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JOURNAL OF HORTICULTURAL SCIENCES

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Short Communications

In this issue…

Horticulture contributes 33% to the agriculture Gross Value Added (GVA)making a very significant contribution to the Indian economy.Current production is 320.48 million tonnes produced in 25.66 million ha with productivity of 12.49 tonnes per ha. This has been made possible due to the sustained efforts by the scientific community by carrying out basic and applied research in various disciplines and resulting in the accumulation of knowledge about horticultural crops. Journal of Horticultural Sciences attempts to attract the knowledge gained from the scientific community and share with the peers. The number of articles have increased and we are proud to see thirty-five articles being published in this issue. The editorial team gratefully acknowledges the authors, subscribers as well as members of Society for Promotion of Horticulture for their constant support.

Horticulture plays important role in nutritional security. An overview of biochemical and molecular considerations with respect to Nutraceutical Horticulture by ***Mohankumar*** *provides an insight into the role of dietary nutraceuticals derived from fruits, vegetables, and spices in sustaining health at molecular level. Another review by* ***Pandey et al.*** *narrates how mushrooms can be part of the integrated and balanced nutrition.* ***Biradar et al.*** *emphasizes the need for food-system transformation that requires a paradigm shift towards nature-friendly nutrition-rich diverse fruits and vegetables, and it should constitute at least 30-50% of our food plate from the current average of less than 10%. These three reviews give us the confidence and guidance to progress in achieving our nutritional security goals.*

Male sterile line development is an important aspect in crop improvement. ***Varalakshmi et al.*** *report the development of male sterile and maintainer lines in ridge gourd. They can be used to develop F1 hybrids with different genetic back grounds such as green/dark green fruit colour and short/medium long/long fruit length.* ***Barik et al.*** *studied the yield attributing traits of brinjal through six generation mean analysis and report that recurrent selection followed by bi-parental mating and selection during the later stage of generations is advisable to increase the occurrence of favorable alleles and accumulation of desirable genes.* ***Sajid et al****. has assessed the genetic diversity of squash genotypes in Bangladesh and found that First Runner was the best suited in their agroclimatic conditions. Similarly diversity analysis in carrot by* ***Manisha et al.*** *has given an indication about the genetic variation among the carrot accessions which will prove useful in selection of diverse parents in crop improvement programme.* ***Usha et al.*** *studied the impact of pollination strategies on fruit set and fruit growth attributes in jasmine and documented the response of different species of jasmine to the pollination methods adopted. This will help in different breeding programmes in jasmine. Diversity analysis of phenotypic traits in okra by*

Ranga and Darvhankar *has indicated that lines EC359637 and IARI Selection 2 can be used for overall improvement in further crop breeding.*

A promising method for preparing metaphase spread for counting the number of chromosomes from the emerging shoot tissue of Citrus has been described by ***Vijayakumar et al.*** *Molecular markers developed by* ***Amulya et al.*** *can be successfully utilized for determining genetic diversity and relationship of bael trees for varietal improvement.Similarly,* ***Akshita et al.*** *developed RAPD and SSR markers to study the variability in ginger.* ***Shareefa et al.*** *report the occurrence of in vitro flowering in coconut during tissue culture and this may help in understanding the physiological, hormonal and molecular regulation of flowering and such information gathered can be used to save time in future genetic improvement programs.****Sivaranjani and Zachariah*** *studied the effect of externally applied elicitors like phenylalanine and chitosan on the induction of essential oil constituents in turmeric and observed that phenylalanine application increased the essential oil components.*

Adak et al. *demonstrated that yield and quality of Dashehari mango can be improved with foliar spray of Zn in sandy loam soil. Genotype variations in biomass production and nutrient removal pattern in gladiolus raised from cormels has been documented by* ***Sujatha et al.*** *Performance of parthenocarpicand non-parthenocarpic grafts of cucumber was studied by* ***Gowda et al.*** *and they have identified better graft combinations. Heat unit requirement of litchi under Sub-Himalayan terai region of West Bengal has been studied by* ***Subba and Bhowmick*** *and they found that Bedana variety performed well in that region.* ***Dhayalan and Sudalaimuthu*** *identified the beneficial microbes in vegetable growing areas Anamalai block in Tamil Nadu that will enhance the plant growth. Rambutan fruits harvested from trees of different ages were assessed for total fruit weight, pulp weight, pericarp weight, seed weight, percentage seed emergence, seedling plant height and number of leaves at monthly intervals by* ***Tettech et al.*** *and they observed that fruits harvested from 8, 10, 25 and 40 years old trees did not show significant difference in fruit characteristics and seed emergence.*

