6. Iron analysis of rasam with iron fortified mushroom

Sl. No.	Treatment	Iron content(mg/100g dry powder)
1	Rasam without dal + with iron fortified mushroom	30.8[a]
2	Rasam with dal + iron fortified mushroom	29.2[a]
3	Rasam without dal+ non fortified mushroom	11.36[b]
4	Rasam with dal+ non fortified mushroom	12.06[b]
5	Rasam without mushroom (traditional recipe)	11.83[b]
	Coefficient of variation	8.589
	CD (0.01)	4.234
	CD (0.05)	2.977

As evident from Table 6, the iron content of the product Arka Rasam Powder made with and without legumes but with iron fortified mushroom was statistically at par and significantly higher as compared to the one made with non-fortified mushroom or the traditional rasam without mushroom. The iron content of the rasam with and without non fortified mushroom and without dal were statistically at par. This result is of significance as the bioavailability of iron is higher as depicted in the animal model studies earlier. Bioavailable iron in 10g Arka rosam powder made with iron fortified mushrooms @ 21.68% bioavailability is 0.667m and it takes cae of 28.02% requirement of bioavilable iron (2.3 mg/day) iron measurating women.

Conclusion

The high bioavailability of iron from both non fortified and iron fortified *Hypsizygus ulmarius* is of immense significance as the predominantly vegetarian population of India suffer with iron deficiency due to very poor bioavailability of 5-8% of non-heme iron from plant sources. The bioavailability of heme iron from meat sources varies from 18-25%. The present data of the bioavailability of 21.68% from iron fortified mushroom can play a significant role in mitigating iron malnutrition in the country. The delivery system standardized in the form of Arka Mushroom Rasam powder was not only significantly higher in

iron content with high bioavailability but also showed enhanced nutritional values in terms of protein, carbohydrate, fiber and B vitamins like biotin, niacin, pantothenic acid, riboflavin and thiamin. Thus, this product can play an important role in mitigating iron malnutrition as it fits into the taste, flavor and aroma of an already well accepted form of daily food accompaniment already in vogue.

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Original Research Paper

Amino acid profile of eighteen isolate of different edible macrofungal species

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ABSTRACT

Edible mushrooms from India (18 isolates belonging to 4 species) were profiled for protein, free and bound amino acids (AA). The protein content (range of 9.5-32.6%) was highest in *Pleurotus cintrinopileatus* and *P. sajor-caju*; free AA (range of 11.6-73.1 mg/g DW) was higher in *Hypsizygus tessulatus* and *Agrocybe aegerita*, bound AA (range of 57.4-171.9 mg/g DW) was also high in *H. ulmarius*, *P. djamor*, *P. florida*, *P. sajor-caju*. The essential free and bound AAs and chemical scores of isoleucine, tryptophan, phenylalanine was highest, higher in *Hericium erinaceus*, *P. cystidiosus*, *P. eryngi*, *P. sajor-caju*. The isoleucine (Ile) score in the free fraction of selected mushrooms were comparable or higher than the best five plant sources, while tryptophan (Trp) scores were almost double. Thus, these mushrooms are good sources of Ile, Trp and aromatic amino acids. The conditionally-essential and nonessential AAs were also quantified. This study reveals the diversity in protein and AA and nutritionally superior mushroom species.

Key words: Mushrooms, Nutrition, Free amino acids, Bound amino acids and Amino acid score

INTRODUCTION

Protein energy malnutrition (PEM) is an important public health concern among children in India which leads to a very high incidence of underweight, stunting and wasting. Verma and Prinja (2008) reported that intake of protein among 90% of the children was less than 80% of recommended dose in many regions of India. Edible mushrooms are increasingly being recognized for their high nutritional content and medicinal values. Mushrooms are protein dense food, also rich in minerals and fibre, and among the only vegetarian sources providing substantial vitamin D; in addition, they are low in carbohydrates and fat, and therefore low in calories. The therapeutic properties of mushrooms are attributed to its unique bioactive compounds such as heteropolysaccharides, statins *etc.* Worldwide production of cultivated mushrooms is estimated at 102.42 lakh tons and the leading countries are China, the US, the Netherlands and France (FAO, 2013). India currently produces around 0.982 lakh tons of mushrooms of which 85% are button mushroom. This scenario is however changing worldwide and in India as well due to the introduction of nutritionally and medicinally superior species which are geographically better suited like the Oyster, Shiitake, Milky and Paddy straw mushrooms. India produces about 98.4 million tons of surplus crop residues annually which are burnt causing environmental pollution (Jain *et al.*, 2014). A mere 10% utilization of this surplus agro-waste to grow mushrooms can result in the production of 4.92 million tons of fresh mushrooms per annum, leading to the production of 123 thousand tons of protein per annum, which in turn can meet the protein requirement of 56 lakh people per annum, besides creating year-round employment for 2.02 million people and organic manure production to the tune of 5.9 million tonnes per annum (Pandey and Kumaran, 2018).

Amino acids are constituent building blocks of proteins, the latter having diverse structural and functional roles in the body. Deficiency of amino acids can therefore compromise normal health. The variety in the diet today ensures access to essential amino acids in most people, and deficiency symptoms are

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rare. Of the 20 different amino acids required by the body to maintain good health, nine of these must be obtained from the diet as they cannot be synthesized by the human body, and therefore are essential amino acids. These are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. All these amino acids have specific roles and the deficiency symptoms also vary. Good dietary sources of essential amino acids are dairy, meat, eggs, nuts, seeds, whole grains *etc.,* thus plant- and animal-based foods contain essential amino acids. A healthy body can synthesize the other 11 amino acids, and therefore classified as the non-essential amino acids. Of these, arginine, cysteine, glycine, glutamine, proline serine, are conditionally essential in the human diet, where their synthesis can be limited under certain pathophysiological conditions. Amino acids occur in the free or the bound form in foods, where they form the building blocks of proteins.The objectives of this study were to profile and characterize the diversity in amino acid content in 18 indigenous isolates belonging to 14 species of edible oyster mushrooms collected across India, and identify superior species in terms of their protein and essential amino acids content which can help in the formulation of nutritionally superior and complete food combinations for vegetarians, and in addition help in reduction of consumption of meat

MATERIALS AND METHODS

Mushroom samples

The mushroom species/isolates used in this study are listed in Table 1. These mushrooms were collected, characterized, conserved and maintained at the germplasm repository of ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India. The mushrooms were cultivated on paddy straw or sawdust-basedspecies-specific substrates. The details of substrate requirement and environmental conditions maintained during growth are described in Table 2. Mushrooms after harvest were dehydrated at 48-50 °C in a commercial Tray dryer (5 kg capacity), for 14-16 h, to a moisture level of 10-11%, powdered in a laboratory blender and used for all nutrient assays.

Table 1. Mushroom species/ isolates studied for its protein and amino acid content

S. No.	ICAR-IIHR germplasm repository code	Scientific name
1	ICAR-IIHR-AA1	*Agrocybe aegerita*
2	ICAR-IIHR-BS1	*Hypsizygus tessulatus* (Brown coloured)
3	ICAR-IIHR-WS1	*H.tessulatus* (White coloured)
4	ICAR-IIHR-HU1	*H. ulmarius*
5	ICAR-IIHR-CA1	*Calocybe indica* - West Bengal isolate
6	ICAR-IIHR-CAJ	*Calocybe* spp. - Gujarat isolate
7	ICAR-IIHR-PCYST1	*Pleurotus cystidiosus*
8	ICAR-IIHR-DJ2	*P. djamor* - Karnataka, Western Ghats Shimoga pink coloured
9	ICAR-IIHR-DJ3	*P. djamor* - Madhya Pradesh (Pink coloured)
10	ICAR-IIHR-DJ4	*P. djamor* - Madhya Pradesh (White coloured)
11	ICAR-IIHR-PE1	*P. eryngi*
12	ICAR-IIHR-PE2	*P. eryngi*
13	ICAR-IIHR-PFL1	*P. florida*
14	ICAR-IIHR-PFL2	*P. floridanus* - Tamil Nadu isolate
15	ICAR-IIHR-YO1	*P. cintrinopileatus*
16	ICAR-IIHR-PSC1	*P. sajor-caju*
17	ICAR-IIHR-HE1	*Hericium erinaceus*
18	ICAR-IIHR-LE4	*Lentinula edodes*

Table 2. Cultivation conditions of different mushroom species/isolates

Cultivation conditions	All *Pleurotus* species, *Agrocybe aegerita*, *Hypsizygus ulmarius*	*Calocybe indica* (ICAR-IIHR-CA1) and *Calocybe* spp. (ICAR-IIHR-CAJ)	*Lentinula edodes*, *Pleurotus eryngi*, *Hypsizygus tessulatus*, *Hericium erinaceus*
Cultivation substrate	Shredded paddy straw having 65-67% moisture, filled @1 kg wet substrate per bag and sterilized at 121 °C, 18 lb pressure, 30 min		Hardwood sawdust supplemented with 25% rice bran, having 65-67% moisture, filled @1 kg wet substrate per bag and sterilized at 121 °C, 18 lb pressure, 120 min
Containers for growth	Polypropylene (PP) bags, (50 µm thick; 160 x 120 mm)		PP bags (50 µm thick; 120 x 100 mm)
Seed rate	5% of wet substrate		
Conditions for vegetative growth	Temperature 24-28 °C, Ambient humidity, No light	Temperature 30-38 °C, Ambient humidity, No light	Temperature 22-28°C, Ambient humidity, No light
Period of vegetative growth (days)	15-25 days (species dependent)	30-40 days	40-80 days (species dependent)
Conditions for mushroom formation	Slitting of bags, Temperature 24-28 °C (variety dependent), Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation	Casing with pasteurized soil, Temperature 30-38 °C, Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation	Cold water (10-12°C) shock treatment for *Lentinula edodes*, slitting of bags for other species, Temperature 18-20 °C, Humidity 80-85%, 12 h diffused natural or artificial light, Proper cross ventilation
Period of mushroom harvest (days)	6-20 days (species dependent) after bag slitting	20-25 days after casing	15-30 days after cold water shock treatment or slitting of bags
Total cropping period (seeding to last harvest) (days)	24-50 days (variety dependent)	65-70 days	60-110 days

Chemicals

The amino acid standards were purchased from Sigma Chemical Co. (USA), solvents used for liquid chromatography were of chromatographic/ MS grade, the reagents were of analytical grade, and Milli-Q (Millipore and system) water was used to prepare standards and mobile phases. Mobile phases were filtered through 0.45 µm pore size membranes before use.

Estimation of total free amino acids

The concentration of the total free and bound amino acids in the mushroomisolates was quantified by a modified method of Moore and Stein (1948). For the estimation of free amino acids, dried, homogenized mushroom sample (100 mg) was extracted in 5 ml of 80% ethanol. An aliquot of the extract was reacted with ninhydrin reagent, boiled for 20 min, cooled, and diluted with 1:1 v/v of n-propanol: water. After 15 min, the absorption was read at 570 nm in a spectrophotometer (PG Instruments T80+, UK). The amino acid concentration was calibrated against a phenylalanine standard (100 to 500 µg).

Estimation of total bound amino acids

Dried, homogenous mushroom sample (100 mg) was digested in 2 N KOH, followed by acid hydrolysis in 2 N HCl in boiling water bath; the excess acid was neutralized with KOH and an aliquot of this solution was subjected to amino acid estimation as described above (Moore and Stein, 1948).

Extraction of free amino acids for profiling

Dry samples of mushroom (0.5 g) were homogenized in 0.1% formic acid in 20% v/v methanol. The homogenate was diluted, filtered through 0.2 µm nylon filter membrane and 5 µl of the supernatant injected into UPLC-MS/MS (Nimbalkar *et al.*, 2012).