Recent years have witnessed a good progress in research efforts to reduce the post-harvest losses in fruits and vegetables. ***Ahmed and Langthasa****evaluated different drying methods to retain the quality of drumstick power and conclude that both shade and sun drying may be considered best for preserving nutrient and also from the point of view of cost involvement. Studies by* ***Viswakarma et al.*** *indicated that pre-harvest spray of calcium chloride is eco-safe and could be done for improving shelf life of mango fruits for better marketability. Time of harvest in dragon fruit is important.* ***Deep Lata et al.*** *report that harvesting dragon fruits between 31-36 days after flowering (DAF) was found ideal for optimum maturity and quality. Both red and white pulp*

fruits harvested at 31 DAF showed better quality in terms of physic-chemical and sensory attributes. ***Nayaka et al****. describe the effect of sugars and amino acids, influencing the biochemical and nutritional attributes that are responsible for browning in guava fruit leather.* ***Sahel et al.*** *attempted modified atmosphere package on physico-chemical properties of pomegranate fruitsfound that MAP extended the shelf life of the fruits.* ***Neeraj et al.*** *report the morphological, physiochemical and colour characteristics of fresh and cured tubes of starch in different potato varieties.*

Bayogan et al. *in Philippines developed a brick-walled evaporative cooler for storage of tomato and report 27.17% higher annual benefit over cost than the ambient storage conditions. Onion detopping machine developed by* ***Rathinakumar and Senthilkumaran*** *has shown promise in the post-harvest management of onion in Karnataka in India. The report by* ***Yella Swami et al.*** *describes the constraints in dry chilli cultivation practices and emphasize the need for mechanization of harvesting.*

Saidulu et al. *studied the biochemical changes in defense responses in rose genotypes that were artificially inoculated with black spot pathogen Diplocarpon rosae and found that induction of defence compounds was in resistant line Knock Out and moderately resistant Arka Nishkant.* ***Sangeetha et al.*** *report the occurrence of algal stem blotch in ber under coastal Odisha conditions in India and they have confirmed the identity of the causal agent as Trentepohlia arborum.* ***Sindhu et al.*** *observed that cropping duration and non-rhizomorphic mycelial phenotype of Pleurotus djamor woody1 co-segregate in the hybrid progenies of this mushroom.*

The help rendered by PhD scholars H.N. Lavanya, Sangeetha Priya, A. Mounika, M.L. Supriya and B.S.Susmitha in the editorial process for this issue is gratefully acknowledged. The contribution of reviewers and section editors has been immense. The editorial team gratefully acknowledges the help rendered by contributions and reviewers.

S. Sriram
Editor in Chief

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Review

Nutraceutical Horticulture : An overview of biochemical and molecular considerations

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The major components of our diet, namely, carbohydrates, fats, proteins, vitamins, and minerals provide for the building blocks besides serving as metabolic fuel to fulfil the bioenergetic needs. Since they serve the basic cellular needs, they are considered as 'primary metabolites'. The molecular and biochemical pathways modulated by the major food components of our diet are well-established. Many phytochemicals referred to as 'secondary metabolites' and not considered as an 'essential part' of our diet, also find their way into the digestive tract along with the major food components. Interest in the role played by the 'non-essential' or 'minor' components of our diet in preventing the initiation or progression of metabolic disorders has gained momentum. The metabolic disorders, by and large, are non-pathogenic in nature and originate as a consequence of derailed cellular metabolism.

Plants produce over 50,000 phytochemicals belonging to the major groups of secondary metabolites such as phenolics, alkaloids, saponins and terpenes. The secondary metabolites serve several functions in plants and tend to accumulate in various plant parts in response to biotic and/or abiotic interactions. Many of the secondary metabolites are used by the pharmaceutical industry either in the formulation drugs directly, or as precursors for active ingredients in the drug formulations.