Extraction of bound amino acids for profiling (Acid/Alkaline Hydrolysis):

Dry, homogenous samples of mushroom (0.5 g) were hydrolysed in 2 N KOH in a Thumburgs tube at 100 °C, for 6 h, followed by acid hydrolysis in 2 N HCl at 100 °C, for 6-8 h, under vacuum (McGrath, 1972). The mixture was cooled, diluted, an aliquot of the centrifuged supernatant was dried completely, dissolved in 5 ml of 0.1% formic acid in 20% methanol. This solution was filtered through 0.2 µm nylon filter membrane and 5 µl was injected into UPLC-MS/MS system.

UPLC-MS/MS conditions

An Acquity UPLC-H class coupled with TQD-MS/MS (Waters, USA) with ESI source and diode array detector was used for identification and quantification of amino acids. The methodology and for amino acid profiling was validated and from its linearity, detection and quantification limits and repeatability. The most sensitive detection mode, Multiple Reaction Monitoring (MRM), was employed (Fig.S1, Supplementary file). The operational parameters of the MS/MS system were optimized for each amino acid by direct sample infusion to select the most abundant mass-to-charge ratio (m/z); mass spectra were obtained at positive ionization mode (ES^+), where the full scan showed the most abundant forms of protonated $[M+H]^+$ amino acids molecules, and the same confirmed as precursor ions of the corresponding amino acids for the collision induced decomposition (CID) fragmentation. Based on the precursor ions and product ions, the MS-MS parameters - capillary voltage, extractor voltage and RF lens were set at 3.2 kV, 4 V and 0.1 V respectively; the gas flow for desolvation and cone was set at 650 and 50 L/h. The MRM variables used to calibrate the UPLC-MS/MS such as cone voltage and the collision energy for individual amino acid standards were optimized as described in Table 3.

Table 3. MRM of amino acids standards

Sl. No.	Compounds	Formula/ Mass	Parent ion (m/z) $[M+H]^+$	Daughters	Cone voltage (V)	Collision energy (eV)	Ion mode
1	Asparagine	132.00	133.03	74.02	8	16	ES+
2	Aspartic Acid	133.00	134.03	74.02	8	14	ES+
3	β-3,4-Dihydroxy phenylalanine	197.20	198.10	152.06	10	15	ES+
4	Citrulline	175.01	176.04	70.04	8	22	ES+
5	Cysteine	121.03	122.00	75.99	26	15	ES+
6	Alanine	89.10	90.13	44.09	10	8	ES+
7	Arginine	174.00	175.03	70.04	14	20	ES+
8	Ethionine	163.02	164.12	56.06	10	15	ES+
9	Methionine	148.98	150.01	104.02	8	10	ES+
10	Proline	115.05	116.02	70.03	12	12	ES+
11	Threonine	119.11	120.08	74.09	8	10	ES+
12	Glutamic Acid	147.03	148.06	84.04	12	12	ES+
13	Glycine	75.01	76.17	30.08	10	12	ES+
14	Histidine	155.06	156.22	110.14	12	12	ES+
15	Isoleucine	131.18	132.21	86.15	10	10	ES+
16	Leucine	131.18	132.15	86.08	10	10	ES+
17	Lysine	146.13	147.16	84.13	8	14	ES+
18	Norleucine	131.11	132.14	86.14	8	8	ES+

19	Norvaline	117.11	118.08	72.09	8	8	ES+
20	Phenylalanine	165.00	166.10	120.13	10	12	ES+
21	Serine	105.04	106.07	60.10	8	12	ES+
22	Tryptophan	204.11	205.14	188.09	10	12	ES+
23	Tyrosine	181.11	182.14	136.19	8	14	ES+
24	Valine	117.22	118.06	72.15	8	8	ES+

The mobile phase consisted of the aqueous Solvent A, 0.1% formic acid in water, and the organic Solvent B, methanol: water (50:50) with 0.1% formic acid, with a gradient elution program as detailed in Table 4, at a flow rate of 0.1 mL/min. The analytical column was 2.1x50 mm UPLC BEH C18 column (Waters) of 1.7 µm particle size, protected by a vanguard BEH C18 with 1.7 µm. The column temperature was maintained at 25 °C. The eluted amino acid was monitored directly without any split in the TQD-MS/MS (Waters, USA) system, optimized for the amino acids analysis.

Table 4. LC-MS gradient elution program for amino acid profiling

Time (min)	Aqueous mobile phase A (%)	Organic mobile phase B (%)
0	98	5
1	95	5
10	80	20
11	80	20
15	60	40
15.5	60	40
19	95	5
20	95	5

Statistical analysis

Quantification of total free and bound amino acids and their component amino acids in 18 oyster mushroom isolates were carried out in triplicates. The data were analysed by one-way analysis of variance (ANOVA). Tests of significant differences were determined by Duncan's multiple range tests at $p<0.05$. Multivariate analysis was carried out by applying principal component analysis.

RESULTS AND DISCUSSION

In this study, it was possible to establish the significant variability among the 18 mushroom isolates in their content of total protein, free and bound amino acids (Table 5), as also in the amino acid profile of the free and bound amino acids.

Protein content:

The protein content was obtained from nitrogen using 6.25 as the conversion factor, on dry weight basis; it ranged between 9.51 to 32.61%. The genus *Calocybe* showed lowest protein values of 9.51 and 14.5% in *Calocybe indica* (IIHR-CA1) and *Calocybe* spp. (IIHR-CAJ) respectively. The protein values of this genus also showed variability with the isolate IIHR-CAJ from Gujarat showing higher protein content as compared to IIHR-CA1 from West Bengal. Among the isolates of the genus *Pleurotus, P. cintrinopileatus* (IIHR-YO1) and *P. sajor-caju* (IIHR-PSC1) contained the highest protein content of 32.61 and 31.78% respectively. Among *P. djamor* isolates, the pink isolate from Madhya Pradesh (IIHR-DJ3) showed significantly higher protein content (28.7%) compared to the pink isolate from Western Ghats - IIHR-DJ2 – (20.62%). The white isolate from Madhya Pradesh (IIHR-DJ4) had intermediate protein content (24.16%). There was significant difference in the protein content of the two isolates of *P. eryngi,* IIHR-PE1 (20.51%) and IIHR-PE2 (16.55%). The variation in protein content among

Hypsizygus species was statistically significant with 25.63% in *H.ulmarius* (IIHR-HU1) compared to 24.55% in brown isolate of *H.tessulatus* (IIHR-BS1) and 20.47% in white isolate (IIHR-WS1). Among the six genera studied, the genus *Pleurotus* had the highest protein content followed by the genus *Agrocybe* and *Lentinula*. All the mushrooms used in the present study were grown under similar lab conditions. Hence the variations occurring in the protein content across the genera, species and isolates of the same species can be attributed to both the genetic makeup of the isolate and the external growing substrates.

Free and bound amino acids:

In foods, amino acids are present in both the free and the bound forms (where it is present as building blocks of proteins). The free and bound amino acid content varied significantly among the 18 isolatesof mushroom studied, and the total bound amino acids content was almost 3.6 times higher than that of free amino acids content (Table 5). Content of total free amino acids was highest in *H. tessulatus*, (IIHR-BS1 and IIHR-WS1), these isolates were different only in their colour (i.e.) brown and white respectively,and *A.aegerita* (IIHR-AA1). It was least in *P.djamor* (IIHR-DJ2 and IIHR-DJ3), both pink coloured isolates, collected from different states of India – Western Ghats of Karnataka and Madhya Pradesh respectively. As observed in the content of free amino acids, the total bound amino acids content was also higher in *H. tessulatus* isolates IIHR-BS1 and IIHR-WS1, and *A. Aegerita* (IIHR-AA1). It was high in *H. ulmarius* (IIHR-HU1) also. Both free and bound amino acids were high also in *P. florida* (IIHR-PFL1)and *P. sajor-caju* (IIHR-PSC1). The *Calocybe* spp (IIHR-CAJ) recorded the least free and bound amino acid content. But in contrast to the content of free amino acid, *P. djamor* from Western Ghats of Karnataka IIHR-DJ2 (151.32 mg Phe eq./ g DW) and Madhya Pradesh IIHR-DJ3 (168.42 mg Phe eq./ g DW) (both pink coloured isolates)had significantly higher bound amino acids. *L.edodes* (IIHR-LE4), had low contents of free and bound amino acids.

In a study in developing mutant rice seed with enhanced protein and grain Lys, Schaeffer and Sharpe (1997) found some free amino acids of developing seed to be inversely correlated to total amino acids in proteins of the mature grain. Asp, Thr, Met and Lys were enhanced in protein, but were conspicuously lower in the free amino-acid pool, probably due to the mutant-developing grains processing Asp more rapidly than control. Conversely, Arg, Val and Glu/ Gln accumulated as free amino acids more in mutants than in control. In this study, we were unable to draw such correlations.

Table 5. Total protein, free and bound amino acid contents in 18 species/ isolates of mushrooms

Sl. No.	ICAR-IIHR germplasm repository code	Scientific name	N (%)	Protein (%)	Free amino acids[$]	Bound amino acids[$]
1	ICAR-IIHR-AA1	*Agrocybe aegerita*	4.18	26.13	67.95[ab]	168.44[a]
2	ICAR-IIHR-BS1	*Hypsizygus tessulatus* - Brown isolate	3.93	24.55	73.11[a]	169.50[a]
3	ICAR-IIHR-WS1	*H. tessulatus* - White isolate	3.28	20.48	65.91[b]	165.19[ab]
4	ICAR-IIHR-HU1	*H. ulmarius*	4.06	25.36	55.49[c]	171.85[a]
5	ICAR-IIHR-CA1	*Calocybe indica* - West Bengal isolate	1.52	9.51	19.75[fg]	63.26[h]
6	ICAR-IIHR-CAJ	*Calocybe* spp. - Gujarat isolate	2.32	14.50	22.11[fg]	57.41[h]
7	ICAR-IIHR-PCYST1	*Pleurotus cystidiosus*	2.82	17.64	23.31[f]	96.91[e]
8	ICAR-IIHR-DJ2	*P. djamor* - Karnataka, Western Ghats Shimoga pink coloured	3.30	20.63	11.56[h]	151.32[c]

9	ICAR-IIHR-DJ3	*P. djamor* - Pink isolate Madhya Pradesh	4.59	28.70	12.21^{h}	168.42^{a}
10	ICAR-IIHR-DJ4	*P. djamor* - White isolate Madhya Pradesh	3.87	24.16	37.97^{e}	87.74^{gf}
11	ICAR-IIHR-PE1	*P. eryngi*	3.28	20.51	24.13^{f}	132.48^{d}
12	ICAR-IIHR-PE2	*P. eryngi*	2.76	17.24	39.17^{e}	96.94^{e}
13	ICAR-IIHR-PFL1	*P. florida*	4.04	25.24	35.88^{e}	164.08^{ab}
14	ICAR-IIHR-PFL2	*P. floridanus* - Tamil Nadu isolate	4.77	29.80	16.70^{hg}	146.19^{c}
15	ICAR-IIHR-YO1	*P. cintrinopileatus*	5.22	32.61	22.58^{fg}	128.83^{d}
16	ICAR-IIHR-PSC1	*P. sajor-caju*	5.09	31.79	45.86^{d}	159.22^{b}
17	ICAR-IIHR-HE1	*Hericium erinaceus*	3.89	24.33	47.30^{d}	91.98^{ef}
18	ICAR-IIHR-LE4	*Lentinula edodes*	4.26	26.65	18.58^{fg}	82.38^{g}
Mean			3.73	23.32	35.53	127.90
Range			1.52-5.22	9.51-32.61	11.56-73.11	57.41-171.85
SEd			0.468	5.62	7.16	
CD (0.05)				0.934		
CV%				3.18		

$ in (mg Phe eq./ g DW)

Amino acid profile

The profile of the free and bound amino acid in the 18 mushroom isolates was analysed. The essential amino acids, histidine (His), isoleucine (Ile), Leucine (Leu), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), valine (Val) and lysine (Lys), available in the free amino acid fraction one given in supplementary data. It was higher in *A. aegerita* (IIHR-AA1) and *P. sajor-caju* (IIHR-PSC1), followed by *H. ulmarius* (IIHR-HU1 and *H. erinaceus* (IIHR-HE1); while it was least in *L. edodes* (IIHR-LE4) and *H. tessulatus* (IIHR-WS1, a white coloured isolate). From the mean values it can be seen that Ile was present in the highest amount (1194.29 mg/100 g DW), followed by Trp (267.48 mg/ 100 g DW), Phe (91.5 mg/100 g DW); Leu and Tyr were at par (24.11, 21.24 mg/100 g DW respectively). The remaining essential amino acids were present in low quantities and at par supplementary data available online.

A. aegerita (IIHR-AA1) had the highest Ile content (2429.11 mg/100 g DW) among the 18 isolates of mushroom studied, followed by *H. ulmarius* (IIHR-HU1) (1950.2 mg/100 g DW), and least in *L. edodes* (IIHR-LE4) (535.24 mg/100 g DW). Trp was more in IIHR-PSC1 (852.8 mg/100 g DW) and IIHR-HE1 (827.1 mg/100 g DW), followed by IIHR-PCYST1 (580.8 mg/100 g DW) and IIHR-PE2 (529.6 mg/100 g DW); and least in IIHR-DJ2, IIHR-DJ3, IIHR-DJ4, IIHR-PFL1, IIHR-HU1. Phe was highest in IIHR-AA1 (161.87 mg/100 g DW), followed by IIHR-HU1 (139.1 mg/100 g DW) and IIHR-PSC1 (133.87 mg/ 100 g DW), and least in IIHR-LE4. Leu was high in IIHR-BS1 (82 mg/100 g DW), and least in IIHR-CA1, (2.4 mg/100 g DW). Tyr was highest in IIHR-PE2 (72.47 mg/100g DW), and least in IIHR-LE4. The contents of His, Thr, Lys and Val were negligible (mean values of 2.31, 6.19, 6.61 and 7.75 mg/ 100 g DW respectively).

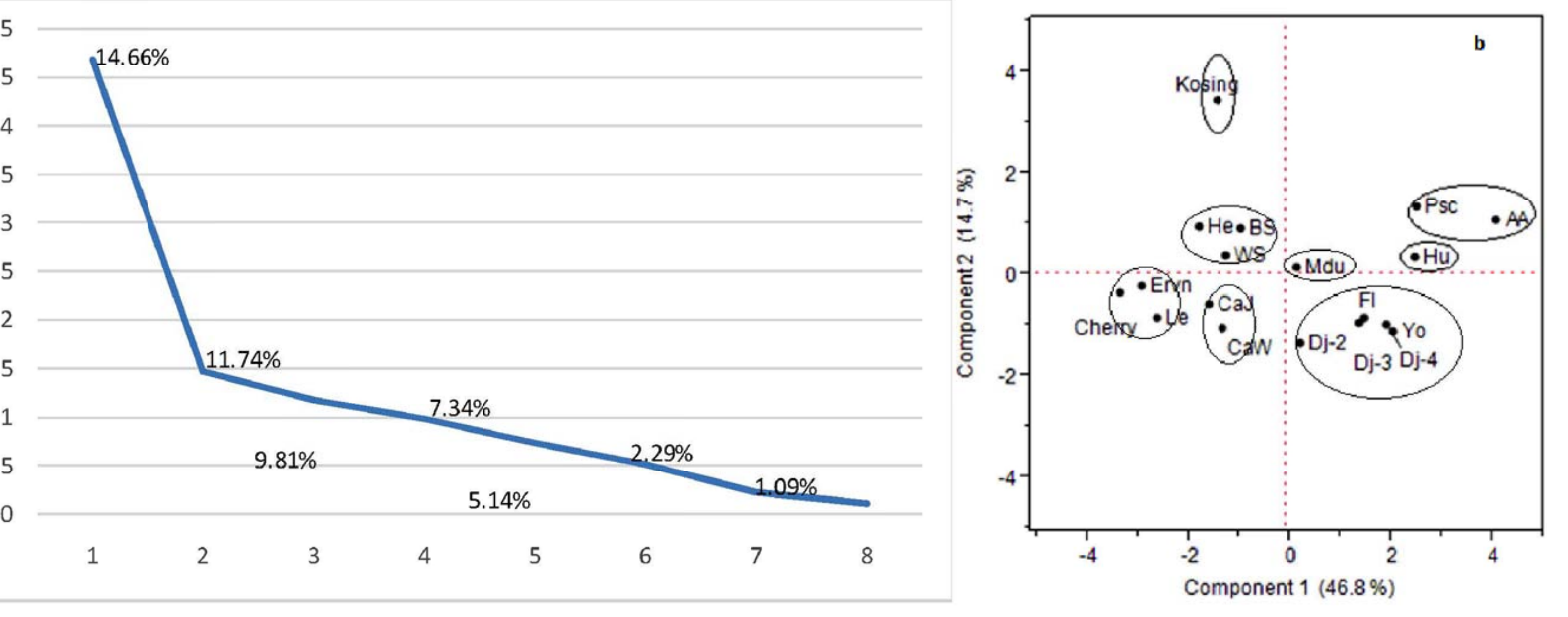
The principal component analysis (PCA) revealed that four principal components (PCs) accounted for 82.98% of the total variability in free, essential amino acid content in the 18 mushroom species/ isolates (Fig. S2, Supplementary file). The first PC had the

highest Eigen value (4.68), and accounted for 46.77% of the total variability in the whole data set, while the second PC had Eigen value of 1.47 and accounted for 14.66% of the total variability, and the third PC contributed 11.74% to the total variability. The next four PCs contributed 9.81 to 2.29% to the total variability and the remaining three PCs had <1 Eigen values, and accounted for <2.26% variability and are therefore insignificant. The components PC1 and PC2, together accounting for 61.4% of variability, were plotted to reveal eight distinct groups (Fig 1). *A.aegerita*, AA (IIHR-AA1) and *P. sajor-caju*, Psc (IIHR-PSC1) with the highest free essential amino acid contents, followed by *Hysizygus ulmarius*, Hu (IIHR-HU1) occupied distinct groups in I quadrant. The mushroom isolates with moderately high free amino acid content, *H. erinaceus*(IIHR-HE1),*H. tessulatus* (IIHR-BS1,brown coloured) and*H. tessulatus* (IIHR-WS1,white coloured) formed a distinct group, as also *P. djamor* (IIHR-DJ2 and IIHR-DJ3, bothpink coloured) and *P. djamor* (IIHR-DJ4,white coloured), *P. florida* (IIHR-PFL1) and*P. citrinopileatus* (IIHR-YO1). Mushrooms with high contents of specific amino acids, such as *P. floridanus* (IIHR-PFL2) (high Thr, Phe, Trp and Tyr contents) and *P. eryngi* (IIHR-PE2) (high Trp, Tyr and Leu contents) formed independent groups. The mushrooms with low contents of most free essential amino acids *viz*. *L. edodes* (IIHR-LE4), *P. eryngi* (IIHR-PE1) and *P. cystidiosus* (IIHR-PCYST1) (though high in Trp), and *Calocybe* spp. (IIHR-CAJ) and *Calocybe indica* (IIHR-CA1) grouped separately.

These values were computed against the essential amino acid score recommended by FAO (2013) for children, adolescents and adults, to obtain the amino acid scores of individual mushroom isolates, for each essential amino acid The free amino acid scores were higher in IIHR-HE1, IIHR-PCYST1 and IIHR-PE2, followed by IIHR-PSC1; it was least in IIHR-YO1 and IIHR-PFL1. These mushroom isolates had higher scores of Trp and Ile, with mean values of 182.7 and 174.4% respectively; followed by the aromatic amino acids (Phe+Tyr) 12.6%. Trp was highest in IIHR-HE1 (515%), IIHR-PCYST1 (498.9%), IIHR-PE2 (465.4%) and IIHR-PSC2 (404.5%), but not detectable in IIHR-DJ2, IIHR-DJ3, IIHR-DJ4 and IIHR-PFL1. Ile was high in IIHR-CA1 (312%), IIHR-AA1 (309.9%), IIHR-HU1 (256.3%) and IIHR-DJ4 (211.1%); the aromatic amino acids were also high in IIHR-CA1 (27.5%) and IIHR-AA1 (17.7%), and in IIHR-PE2 (21.7%) additionally. Both Ile and the aromatic amino acids were least in IIHR-LE4. When compared to the FAO amino acid scores, the mean values of the free essential amino acids of the mushrooms could provide over 27 times the recommended scores of Trp and almost 6 times that of Ile, 30% of aromatic amino acids, and 1-8% of the remaining free essential amino acids. Thus, the mushrooms studied are promising vegan sources of essential amino acids.

Of the conditionally essential free amino acids, Asn content was higher, and among the isolates IIHR-PSC1 (286.5 mg/100 g DW), followed by IIHR-PFL2 and IIHR-AA1 had higher contents of Asn. Of the non-essential amino acids, Ala (18.58 mg/ 100 g DW), followed by Glu were in higher content (7.19 mg/100 g DW); more in IIHR-AA1 and IIHR-BS1 respectively.