The secondary metabolites associated with our diet are known to influence cellular function upon assimilation. Such dietary phytochemicals capable of sustaining normal cellular function and sustain health are known as 'nutraceuticals'. Although phytochemicals of nutraceutical value occur widely, horticultural crops such as fruits, vegetables and spices are particularly rich in nutraceuticals. The interest in the nutraceuticals is on the increase as can be ascertained from number of books and journals featuring research articles on nutraceuticals (Fig. 1, 2). This is an overview of the role of dietary nutraceuticals derived from fruits, vegetables, and spices in sustaining health *via* their interaction with the biochemical/molecular components in our cells.

Cellular components interacting with nutraceuticals

The biochemical pathways modulated by the dietary nutraceuticals are many and complex. However, the major player interacting with nutraceuticals appears to be the nuclear transcription factor (NF-KB). Many dietary nutraceuticals exhibit inhibitory effect on NF-kB (Fig. 3). In addition, nutraceuticals are capable of inhibiting NF-kB activation, mediated by the tumor necrosis factor-alpha (TNF-α), a cell-signaling molecule. The activation of NF-kB transcribes genes that mediate the initiation and progression of several metabolic disorders.

Transcription factors

Transcription factors are proteins that bind to DNA to effect transcription. Over 1600 transcription factors exist in mammalian cells. One such transcription factor of importance is NF-kB. As many as 133, 517 citations (Oct 2021; PubMed Central, National Center for Biotechnology Information, NCBI) exist on various aspects of NF-kB. It is a transcription factor of relevance to the initiation and progression of diseases. Therefore, inhibition of NF-kB and/or its endogenous activators (see below) are considered valuable targets for drug development.

NF-kB was discovered in 1986 by Ranjan Sen and David Baltimore. It is ubiquitous to all mammalian cells and exists in the cytoplasm. NF-kB is expressed constitutively and remains inactive when

bound to its inhibitory peptide, IkBα. The list of activators of NF-kB is large and include biotic as well as abiotic factors such as viral antigens, free-radicals (FRs), carcinogens, environmental pollutants, alcohol, to name a few. In addition, a family of endogenous peptides known as tumor necrosis factors (TNFs), play a crucial role in the activation of NF-kB. Upon binding to its activators, TNF promotes degradation of its inhibitory peptide (IkBα) resulting in the activation of NF-kB. The active NF-kB then enters the nucleus and binds to the response elements (RE) of DNA to promote transcription. In fact, active NF-kB has potential to transcribe over 150 genes with a potential to deregulate cellular function.

Tumor Necrosis Factor

TNF is a transmembrane protein that plays a crucial role in the activation of NF-kB. It was first isolated in 1984 and identified as an endogenous tumor regression factor. Therefore, it was designated as a tumor necrosis factor. However, over the years, the TNF was identified as a pro-inflammatory cytokine (cell-signaling peptide) with an ability to initiate several inflammation-induced metabolic disorders upon binding to its elicitors. Thus, TNF has a dual role in cell metabolism and often described as a 'double-edged sword'. The localized and controlled expression of TNF-mediated inflammatory reaction has therapeutic significance. However, its uncontrolled expression leads to chronic inflammation and contribute toward metabolic disorders. For example, in cancer cells, TNF is expressed constitutively. TNF plays a crucial role in pathogenesis of several diseases and hence has attracted a greater research interest. Several synthetic FDA approved drugs as inhibitors of TNF are currently available. The TNF inhibitor drug industry is expected to reach 42.1 billion US $ in the year 2025.

Biochemical/molecular pathways modulated by the active TNF and NF-kB

As described above, activation of TNF and NF-kB has potential to result in far-reaching consequences through their abilities in initiating transcriptions detrimental to the normal cellular function. Such transcriptional changes are significant to derail cells from their normal function by activating pro-inflammatory pathways. Although localized and regulated inflammation is beneficial in containing the disease progression, chronic inflammation contributes toward a number of diseases. In fact, most disease names ending with suffix "*itis*" (bronchitis, hepatitis, meningitis…) suggest inflammatory origin (*itis:* inflammation).

By inhibiting apoptosis (programmed cell death) and promoting angiogenesis (development of new blood vessels), NF-kB confirms immortality to abnormal cells. Factors that inhibit apoptosis promote proliferation of cells with undesirable function. NF-kB also promotes development of new blood vessels (angiogenesis). Among several factors that promote angiogenesis, vesicular endothelial growth factor (VEGF) plays a major role in the development of blood vessels. Developing tumor cells promote angiogenesis mediated by VEGF. Inhibition of angiogenesis is therefore desirable for containing tumor growth. To date, over 14 FDA approved angiogenesis inhibitors are available.