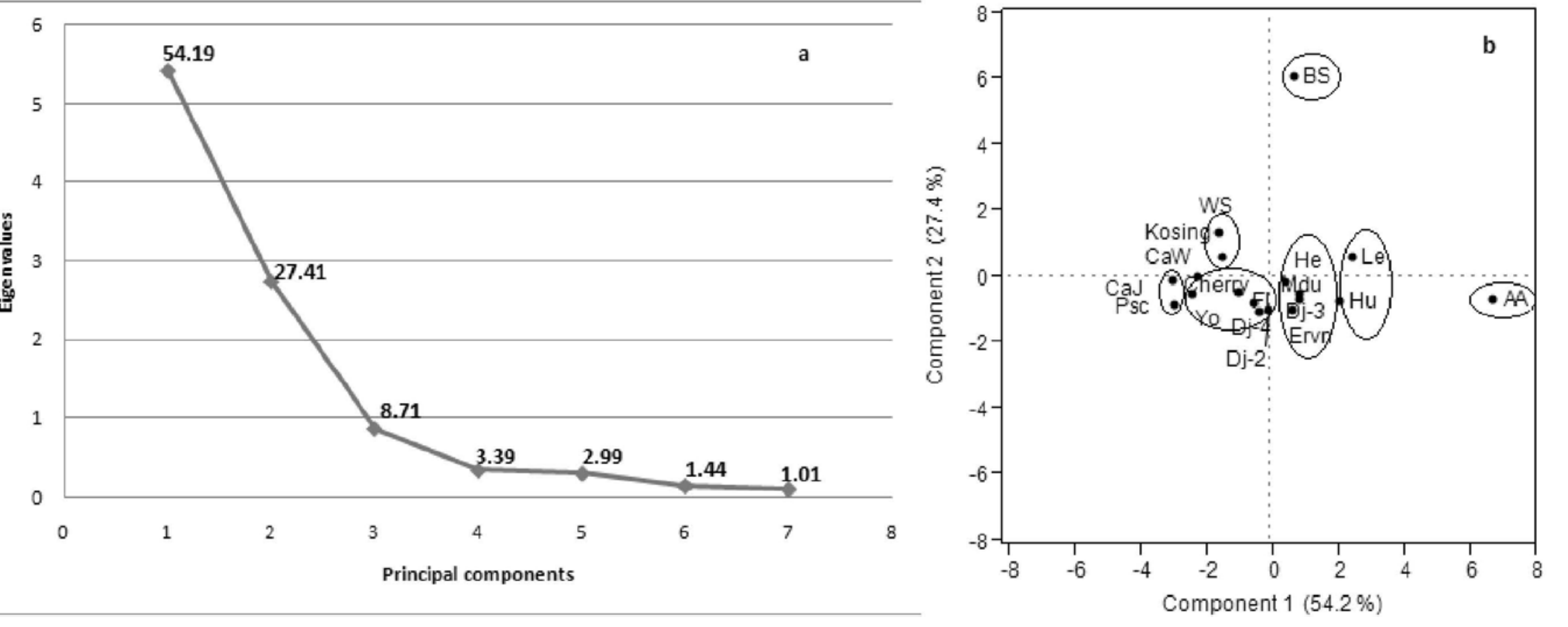
As in the free amino acids, Ile content (mean of 341.92 mg/100 g DW) was higher among the essential bound amino acids also, though the former was 3.5 times more than the latter (Table 9). The other amino acids in high content included Phe (129.8 mg/100 g DW), Trp (63.9 mg/100 g DW) and Leu (59 mg/100 g DW). These amino acids were also high in the free fraction. Ile was more in IIHR-BS1, IIHR-AA1, IIHR-WS1 (703.6, 556.2, 539.8 mg/100 g DW respectively) and less in IIHR-PSC1 and IIHR-CAJ (121.7, 169.69 mg/ 100 g DW respectively); Phe was high in IIHR-HU1, IIHR-AA1 and IIHR-LE4 (170 to 176 mg/ 100 g DW), and again low in IIHR-PSC1 and IIHR-CAJ (78.9, 84.98 mg/100 g DW respectively). Trp content was high only in IIHR-BS1 (1123 mg/ 100 g DW), and negligible in the other species. Bound Leu was high in IIHR-AA1 and IIHR-LE4 (211, 154.9 mg/100 g DW respectively), but low in IIHR-PSC1, IIHR-YO1 and IIHR-CAJ (17.52, 16.26 and 19.34 mg/100 g DW respectively). IIHR-BS1 and IIHR-AA1 had higher bound amino acid content; while IIHR-CAJ and IIHR-PSC1 had the least among the 18 mushroom isolates studied.



2a. Given value of major components for different oyster mushroom isolates

2b. Components score plot for PC1 and PC2 for free, essential amino acids in 18 Oyster mushroom / isolates

Code	Species/ isolates	Code	Species/ isolates	Code	Insert species isolates
He	*Hericium erinaceus* (IIHR-HE1)	CaJ	*Calocybe* spp. Gujarat isolate (IIHR-CAJ)	Hu	*Hypsizygus ulmarius* (IIHR-HU)
BS	Brown *Hypsizygus tessulatus* (IIHR-BS1)	CaW	*Calocybe indica* West Bengal isolate (IIHR-CA1)	Fl	*Pleurotus florida* (IIHR-PFL1)
WS	White *Hypsizygus tessulatus* (IIHR-WS1)	Kosing	*Pleurotus eryngi* (IIHR-PE2)	Dj2	*Pleurotus djamor*- Pink-Western ghats isolate (IIHR-DJ2)
Ervn	*Pleurotus eryngi* (IIHR-PE1)	Mdu	*Pleurotus floridanus* (IIHR-PFL2)	Dj3	*Pleurotus djamor*- Pink-Madhya Pradesh isolate (IIHR-DJ3)
Cherry	*Pleurotus cystidiosus* (IIHR-PCYST-1)	Psc	*Pleurotus sajor-caju* (IIHR-PSC-1)	Dj4	*Pleurotus djamor*- White-Madhya Pradesh isolate (IIHR-DJ4)
Le	*Lentinula edodes* (IIHR-LE4)	AA	*Agrocybe aegerita* (IIHR-AA1)	Yo	*Pleurotus citrinopileatus* (IIHR-YO1)



3a. Eigenvalue of major components for different mushroom / isolates

3b. Components score plot for PC1 and PC2 for bound, essential amino acids in 18 Oyster mushroom / isolates

Code	Species/ isolates	Code	Species/ isolates	Code	Species/ isolates
He	*Hericium erinaceus* (IIHR-HE1)	CaJ	*Calocybe* spp. Gujarat isolate (IIHR-CAJ)	Hu	*Hypsizygus ulmarius* (IIHR-HU)
BS	Brown *Hypsizygus tessulatus* (IIHR-BS1)	CaW	*Calocybe indica* West Bengal isolate (IIHR-CA1)	Fl	*Pleurotus florida* (IIHR-PFL1)
WS	White *Hypsizygus tessulatus* (IIHR-WS1)	Kosing	*Pleurotus eryngi* (IIHR-PE2)	Dj2	*Pleurotus djamor*- Pink-Western ghats isolate (IIHR-DJ2)
Ervn	*Pleurotus eryngi* (IIHR-PE1)	Mdu	*Pleurotus floridanus* (IIHR-PFL2)	Dj3	*Pleurotus djamor*- Pink-Madhya Pradesh isolate (IIHR-DJ3)
Cherry	*Pleurotus cystidiosus* (IIHR-PCYST-1)	Psc	*Pleurotus sajor-caju* (IIHR-PSC-1)	Dj4	*Pleurotus djamor*- White-Madhya Pradesh isolate (IIHR-DJ4)
Le	*Lentinula edodes* (IIHR-LE4)	AA	*Agrocybe aegerita* (IIHR-AA1)	Yo	*Pleurotus citrinopileatus* (IIHR-YO1)

PCA revealed three PCs accounting for 90.31% of the total variability in bound, essential amino acid content in the 18 mushroom isolates (Fig. S3, Supplementary file). The first PC had the highest Eigen value (5.42), resulting in 54.19% of the total variability, the second PC with 2.74 Eigen value accounting for 27.41% to the total variability, and the third PC contributed 8.71% to the total variability. The next two PCs contributed 3.39 and 2.99% to the total variability and the remaining five PCs had <2 Eigen values, and accounted for <3.31% variability and are therefore insignificant. The components PC1 and PC2, together accounting for 81.6% of variability, were plotted to reveal eight distinct groups (Fig.2). BS (IIHR-BS1) with the highest bound essential amino acid and the only mushroom of the 18 isolates with considerable Trp contents formed a separate group in I quadrant; *A. aegerita* (IIHR-AA1) with the next highest content also formed a separate group. *L. edodes* (IIHR-LE4) and *H. ulmarius* (IIHR-HU1) formed a distinct group, *L.edodes* (IIHR-LE4) was high in Phe and Leu, and *H. ulmarius* (IIHR-HU1) was high in Phe and Lys. The mushrooms with the next highest contents, were *H. tessulatus* (IIHR-WS1) with high Ile and *P. eryngi* (IIHR-PE2) with high Ile and Trp formed a separate group. The mushroom isolates with moderately high bound amino acid content, *H. erinaceus* (IIHR-HE1), *P. floridanus* (IIHR-PFL2), *P. djamor* (IIHR-DJ3) and *P. eryngi* (IIHR-PE1) formed a distinct group. The isolates with lesser contents *P. florida* (IIHR-PFL1), *P. djamor* (IIHR-DJ2), *P. djamor* (IIHR-DJ4), *P. cystidiosus* (IIHR-PCYST1), *C. indica* (IIHR-CA1) and *P. citrinopileatus* (IIHR-YO1) grouped together, though *P. citrinopileatus* was high in Trp. *Calocybe* spp. (IIHR-CAJ) and *P. sajor-caju* (IIHR-PSC1) with lowest contents of these amino acids grouped together.

IIHR-AA1 and IIHR-HU1 were high in both free and bound essential amino acids, while IIHR-CAJ and IIHR-CA1 were both low. IIHR-PE2 had high Trp contents in both free and bound amino acids. IIHR-LE4 which was low in free essential amino acids was high in bound essential amino acids, and the reverse was true of IIHR-PSC1 mushroom. IIHR-PFL1, IIHR-DJ-2 and IIHR-DJ4 grouped together in both free and bound amino acids; but unlike in free amino acids, IIHR-HE1, IIHR-BS1 and IIHR-WS1 were in distinct groups in bound amino acids.

Score of bound amino acids of Ile was higher, followed by the aromatic amino acids Phe and Tyr, and marginal scores of Leu and Met (Table 10). Ile was high in IIHR-BS1, IIHR-WS1, IIHR-CA1 and IIHR-PE2 (82 to 96%); Met score was high in IIHR-DJ-2, IIHR-AA1, IIHR-HU1, IIHR-PE1 (1.6 to 1.9%); while *H. tessulatus* (IIHR-BS1 and IIHR-WS1), IIHR-YO1 and IIHR-PSC1 had low scores of Met. Leu score was highest in IIHR-AA1 (13.2%), followed by IIHR-LE4 (9.5%); IIHR-YO1 (0.8%) and IIHR-PSC1 (0.9%) had low scores. When compared to the FAO amino acid scores, the mean values of the bound essential amino acids of the mushrooms could provide over 1.8 times the recommended scores of Ile and 37% of aromatic amino acids, 12% of Trp, and 5-7% of Leu and Met; the scores of the remaining amino acids was insignificant.

Kayoden *et al*. (2015) have reported that the chemical score of essential amino acids of the commercially cultivated oyster mushroom (*P. sajor-caju*), ranged from 55.9% Met to 150.3% Ile, and this was comparable to standard dietary reference intake requirement. In our *P. sajor-caju* isolate (IIHR-PSC1), the Ile score was comparable at 186.5%, but the Met score was very low (3.7%). Oyetayo *et al.* (2007) analysed the cap and stalk of two cultivated *P. sajor-caju* varieties obtained from the wild, where they observed that the cultivated mushroom cap accumulated more crude protein than the wild. The mushroom was a rich source of the essential amino acid Leu, while Met and Cys chemical scores were low, probably the reason for the low Met content in IIHR-PSC1.

Manzi *et al.* (1999) have compared the nutritional content in several isolates of edible mushrooms, *P.ostreatus, P. eryngii, P. pulmunarius* and *L. edodes.* They have reported total nitrogen ranging from 3.47 to 7.93% (dry basis), (values comparable to this study), with *P. ostreatus* isolates exhibiting the largest variability. They reported that in *P. pulmunarius* and *L. edodes*, Glu (12.8%), Asp (9.1%) and Arg (3.7%) were the most abundant amino acids, but with low Arg content. The chemical score ranged between 96 to 110%, the limiting amino acid being Leu and/ or Lys. In this study also Leu and Lys scores were low, as also His, Met and Thr. Thus, the amino acid composition varies significantly among mushroom isolates.