Inhibition of TNF, NF-kB and associated cellular events by nutraceuticals from fruits, vegetables, and spices

By their ability to inhibit activation of TNF and NF-kB, nutraceuticals derived from fruits, vegetables, and spices modulate inflammation, apoptosis, and angiogenesis (Fig. 2, 3). These molecular/biochemical events promote metabolic disorders such as cancer. A significant body of knowledge exits pertaining to the potential and mode of action of nutraceuticals in containing diseases such as prostate, breast, colon cancer and Alzheimer's disease. To date, information pertaining to the nutraceutical benefits of curcumin (turmeric), quercetin (onion), resveratrol (red grapes, peanut seed coat), sulforaphane (cole crops) and capsaicin (chilies) appear prominently (Fig. 4abc and 5ab). It is evident from the published literature that studies on the nutraceutical benefits of other horticultural crops are actively pursued.

An interdisciplinary course covering the nutraceutical aspects of horticultural crops will be a very useful addition to the undergraduate or graduate curriculum. Such a course deriving appropriate content from horticulture, biochemistry, molecular biology, food science, pharmacology, and human physiology will be valuable to advance awareness on the scientific basis for the health sustaining benefits of fruits, vegetables, and spices.

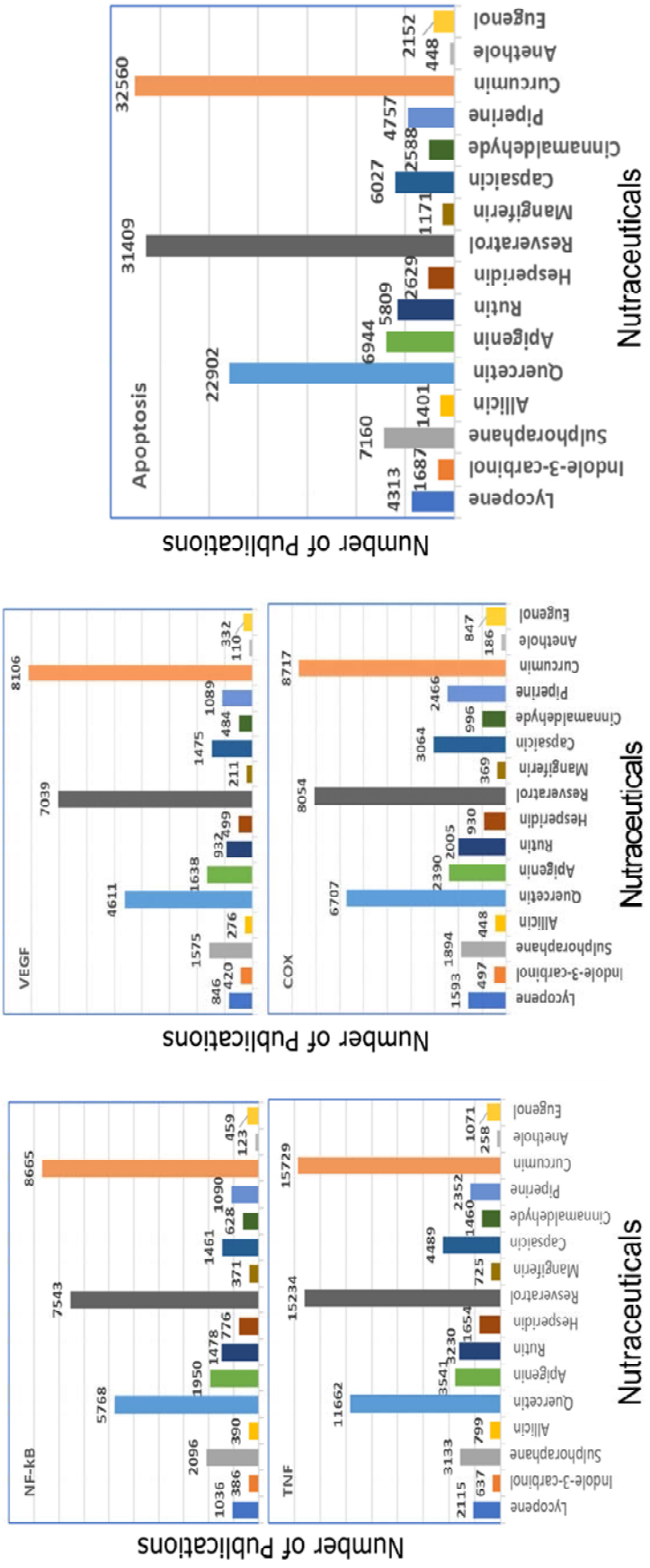

Fig. 1. Number of publications pertaining to the nutraceutical phytochemicals of selected fruits, vegetables and spices capable modulating inflammation, apoptosis (programmed cell death) and angiogenesis by their ability to inhibit activation of TNF (tumor necrosis factor) and NF-kB (nuclear transcription factor). VEGF : Vascular endothelial growth factors; Cox : Cyclo oxygenase.
Source: National Center for Biotechnology Information, USA.

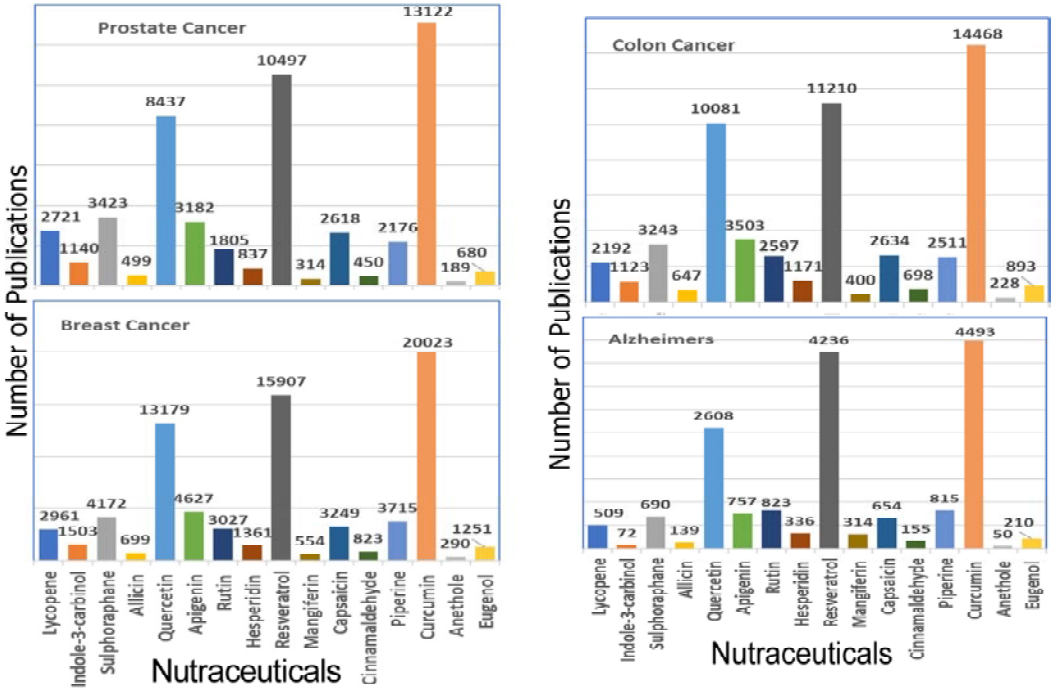

Fig. 2. Number of publications pertaining to the nutraceutical phytochemicals of selected fruits, vegetables, and spices capable of preventing or containing diseases such as prostate, breast and colon cancer, and Alzheimer's disease through their ability to inhibit activation of TNF (tumor necrosis factor) and NF-kB (nuclear transcription factor). *Source:* National Center for Biotechnology Information, USA.

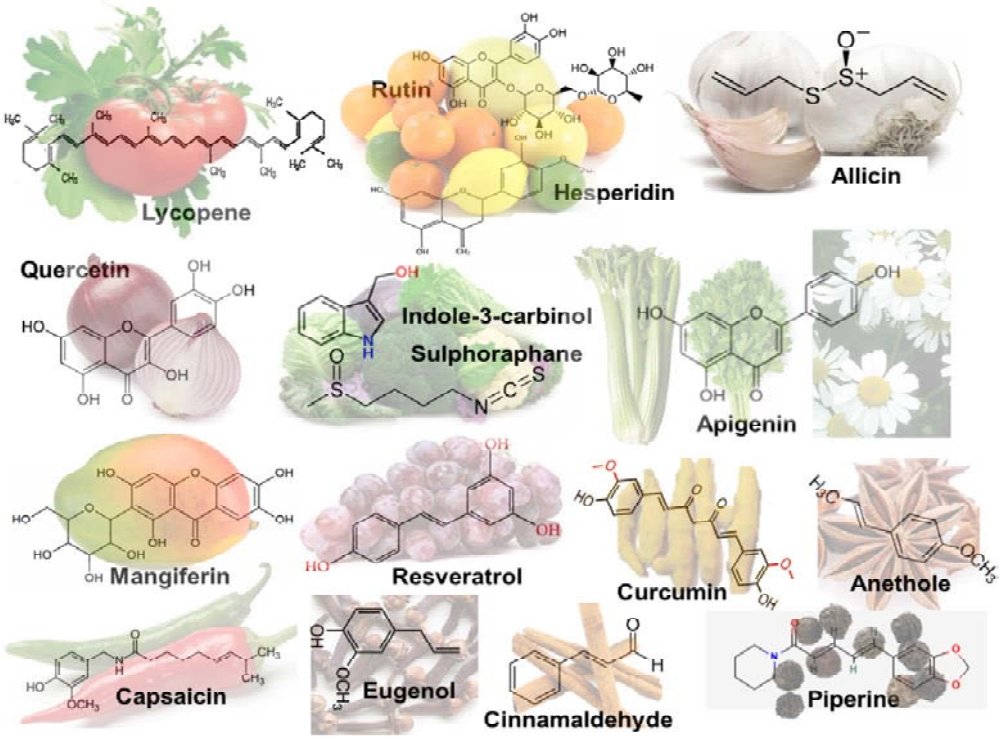

Fig. 3. Nutraceutical components of selected fruits, vegetables, and spices capable of inhibiting nuclear transcription factor and tumor necrosis factor (NF-kB/TNF). The activation of TNF/NF-kB has negative effects on cellular function.

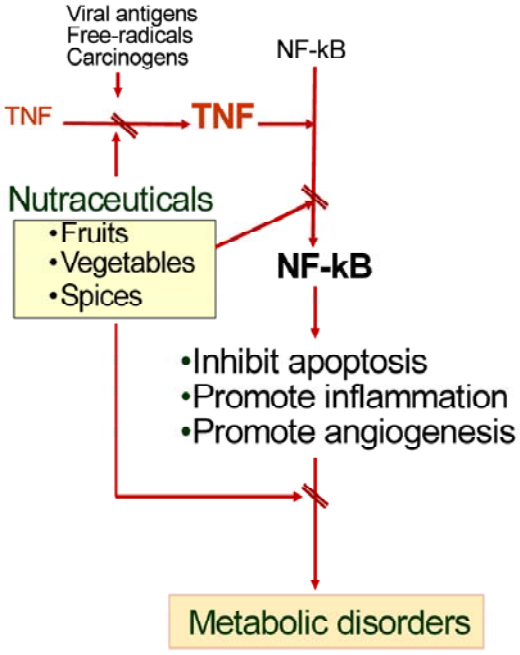

Fig. 4. Simplified schematic presenting the mechanism of activation of tumor necrosis factor (TNF) and nuclear transcription factor (NF-kB). Nutraceuticals derived from fruits, vegetables and spices play a role in the inhibition of NF-kB and suppress cellular processes (inflammation, angiogenesis, and apoptosis) that lead to metabolic disorders (Bold faced TNF and NF-kB represent active forms).

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Review

Mushrooms for integrated and diversified nutrition

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Mushrooms were considered as "Objects of mystery" by the primitive man, and were realized as food much before civilization. Mushrooms have been variously used in different cultures from being priced as "Food of Gods" in the Roman culture to tools of psychological sedation for the Mexican warriors. Most of the edible mushrooms are saprophytic growing on decomposed organic plant matter. Mushrooms play varied and important roles in human nutrition and health. They are unique nutrition dense vegetables with quality high protein, very low fat, zero cholesterol, low carbohydrates, low glycemic index, high fiber, good cardiac friendly sodium to potassium ratio and some unique bioactive compounds like ergothioneine and polysaccharides. These unique nutritive properties of mushrooms make them a recommended food for diabetics, body weight management, hypertension and cardiac well-being. The concept of selenium rich mushrooms for slowing down the progress of AIDS has been gaining importance in the recent years. Apart from being a healthy vegetable, the unique lignocellulosic waste-based production system of mushrooms makes them the most eco-friendly zero waste green technology with immense environmental benefits (Gupta *et al*., 2004, Jain *et al*., 2014 and & Pandey *et al.,* 2014). Despite many environmental and nutritional benefits; mushrooms yet have not become a part of daily nutrition in the Indian diet and the per capita consumption still remains very low at 70 grams per annum. There is a need to educate and enhance the awareness among the people about the nutritional and health potential of mushrooms. There is also the need to draw the attention towards integrating mushroom technology in successful agro-residue management programs, livelihood programs, national nutrition programs and women empowerment and rural development schemes which ultimately culminate in providing better daily nutrition.