Musieba *et al.* (2013) reported the proximate composition and amino acids contents of indigenous *P. citrinopileatus* collected from Kakamega forest in Western Kenya, which is an integral part of their traditional food system; eight essential amino acids have been reported in decreasing order of abundance: Leu > Val > Thr > Lys > Phe > Ile > Met > Trp. The non-essential Glu was also present in high proportion. The results presented here vary much from their report. The variability of amino acids in white button mushrooms *(Agaricus bisporus)* and oyster mushrooms (*Pleurotus pulmonarius*) was reported by Rana *et al.* (2015). Soluble protein content of *A. bisporus* and *P. pulmonarius* was 3.3% and 1.8%, respectively, the *Pleurotus* isolates studied here had much higher protein content. *A. bisporus* contained nine essential amino acids, while *P. pulmonarius* contained only five, among the 17 amino acids identified by thin layer chromatography. In an early study by Zakia *et al*. (1963) *Pleurotus* spp. had 2.8% protein and 0.1% non-protein nitrogen on freshweight basis. Seventeen amino acids were identified, including all the essential amino acids, with all except Met and Phe being present in high concentration.

Sharma *et al*. (2012) studied five wild edible species of *Lentinus*, *L. sajor-caju*, *L. connatus*, *L. torulosus*, *L. cladopus* and *L. squarrosulus* from Northern India. Asp, the predominant amino acid and Pro which were more in *L. squarrosulus*, Arg and Ala were maximum in *L. torulosus*, Tyr was maximum in *L. cladopus*.

A study by Jaworska and Bernas (2013) demonstrates how processing treatments affect chemical composition of mushrooms. Compared to fresh mushrooms, most amino acids except Gln and Ile were lesser in frozen mushrooms. The decrease was more in *A. bisporus* soaked and blanched in citric and L-ascorbic acid solutions, and in *Boletus edulis*, soaked and blanched in pectin solution. Blanching treatment alone resulted in higher Asn, Gln, Ile and Lys than those soaked and blanched.

Of the conditionally essential bound amino acids, Pro and Ser were present marginally and of the non-essential amino acids Glu and Ala, as reported above for free amino acids. Pro was high in IIHR-AA1, IIHR-PCYST1, IIHR-CA1, IIHR-PFL2 (10 to 16mg/ 100 g DW); Ser was more in IIHR-AA1, IIHR-HU1 (>6mg/100 g DW). The non-essential amino acids were high in IIHR-BS1, IIHR-AA1and IIHR-LE4. These amino acids were less in IIHR-WS1and IIHR-CAJ (Table 11). The non-proteinogenic amino acids like ethionine, citrulline, β-3,4-dihydroxy phenylalanine were negligible in all the mushrooms studied.

High protein foods, such as tofu and quinoa, contain significant amounts of essential amino acids. A comparison was made of the amino acid scores reported from the five best plant sources (from FAO data compiled from among 50 plant sources), to the free and bound amino acid scores in selected mushroom isolates used in this study, rich in essential amino acids (Table 12). The best plant sources were soybean, cowpea, quinoa, turnip seed and sword bean, with protein content ranging between 6.6-38.0%, while in the mushrooms the protein content ranged between 9.51-31.79%. These mushrooms had significantly higher Ile and Trp contents. The Ile score, especially in the free fraction of selected mushrooms (66-312%) were comparable or higher than the best five plant sources (152-186%); though Ile was lower in the bound fraction (12-96%). Similarly, Trp scores in the plant sources ranged between 104-264%, while in the free fraction of a few mushrooms it was almost twice as much, in IIHR-HE1 it was as high as 515%; the content in the bound fraction was much lower. The total aromatic amino acids score was ~1/10th of the plant sources listed. However, the scores of the remaining amino acids were not appreciably high. Thus, the mushrooms listed in table 12 are an excellent source of Ile in the free fraction (IIHR-CA1, IIHR-AA1, ICAR-IIHR-HU1), good source of Trp (IIHR-HE1, IIHR-PCYST1, IIHR-PE2, IIHR-PSC1) and moderate sources of the aromatic amino acids (Phe, His, Tyr) in the bound fraction (IIHR-CA1, IIHR-AA1).

CONCLUSION

From the results of this study it can be concluded that the indigenous mushrooms collected from various parts of India, reveal a great diversity in their protein content (9.51-26.13%; highest in *P. cintrinopileatus* and *P. sajor-caju*), contents of free amino acids (11.56-73.11 mg Phe eq./ g DW; higher in *Hypsizygus tessulatus* and *Agrocybe aegerita*) and bound amino acids (57.41-171.85 mg Phe eq./ g DW; higher in *H. tessulatus*, *A. aegerita, H. ulmarius*, *P. djamor*, *P. florida*, *P. sajor-caju*), as also in the amino acid profiles. Of the essential amino acids, Ile

and Trp were significantly higher especially in the free fraction, comparable or higher than the best five plant sources. The free amino acid scores were higher in *H. erinaceus* (IIHR-HE1), *P. cystidiosus* (IIHR-PCYST1), *P. eryngi* (IIHR-PE2), *P. sajor-caju* (IIHR-PSC1); *H. tessulatus* (IIHR-BS1) and *A. aegerita* (IIHR-AA1) had higher bound amino acid content. Score of bound amino acids of isoleucine was higher, followed by the aromatic amino acids, phenylalanine and tyrosine. Thus, the mushrooms studied are an excellent source of Ile in the free fraction (IIHR-CA1, IIHR-AA1, ICAR-IIHR-HU1), good source of Trp (IIHR-HE1, IIHR-PCYST1, IIHR-PE2, IIHR-PSC1) and moderate sources of the aromatic amino acids (Phe, His, Tyr) in the bound fraction (IIHR-CA1, IIHR-AA1). Principal component analysis helped group the 18 mushroom isolates according to their free and bound essential amino acid content. The content of the conditionally essential and non-essential amino acids also revealed significant diversity. The data generated in this study has identified nutritionally superior mushroom isolates belonging to *H. erinaceus*, *P. cystidiosus*, *P. eryngi*, *P. sajor-caju, H. tessulatus* and *A. aegerita* by their amino acid profile, for commercialization.This can aid nutritionists and dieticians to formulate designer foods blending mushroom with vegetables/ grains/ millets for better health and nutrition, which can go a long way towards nutritional security.

Compliance with Ethical Standards: This article does not contain any studies with human participants or animals performed by any of the authors.

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Short Communication

A promising new tamarind selection - Lakshamana : Linking biodiversity and livilihood

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ABSTRACT

Tamarind is a well-known commodity of Indian cuisine having medicinal and industrial uses. It is a nutritious tree crop of widespread occurrence growing on marginal lands in semi-arid and sub-humid tropical climates of India, making it highly valuable in ensuring food security for rural poor. Given the great potential of this neglected and underutilized species to address global challenges such as hunger, poverty and climate change adaptation, there is a need to revisit research and development priorities in its favor and to develop strategies together with stakeholders to increase its utilization. In the present study, a survey was undertaken in Tumkur district of Karnataka to characterize the variability available in tamarind for pod and tree characters and identify superior trees using horticultural traits. A farmer's tamarind selection "Lakshamana**" emerged from participatory breeding having significantly better traits compared to local tamarind.**

Key words: Tamarind, Selection, Pulp recovery and Yield

Tamarind (*Tamarindus indica*) is an evergreen tree legume, distributed all over the world in tropical and sub-tropical countries. The tree produces fruits in pods which consist of a brittle outer shell encapsulating the pulp and enclosed seeds. Once established, the tree develops a large tap root which protects it from strong winds and cyclones, making it well suited to the region prone to such weather phenomena. It is also considered to be a suitable tree for inter-planting with other commercial forest species. Tamarind starts bearing from 6-8 years and has productive life of 50-70 years after which it declines. The normal life span of the tree is 150 years. A typical established tree yields between 50-100Kg of fruit which is harvested during multiple picks over an 8-10-week period between February and April. Apart from tamarind pulp other by-products such as seed, shell, fiber is also useful for various purposes. Tamarind comes in two main types; sweet and sour. Sweet tamarind is harvested ripe and usually consumed fresh, while the sour tamarind is processed into a range of value-added products. Some of the most common products prepared from tamarind include juice, pulp, powder, chutney, pickles, sauces, sugar coated candies and Tamarind Kernel Powder (TKP). TKP is an important sizing material for the jute and textile industry and tamarind seeds are gaining importance as a rich source of proteins and valuable amino acids. India is the world's largest producer of tamarind; it is estimated that 300,000 tons are produced annually. It is also an exporter of tamarind, mainly to Europe and Arab countries (Spice Board, 2018)

Recently, there has been an increased interest in ûnding alternative, potentially high-value cash crops to improve the income of small farmers who are currently depending upon growing and selling traditional cereal crops. Tamarind has a wide range of genetic variation in India, according to the phenotype and genetic characteristics which could facilitate identification of superior and desirable types. Being a highly cross-pollinated crop, and propagated from time immemorial by seeds, considerable amount of variability exists in the trees growing in different regions. Selection is the crop improvement method widely adopted in tamarind and varieties are being released using this method. The consumer preference is for traits such as broad, brown pulp with good pulp recovery which is currently not being met by the few released varieties. The present study was undertaken keeping in view the emerging

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importance of the crop with the objective of identifying superior quality combined with high pulp recovery. In this context, a survey was undertaken in Tumkur district of Karnataka to characterize the variability available for pod and tree characters and identify superior trees using horticultural traits. *In situ* analysis of the samples collected from this region was carried out at ICAR-Indian Institute of Horticulture Research, Bengaluru and an elite tamarind variety was identified having broad pods with good pulp colour and recovery. Farmer's tamarind selection "**Lakshamana**" emerged from participatory breeding having significantly better traits compared to local tamarind.

Lakshamana -A promising tamarind selection

This is an accession identified from Nandihalli village of Tumkur district Karnataka, having passport data: latitude 13.52° N, Longitude-76.74° E and 860 m MSL growing in field of Shri Laxmannappa. It was found to be superior with better yield and pod characters compared to local and registered mean annual yield (4 years from 2016-2020) of 251.4 kg/ tree as against 165.0 kg/tree in local trees (Table 1).

Table 1. Economic traits of promising selection Lakshamana in comparison to local check

Sl.No.	Traits	Lakshamana	Local
1.	Fruiting season	Feb-March	Feb-March
2.	Fruit bearing position	Terminal	Terminal
3.	Fruit clustering habit	Cluster of 2-3 or solitary	Clusters or solitary
4.	Fruit shape	Long, curved	Small, straight
5.	Pod length (cm)	25.4	13.5
6.	Pod breadth (cm)	3.8	2.36
7.	Number of pods/kg	24	49
8.	Number of seeds/pod	8.2	14.5
9.	Shell wt. (g/kg fruits)	250 (25%)	302 (30%)
10.	Pulp wt. (g/kg fruits)	430 (43%)	280 (28%)
11.	Seed wt. (g/kg fruits)	270 (27%)	380 (38%)
12.	Fiber wt. (g/kg fruits)	50 (5%)	47 (4.7%)
13	Yield per plant (kg per tree)	251.4	165.0

Fig. 1. Close view of tamarind tree and grower

Lakshamana is a 40-year-old tree and regular bearer (Fig.1). It commences flowering in May-June, matures in February-March and harvesting can be done in March-April under Tumkur conditions. This is a lean period in this region when there is less agricultural activity. The farmers can use this time to process and pack the tamarind to get better price in market. The pulp of "Lakshamana" is of superior quality having light brown colour, it is broader in shape which is desirable for marketing and has less fiber content (Fig.2). The inner cavity is silvery and this encloses the seeds. The pulp recovery is high (43%) as against 28% in local tamarind trees.

Fig.2. Pods, pulp and seed of tamarind Lakshamana

The pulp of Lakshamana has been characterized for nutritional traits (acidity and sugars) and total acidity and total sugar was found to be 20% and 29.78%, respectively. It was also profiled for sugar through liquid chromatography with tandem mass spectrometry (LC-MS/MS) and organic acid by high-performance liquid chromatography (HPLC) (Table 2). Glucose and fructose are the major sugars and accounted for 96.8% of the total sugar content. Beside that small amount (<1%) of mannose, ribose, arabinose, rhamnose, *myo*-inositol, sucrose and maltose were also found. Among organic acid tartaric acid content was highest (18.61%). Although tartaric acid occurs in other sour fruits, but tamarind fruits are reported to be the richest natural source of tartaric acid. Tamarind is known to be simultaneously the most acidic fruit with the sweetest taste because of presence of high levels of reducing sugars (glucose and fructose) and tartaric acid. Combination of organic acid and reducing sugar gives sweet-sour taste to this fruit.

Table 2. Sugar and organic acid profile of Lakshamana with local check

Nutritional traits	Lakshamana (g/100g)	Local (g/100g)
Glucose	20.53	14.5
Fructose	10.64	7.66
Mannose	0.66	0.55
Ribose	0.06	0.06
Arabinose	0.05	0.06
Rhamnose	0.02	0.01
myo-Inositol	0.06	0.02
Sucrose	0.004	0.002
Maltose	0.006	0.013
Tartaric acid	18.61	17.62
Malic acid	2.88	2.65

Harvesting and processing of Lakshamana pods starts from February and lasts up to mid-June. The pods fall down on own or the branches are shaken with help of long poles or a person climbs and shakes the branch to break free the pods. The pods are collected and left out to dry in sun for a few days. Processing which involves breaking the shell and removing seeds is carried out to secure better market value. One person can process 15-20Kg pods per day and earn around Rs 400/day. The whole family of Shri Laxmannappa gets involved during this period for processing thus employment is generated. After the shell is removed, the pulp is inverted to discard the seeds. It is stacked in ring shape in bamboo basket with capacity of 50kg. Each basket fetches 1500 Rs. The seed is also sold at rate of 17 Rs/kg and the shell chips at 2.50 Rs/kg. The seeds of "Lakshamana" tamarind are bold type and 1 quintal of pulp produces approximately 40 kg seeds. Thus, primary processing and value addition activities have potential of improving livelihood. Collective marketing and little primary processing can significantly improve family income from this accession.

In recent years several community-based strategies that focus on documenting local diversity, raising awareness of its status and improving its performance through participatory breeding and selection have been developed. Another way to strengthen on farm conservation is to recognize and support individual farmers who make contribution

to on-farm conservation (Gruberg *et al*. 2013). This strategy coupled with emerging scientific and economic interest to promote and commercialize the tamarind products will be helpful in increasing its value through market-based interventions. Tamarind is well suited for the backyard, dry and waste land farming. Hence, identification of this potential accession besides increasing the area and production could also address the issue of sustainability as the crop is climate resilient and profitable. Further, it also contributes towards rural livelihood security and employment for women during lean periods when there is less agricultural activity. There exists ample scope for area expansion under tamarind with superior fruit types all over India.

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Short Communication

Mexican creeper, *Antigonon leptopus* hook. and arn : An effective bee forage plant to conserve honey bees

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ABSTRACT

Decline in honey bee populations has become a matter of concern and their conservation is very essential to sustain essential ecosystem services. They provide making available continuous supply of floral resources is of immense value in conserving honey bees. The effectiveness of an ornamental creeper, *Antigonon leptopus* Hook. & Arn as a sustainable bee forage plant was evaluated. It attracts four major native species of honey bees *viz*., *Apis cerana, A. florea, A. dorsata* and *Tetragonula iridipennis*. The wild little bee, *A. florea* was the most dominant forager followed by the Indian bee, *A. cerana*. The plant is amenable for easy multiplication through seeds as well as cuttings and meets both aesthetic and ecological needs. Using *Antigonon*, different studies related to honey bees like assessing species diversity, foraging behaviour, temperature driven shifts etc. can be carried out. Popularising perennial bee flora like *Antigonon* would help in conserving honey bees in both natural and urban habitats. Since *Antigonon* attracts all species of honey bees throughout the year, it could be utilized as a potential bioindicator of honey bee populations in a given environment.

Keywords: *Antigonon*, *Apis* spp., Bee flora, Honey bees and Ornamental creeper

Uninterrupted availability of pollen and nectar sources is essential for sustaining honey bee colonies and to take maximum advantage of their ecosystem services like pollination and honey production. Destruction of natural habitats and lack of adequate floral resources have led to significant decline in bee populations both in wild and agro ecosystems. As per an estimate, there was about 40% decline in honey bee populations in India during the last 25 years (Gallai *et al*. 2009). With the advent of intensive agriculture characterized by mono-cropping, clean cultivation and large-scale use of pesticides and ever growing urbanisation, honey bees are deprived of adequate foraging plants as well as congenial nesting sites (Reddy *et al.* 2012). Decline in honey bee populations results in poor pollination and reduced productivity of several food crops including fruits, vegetable and oil seeds as majority of them are dependent on honey bees for pollination. In order to conserve and sustain both wild and managed pollinator populations in cropping as well as urban and peri-urban ecosystems, it is imperative to have bee flora which could provide nectar and pollen and help bees survive during off-season. A large number of flowering plants comprising herbs, shrubs, creepers and trees are credited as bee foraging plants. However, majority of them either have very short blossom period or are not adaptable to wider agro-climatic conditions. Under such circumstances, an ideal bee forage plant is the one which flowers almost throughout the year and produces copious amounts of nectar. At the same time, it should not compete with agriculturally important plants for land and other resources. Having an aesthetically pleasing blossom would be an added advantage. Such bee forage plants can be grown in open fields as well as in urban habitats like parks, boundary walls of institutions or within individual house premises.

This paper reports the efficacy of *Antigonon leptopus* Hook. & Arn, a creeper, (Family: Polygonesiae) as one such plant species which attracts a large number of honey bees besides other beneficial

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insects. Commonly called Coral vine or Mexican creeper or bee bush, *A. leptopus* is a climber, native to Central America. It produces indeterminate axillary racemes of attractive pink flowers of 20-25 mm diameter and blossoms almost throughout the year. Flowers are an abundant source of nectar and pollen to honey bees. Each flower is estimated to produce 1-1.5 µL of nectar with 26-28% sugar and 0.025-0.036 mg of pollen and attracts a wide range of floral visitors mainly social bees (Raju *et al*., 2001; Abrol, 2003). There is also a variant of the same species which produces white flowers but occurs at a low frequency. The plant is also known to possess a wide range of phytochemicals like alkaloids, phenolic compounds, saponins, triterpenoids and glycosides in different parts and is valued for its medicinal properties (Rakshit and Raghavendra, 2018).

Antigonon leptopus can be propagated through seeds as well as semi-hard wood cuttings. The dual reproductive behavior of *A. leptopus* is considered as an adaptation for successful survival in tropical environments (Raju *et al.,* 2001). Our experience shows seeds to be a better means of multiplication. The germination rate was fairly high (80-85%). Seeds were sown in pro trays and a month-old seedlings were planted along the boundary wall of the experimental field of ICAR-Indian Institute of Horticultural Research (ICAR-IIHR), Hesaraghatta, Bengaluru (Fig. 1).

Fig. 1. *Antigonon* creeper grown on the compound wall of IIHR, Bengaluru

The success rate of establishment was more than 90% and seedlings reached flowering stage within five to six months after planting. The plants do not require any special attention except for 3-4 irrigations in summer months in the first year. The *Antigonon* creepers planted at ICAR-IIHR as well as those in a nearby wild habitat were continuously monitored for flowering and honey bee activity for a year. The duration of blooming and species diversity and abundance were recorded. It was observed that the creepers were in peak flowering for 8-10 months with a relatively lower flower density during December – February.

At ICAR-IIHR campus in Hesaraghatta and surrounding places near Bengaluru (13.13° N, 77.47°E), four species of honey bees *viz*., Indian honey bee, *Apis cerana indica* Fab., little bee, *A. florea* Fab., rock bee, *A. dorsata* Fab. and stingless bee, *Tetragonula iridipennis* Smith were found foraging on *Antigonon* flowers throughout the year (Fig. 2). The proportion of different species foraging at a given time was calculated by visually counting different species from 10 plants during peak foraging hours. Among all insects visited flowers of *Antigonon*, honey bees constituted 89.09 per cent while all other insects together (butterflies, moths, wasps, syrphids, calliphorids and ants) constituted the remaining 10.91 per cent. Within four species of honey bees foraged on *Antigonon*, *A. florea* was the most dominant forager (constituting 34.06% of total foragers) followed by *A. cerana* (27.18%), *A. dorsata* (21.34%) and *T. iridipennis* (5.51%). Diurnal variations in foraging activity of different species indicate that though bees were found visiting flowers throughout the day, there were significant variations in the number of worker bees foraging at different periods of the day. The major peak activity was recorded between 6.00 – 10.00 AM followed by a minor peak between 4.00 and 6.00PM. Among three *Apis* species, the wild rock bee was found to maintain relative consistency throughout the day with least variations in their numbers while the foraging activity of other two species had significantly come down during afternoon hours till evening. This is an indication of their sensitivity to higher temperatures. In a related study by Reddy *et al*. (2015), increase in maximum temperature was reported to adversely affect the foraging activity of *A. cerana*.

The major advantage of the creeper is that it flowers throughout the year especially during rainy season (June – September) which is considered as dearth period for honey bees and when desertion rate is high. To save colonies, apiarists generally resort to feed the colonies with sugar syrup and *Antigonon* could

Little bee (*Apis florea*)

Indian bee (*Apis cerana indica*)

Rock bee (*Apis dorsata*)

Stingless bee (*Tetragonula iridipennis*)

Fig 2. Different species of honey bees foraging on *Antigonon* flowers

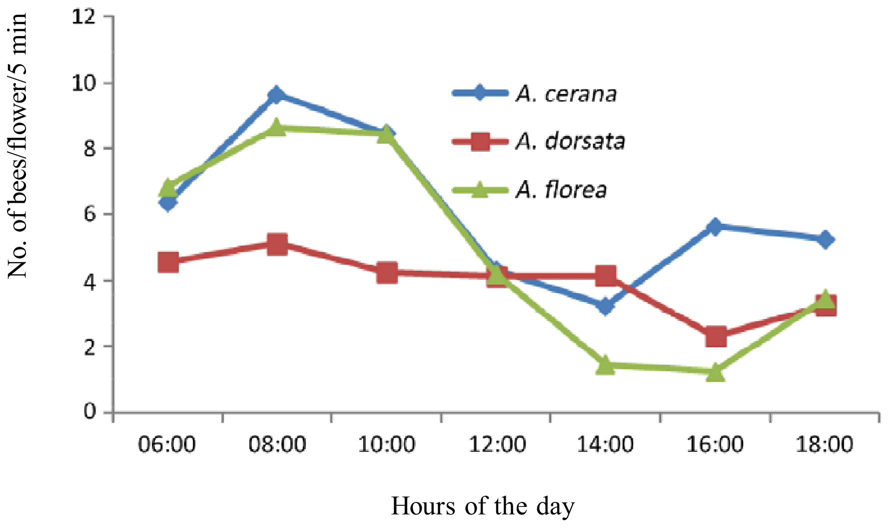

Fig 1. Diurnal variations in the foraging activity of three *Apis* species of honey bees on *Antigonon* flowers

help in saving cost and time of sugar feeding. Pruning once a year helps in preventing creeper from over growing and retaining aesthetic value. Planting of bee plants is all the more important in urban localities as it is a common sight to find bees dying while trying to feed on sweet liquid substances like soft drinks and leftover tea or coffee in paper cups. A study by Chandrasekharan *et al*. (2011) established the detrimental effect of these factors on honey bees in the absence of sufficient floral resources. The Mexican creeper not only adds beauty to premises but also helps in sustaining beneficial insect diversity in general and honey bees in particular. The Mexican creeper is also credited as a beneficial plant attractive to parasitoids of oil palm pests and is preferred to be planted around oil palm plantations (Kamarudin and Arshad, 2016).

Based on data related to species diversity and flowering duration recorded, it could be possible that *Antigonon* creeper could also be used as a bioindicator to monitor honey bee populations and species diversity in a particular location. This can also be used to conduct certain specific studies like fluctuations in bee numbers in relation to seasonal and diurnal variations, temperature influenced shifts in foraging behavior and species-specific foraging behavior. For instance, Gross et al. (2019) used *A. leptopus* to study the interspecific interactions and aggression pattern during foraging of *A. cerana* and *A. mellifera.* Hence *A. leptopus* can be planted and popularized in different landscapes wherever possible to conserve and sustain honey bees.

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Short Communication

First report on honeydew excretion by the melon thrips, *Thrips palmi* karny (Thysanoptera : Thripidae) and its biochemical analysis

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ABSTRACT

Sap sucking insects like thrips, aphids, mealybugs, whiteflies exploit the sugar rich phloem for growth and development. The excess sugar in the phloem sap creates osmotic imbalance leading to loss of water from haemolymph to gut lumen. In order to maintain osmolarity, sap sucking insects have developed structural adaptation (filter chamber) and also excrete excess sugar as honeydew through various orifices. The excreted honeydew is known to play very vital ecological role such as natural enemy calling (attracting parasitoids). In this regard scanty information is available on this important aspect for different sap sucking insects. In this study we are reporting for the first time on the composition of honeydew from the major horticultural thrips, *Thrips palmi* reared on French bean (*Phaseolus vulgaris*). LC-MS-MS analysis revealed the presence of 15 different sugars majorly inositol, fructose, maltose, glucose and sorbitol @ (130.9 ±0.47µg); (95.1±0.45µg); (60.7 ±0.28µg); (54.2 ±0.40µg) and (28.1 ±0.35µg), respectively.

Keywords: Honeydew, LC-MS-MS and Sugars and *Thrips palmi*

Sap sucking insects such as thrips, aphids, whitefly, mealybugs, leafhoppers, psyllids *etc*. feed primarily on the phloem sap which is rich in sugars such as sucrose, fructose, trehalose, maltose, raffinos, meteoritose *etc*.; free amino acids such as asparagine, glutamine, glutamate and serine (Hijaz and Killiny, 2014). Feeding of sugar rich sap leads to differential osmolarity between the hemolymph and gut lumen. To maintain the osmolarity between the gut lumen and the haemolymph, phloem feeders have developed several adaptations such as filter-chamber for efficient water usage and to excrete excess sugars in the form of honeydew through different orifices such as cornicles in aphids and anus in many other sap sucking insects. Honeydew acts as a medium through which insecticides are excreted and thereby contribute to the development of resistance to insecticides. This excretion of copious amount of honeydew on crops serves as substrate for the development of many saprophytic fungi like *Capnodium* sp., which affects photosynthesis (Lin, 2006; Wallace, 2008; Neto, 2011). It is also reported to be involved in natural enemy calling, which is self-inimical and provide food for ants which ensures dispersal and protection from the predators (Leroy *et al*., 2011). It has also been documented that honeydew is also a source of food for parasitoids involved in biological control. Rate of honeydew excretion and its composition by aphids and whiteflies is well studied, but the information on honeydew excretion by thrips is not studied in detail. Hence, a study was conducted to understand the pattern of honeydew excretion by the melon thrips, *T. palmi* due to its significance as a polyphagous pest and an important vector of *Watermelon bud necrosis virus* and *Groundnut bud necrosis virus* in India.

Stock culture of *T. palmi* was maintained on French bean (*Phaseolus vulgaris* CV. Arka Komal) pods at a temperature of 25±2°C and 68±% RH (Rebijith *et al*., 2011). One hundred adults of *T. palmi* were released on fresh French bean pods

This is part of Ph.D. thesis of first authour submitted to Jain (Deemed to be) University , Bengaluru

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of approximately equal size (3cm surface width; 13 cm length and 6 g in weight) which were harvested from the French bean plants grown under insect proof cages. The samples with thrips were placed inside a plastic container (10cm x10cm) and kept at the room temperature (25±2°C, 68±% RH). There were three replicates and same replicates of control (without inoculating *T. palmi*) were also maintained. Observations were made continually on the behavior of *T. palmi* adults and for the excretion of honeydew under the stereo-zoom microscope Stemi 305 (ZEISS, Germany).

Sugars were separated by following modified Steppuhn and Wackers (2004) method. After 24 h observation, the bean pods were washed with 10 mL of 80% ethanol, and the extract was evaporated and re-dissolved in mobile phase containing solvent A and solvent B in 1:1 ratio, filtered and injected to LC–MS/MS for sugar profiling.

Sugar standards *viz.* fructose, sucrose, galactose, glucose, maltose, fucose, rhamnose, xylose, arabinose, mannose, sorbitol, inositol, lactose, ribose and trehalose were purchased from Sigma Chemical Co., USA and calibration curve was prepared using different concentration of individual sugars. The mobile phase used was composed of solvent (A) 80:20 (Acetonitrile: Water) and solvent (B) 30:70 Acetonitrile: water with 0.1% ammonium hydroxide was filtrated through 0.2 µm nylon filter paper and separation was done using gradient elution. The initial gradient was composed of 100% solvent A for one min and at 8th min it was champed to 88% of solution A and 12% of solution B, which was held for 1 mint and a linear gradient was followed by 98% of solution A and 2% of solution B and at 15th mins it was held for 30 sec. The system had returned to initial settings at 19th min and equilibrated for 6 min. before the next injection and the flow rate was 0.1mL/min. The analytical column used was 2.1x10 mm UPLC BEH-Amide (Waters, USA) with 1.7 µm particle size and protected by vanguard BEH-Amide with particle size 1.7 µm. The column was maintained temperature of 25°C.

Study was conducted to understand the pattern of honeydew excretion by the melon thrips. Close observation under the stereo microscope revealed that *T. palmi* adults excrete honeydew which lasted for about 10 sec from initiation bending of abdomen to the release. These events were recorded in VLC format. Analysis of sugars in the honeydew revealed that there was a significant difference between the control samples and samples inoculated with thrips. Among the sugars estimated, inositol was the predominant sugar (130.95 ±0.47 µg/pod) in the honeydew followed by fructose (95.13 ±0.45 µg/pod); maltose (60.700 ±0.28µg/pod); glucose (54.22 ±0.40µg/pod); sorbitol (28.15 ±0.35g/pod) (Fig: 2) followed by less of lactose, mannose, galactose, arabinose, ribose and fucose (Table 1). It clearly indicated that the honeydew excreted by thrips is rich in soluble sugars. There is a single report on honeydew excretion by the red banded thrips, *Selenothrips rubrocinctus* (Giard) (Buss *et. al.,* 2006). Wool *et al.* (2006) reported about 20 sugars in the honeydew excreted by aphids. Glucose and fructose are basic components of the honeydew of sap feeding insects (Fischer *et al.,* 2005; Wool *et al.,* 2006). These sugars are present in honeydew in different proportions depending on the insect species and host plants. Hendrix *et al.* (1992) also observed the differences in sugar composition of honeydew excreted by *Trialeurodes vaporariorum* (Westwood) and *Bemisia tabaci* (Gennadius) feeding on different host plants.

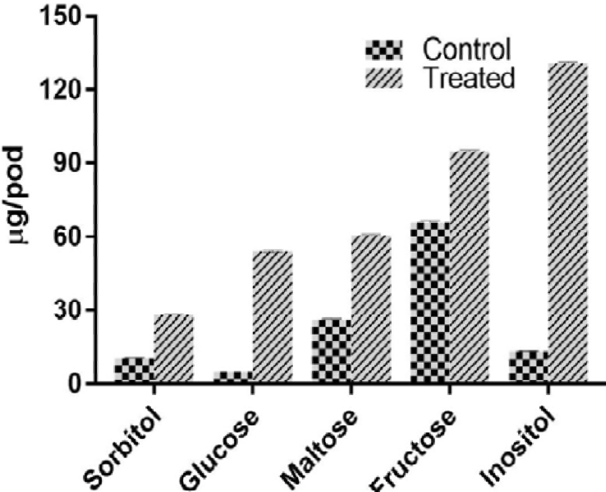

Fig. 1. Contents of major water soluble sugars in beans with or without thrips infestation

Table 1. Biochemical analyses of water-soluble sugars by LC-MS-MS in the bean pods with or without thrips infestation

Sugar	Control (±SE)	Treated (±SE)
Ribose	0.930±0.00	3.860±0.05
Arabinose	0.553±0.00	3.907±0.04
Xylose	3.690±0.08	0.683±0.02
Rhamnose	0.030±0.00	0.163±0.00
Fucose	0.010±0.00	0.020±0.00
Glucose	5.190±0.02	54.227±0.40
Fructose	66.253±0.56	95.137±0.45
Galactose	1.757±0.08	4.293±0.23
Mannose	5.100±0.09	5.253±0.07
Inositol	13.360±0.03	130.957±0.47
Sorbitol	10.660±0.09	28.153±0.35
Maltose	26.420±0.12	60.700±0.28
Lactose	0.907±0.00	3.820±0.04
Sucrose	0.343±0.00	0.890±0.01
Trehalose	0.010±0.00	0.030±0.00
Total	135.213±1.12	392.093±2.46

Differences in chemical composition of honeydew secreted by aphids are explained inter alia by genetic variation between insect populations (Fischer and Shingleton, 2001). However, the host plant sap is a primary factor that influence the diversity in biochemical composition of honeydew. Honeydew composition is an important factor in tri-tropic interaction involving natural enemies and also mediating ant–homopteran mutualisms. However, further studies on sugar composition in relation to species of ants attracted and its impact on predation/parasitism is required in order to have sustainable management of this pest.

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Short Communication

Influence of potting mixture on growth and economics of stone graft of mango cv. alphonso

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ABSTRACT

Konkan is considered as an important mango belt of India. This belt is not only famous for production of king of mango 'Alphonso' but also for supply of quality planting material throughout the country. Soil is the basic medium used in nursery. Availability of quality soil for nursery is getting scared and it is need of the hour to find out light-weight, well aerated media for reducing transport cost and mortality. Hence field experiment was carried out to find the response of mango cv. Alphonso stone grafts in different potting mixture. The treatment cocopeat + leaf manure + compost (1:1:2) was recorded significant increase in plant height (129.40%), girth of grafts (38.08%), highest number of shoot (1.50), number of leaf (22.70), highest absolute growth rate (0.1483 cm/day) and relative growth rate (0.0237 cm/cm/day). Whereas, maximum leaf area (617.03 cm^2) was obtained in soil + leaf manure (1:1) followed by leaf manure + cocopeat (1:3) (610.17 cm^2) leaf manure + cocopeat (1:3). Maximum root length (21.97 cm) and dry weight of root (7.23g) was obtained in treatment cocopeat + leaf manure + compost (1:1:1). Economics involved for different treatments showed that cocopeat + leaf manure + compost (1:1:2) was recorded with highest B:C (1.39) followed by Soil + Cocopeat (1:1) in stone grafting. From the above investigation, it is concluded that potting mixture had significant effect on growth performance and economics of mango grafts. For raising of mango grafts, the media containing cocopeat along with leaf manure and compost was the ideal soilless media.

Key words: Alphonso, B:C ratio, Growth parameters, Mango and Soilless media

Mango (*Mangifera indica* L.) is highly demanded fruit of all class and masses occupy a unique place among the fruits in world. Due to rise in demand from all parts of world for mango and mango based products, area is increasing. Demand for quality planting material of mango has increased in recent years due to adoption of high density planting by farmers. Media play major role in quality production of grafts. Konkan region is not only supplying mango grafts but also supplying other fruits and spices grafts to various parts of India. Demand for basic media i.e. soil is very high in this region. The area is also blessed with large forest and coconut plantation through which leaf manure and cocopeat can be prepared which can be used as light weight media. Considering future opportunity for soilless nursery, the present study was undertaken to understand influence of different potting mixture on survival, growth performance and economics of mango stone grafts.

The present study on influence of different potting media on growth and economics of mango stone graft was carried out at Department of Horticulture, College of Agriculture Dr. B. S. K. K. V. Dapoli, Dist. Ratnagiri (M. S.), India. The experiment was conducted in randomized block design with ten treatments and three replications. The 10 treatments consist of control (Soil + FYM 3:1), Soil + Single Super Phosphate + Rice husk + Organic manure (55:15:15:15), leaf manure (100%), cocopeat (100%), Soil + Leaf manure (1:1), Soil +Cocopeat (1:1), Leaf manure + Cocopeat (1:1), Leaf manure + Cocopeat (1:3), Cocopeat + Leaf manure + Compost (1:1:1) and

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Cocopeat + Leaf manure + Compost (1:1:2). In this experiment morphological parameters such as plant height (cm), girth of graft (mm), number of shoots, number of nodes, number of leaves, leaf area(cm^2), absolute growth rate on height basis (cm/day), relative growth rate on height basis (cm/cm/day), root length (cm) and dry weight of root (g) were recorded, influenced by different potting media. Statistical analysis of the data was carried out by standard method of analysis of variance as given by Panse and Sukhatme (1995). On the basis of survival and final sale of grafts at the end of experiment, net profit and B:C were calculated.

At the end of experiment the per cent increase in plant height was found higher in treatment T_{10}: cocopeat + leaf manure+ compost 1:1:2 (129.40%), at par with T_9: cocopeat + leaf manure+ compost 1:1:2 (120.64%) which found superior over rest of the treatments. The lower per cent increase in plant height was recorded in treatment T_1 control: soil+ FYM 1:3 (105.03%). Similar findings were reported by Parasana *et al.* (2013) in growing media containing soil + sand + FYM (2:1:1) for khirni, Kurava (2015) in soil, FYM and fertilizer media in mango and Ragaji (2017) in media containing soil + cocopeat (1:1) followed by soil + leaf manure + cocopeat (1:1:1). Similarly, significantly highest per cent increase in plant girth was found in treatment T_{10} (38.08%) which was at par with T_6: soil + cocopeat 1:1 (37.19%). The lowest per cent increase in plant girth was found in treatment T_5: soil+ leaf manure 1:1 (23.83%). Grafts containing media mixture with proper aeration, moisture and substantial amount of nutrients, facilitate root absorption for formation of photosynthesis. It helped in cell division, cell elongation and adequate water supply resulted in increase in per cent of girth of grafted plants. Similar findings were reported by Bachubhai (2005) for mango seedling in soil (40%): sand (40%): FYM (20%) and Ragaji (2017) in soil + cocopeat (1:1) for mango.

At 180 days after grafting (DAG), the statistically maximum number of shoot was recorded in treatment cocopeat + leaf manure+ compost 1:1:2(1.50). The minimum number of shoot was recorded in cocopeat 100% (1.27). This was due to availability of moisture and nutrient through media (Ikram *et al.* 2012) resulted in increasing morphological characters like height, girth and number of shoots. The treatment cocopeat + leaf manure + compost 1:1:1 was recorded maximum number of nodes (2.20) which was found superior over rest of the treatments at the end of experiment. The minimum number of nodes was recorded in treatment T_1 (soil + FYM 3:1) (1.45). Similarly, the leaf area was recorded maximum in treatment soil + FYM 1:1 (617.03 cm^2) the minimum leaf area recorded in treatment soil + FYM 3:1 (538.55 cm^2). Soilless media is light in weight and porous (Wilson, 1983) with low salt content, good water holding capacity and ion exchange capacity with optimum pH produced maximum number of nodes. Similar findings were reported by Kurava (2015) in media containing soil, FYM and fertilizer for mango and Kelkar (2016) in top soil + FYM + Vermiphos media for mango cv. Alphonso. At the end of experiment, highest number of leaves was observed in treatment T_{10} which consists of cocopeat + leaf manure+ compost (1:1:2) (22.70) while the lowest number of leaves was observed in control treatment Soil + FYM (1:3) (15.22) followed by T_8 (16.17). Similar findings were also reported by Waseem *et al.* (2013) in soil + leaf mold+ coconut husk (33:33:33) and Ragaji (2017) in media containing leaf manure.

At 180 DAG, absolute growth rate (AGR) on height basis was highest in treatment (T10). cocopeat + leaf manure+ compost 1:1:2(0.1483 cm/day) while lowest AGR was recorded in treatment (T9) cocopeat + leaf manure+ compost 1:1:1 (0.0048 cm/day). The highest relative growth rate (RGR) on height basis was obtained in treatment (T10) cocopeat + leaf manure+ compost 1:1:2 (0.0237 cm/cm/day) whereas lowest RGR was obtained in treatment (T1) soil + FYM (0.0208 cm/cm/day). Similar finding was reported by Kelkar (2016) in top soil + FYM + Vermiphose media for mango and Ragaji (2017) for mango stone grafting in soil + leaf manure (1:1).

At the end of the sixth month, the root length was significantly influenced by the different treatments. The highest root length was recorded in the treatment T_9 (21.97) cocopeat + leaf manure + compost 1:1:1 which was at par with T_2 (20.20 cm) soil + SSP + rice husk + cocopeat (55:15:15:15). The lowest root length was recorded in T_5 (14.57) soil + leaf manure (1:1) which was at par with T_8 (16.37) i.e. leaf manure + cocopeat (1:3). Similar findings were reported by Khot (2017) for bullock's heart in soil + FYM (2:1) and Ragaji (2017) for mango stone grafting in soil + cocopeat (1:1) and leaf manure + cocopeat (1:3) media.

Table No.1: Effect of different potting mixture on morphological characters and B:C

Treat-ments	Plant height (cm)	Girth of graft (mm)	Number of shoot	Number of Node	Number of leaves	Leaf area (cm^2)	Absolute growth rate (cm/day)	Relative growth rate (cm/day)	Root length (cm)	Dry weight of root (g)	Survival (%)	Net profit (Rs)	B:C
T_1	25.68 (105.03)	7.90 (29.78)	1.45	1.45	15.52	538.55	0.0496	0.0208	18.20	4.73	37.33 (37.66)	3.5	1.00
T_2	26.38 (115.90)	8.46 (32.37)	1.33	1.70	18.07	585.58	0.0648	0.0223	20.20	6.12	50.67 (45.38)	334.8	1.17
T_3	27.43 (106.08)	7.69 (26.07)	1.30	1.78	16.92	565.62	0.1089	0.0209	16.80	2.69	36.00 (36.87)	225.5	1.16
T_4	26.61 (107.46)	7.73 (27.14)	1.27	1.67	17.33	564.02	0.0849	0.0212	19.20	3.74	49.33 (44.62)	278.7	1.14
T_5	28.25 (115.90)	8.27 (23.83)	1.37	1.60	18.32	617.03	0.1383	0.0223	14.57	5.68	46.67 (43.09)	388.5	1.23
T_6	26.39 (116.48)	7.88 (37.19)	1.47	1.73	18.13	570.03	0.0658	0.0225	18.53	5.69	52.00 (46.15)	509.8	1.28
T_7	25.95 (111.03)	8.09 (26.61)	1.42	1.53	16.53	608.58	0.0082	0.0216	18.03	5.17	37.33 (37.66)	47.3	1.03
T_8	27.89 (116.86)	8.57 (33.38)	1.48	2.02	16.17	610.17	0.1253	0.0222	16.37	4.69	41.33 (40.01)	48.6	1.03
T_9	28.15 (120.64)	8.23 (29.29)	1.38	2.20	17.98	576.59	0.0048	0.0228	21.97	7.23	42.67 (40.78)	273.5	1.17
T_{10}	29.77 (129.40)	8.69 (38.08)	1.50	1.95	22.70	595.61	0.1483	0.0237	19.53	6.85	50.67 (45.38)	634.7	1.39
Mean	**114.58**	**30.38**	**1.390**	**1.763**	**17.77**	**583.18**	**0.0799**	**0.0220**	**18.34**	**5.29**	**44.40**	-	-
S.E.±	**3.33**	**1.28**	**0.011**	**0.024**	**0.20**	**12.31**	**0.00**	**0.00**	**0.64**	**0.42**	**0.68**	-	-
C.D. at 5%	**9.89**	**3.81**	**0.034**	**0.072**	**0.59**	**36.57**	**0.00**	**0.00**	**1.92**	**1.25**	**2.04**	-	-

(Value in parenthesis indicates per cent increase in plant height and girth and arcsine in survival)

The maximum dry weight of root was obtained in the treatment T_9 (7.23) cocopeat + leaf manure + compost (1:1:1) which was at par with T_{10} (6.85) cocopeat + leaf manure + compost (1:1:2) and T_2 (6.12) soil + SSP + rice husk + cocopeat (55:15:15:15). The lowest root length was obtained in T_3 (2.69) leaf manure 100% which was at par with T_4 (3.74) cocopeat 100%. Similar findings were reported by Panchal *et al*. (2014) for khirni seedlings in soil + cocopeat + FYM (1:1:1) and Ragaji (2017) for mango graft in soil + cocopeat (1:1) media.

The benefit cost ratio (B:C) for mango stone grafts raised in different potting media was shown in Table No. 1. Net profit was calculated on the basis of expenditure incurred and income received from total number of mango grafts survived and sold at the end of experiment. Media mixture Soil: cocopeat (1:1) recorded significantly maximum 52 % survival with 1.29 B:C with net profit of Rs. 509.80 followed by 50.67 % survival in T_{10} (cocopeat + leaf manure+ compost 1:1:2) and T_2 (soil + SSP + rice husk + organic mill (55:15:15:15)). The highest B:C (1.39) was recorded in T_{10} in which net profit received after selling of mango graft was Rs.634.70. Treatment which was used as regular nursery practice T_2 (soil + SSP + rice husk + organic mill (55:15:15:15)) recorded net profit of Rs. 334.80 and B:C 1.17. Lowest B: C (1.00) was reported in T_1 control i.e. Soil + FYM 3:1 with net profit of Rs. 3.50 followed by T_7 Leaf manure + Cocopeat (1:1) and T_8 Leaf manure + Cocopeat (1:03) with net profit of Rs. 47.30 and Rs. 48.60, respectively.

Fig. 1. Comparison of mango stone grafts raised in different potting media at 180 DAG

Conclusion

From the above investigation, it was concluded that potting media had significant effect on growth performance and B:C of mango grafts. Locally available leaf manure, cocopeat, compost can be used as media which serves as alternative to soil in near future. Grafts filled with soilless media reduced weight of bag and helps in easy transportation. The media containing cocopeat along with leaf manure and compost (1:1:2) was the ideal soilless media for raising mango grafts.

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