Mushrooms as part of nutrition sensitive agriculture

Nutrition sensitive Agriculture is a strategy which aims to ensure the sustained production of diverse, nutritious, culturally suitable, safe and affordable food for daily diet. This approach requires action at every stage of the food chain from farm to fork. This approach can result in improving the health through availability of nutritious, safe and diverse food, income generation to enhance accessibility to health services and input efficient technologies (www.fao.org). Among the many principles that are applied in nutrition sensitive agriculture, facilitation for diversified food production, production of nutrient-dense crops, reduction of seasonality, creation of employment and women empowerment are the aspects where mushrooms can play a very important role and bridge the gap in a modest way.

Mushroom for nutrition & health

The food we eat is vital to our health and well-being. Nutritionists and doctors are becoming increasingly aware of the links between diet and major health problems, such as heart disease, obesity, tooth decay *etc*. Diet is recognized as an important contributory factor for well-being. Following a healthy, varied diet in order to reduce the risk of such problems does not just mean ensuring adequate intake of protein or vitamins and minerals but it is a1so important to strike the right balance. The best and most sustainable way to strike this balance is through increasing the diversity of our food plates. One way to enhance this food plate diversity is to include various types of mushrooms in the daily diet.

Nutrition through mushrooms

Protein

Protein is an essential element of nutrition required for efficient physiological functions, vital performance of hormones and enzyme action of the human body. Mushrooms are one of several vegan-friendly sources

Table 1. General nutrition facts of most common mushrooms (Serving size 100 g fresh)

	Button mushroom	Oyster mushroom	Shiitake mushroom
Calories	22, calories from fat 3	33, calories from fat 3	34, calories from fat 4
Total carbohydrate	3 g (1% daily value)	6 g (2% daily value)	7 g (2% daily value)
Sugars	2 g	1 g	2 g
Proteins	3 g	3 g	2 g
Total fat Saturated fat Trans fat	0 g (1% daily value) 0 g 0 g	0 g (1% daily value) 0 g 0 g	0 g (1% daily value) 0 g 0 g
Cholesterol	0 mg	0 mg	0 mg
Dietary fiber	1g (4% daily value)	2 g (9% daily value)	2 g (10% daily value)
Sodium	5 mg (0% daily value)	18 mg (1% daily value)	9 mg (0.5%daily value)
Iron	3%of daily value	7%of daily value	2%of daily value
Vitamin A	0%	1%	~
Vitamin D*	325% of DV	651 % of DV	490 % of DV

*(in 84g of 1 pulse UVB exposed mushrooms; Percent Daily Values are based on a 2000 calorie diet). **Source:** USDA SR23 2010 Nutritional Data and Kalaras, 2012

of protein and contain 2-4% protein on fresh weight basis and 10 - 40% on dry weight basis. It is remarkable that 100 g of dry mushrooms can cover 29.41% to 66.00% of the Recommended Dietary Allowance (RDA) of protein for men and from 35.80% to 80.35% for women. The amino acid profile of common mushroom protein suggests that Protein Digestibility Corrected Amino Acid Score (PDCAAS) is approximately 0.66 which is equivalent to legumes and has a digestibility of 70%. The highest score is 1, which applies to animal protein sources. Wheat has a PDCAAS of 0.44 and those of vegetables at 0.73 (WHO, 2007). Mushrooms are a better source of protein compared to common fruits and vegetables with the exception of some green leafy vegetables like fenugreek or drumstick leaves and cauliflower (Fig1). A combination of these vegetables with mushrooms can become a better source of protein for vegetarians vis-à-vis either of these alone. Legumes (dals) are the main source for protein for the predominantly vegetarian population. Some amount of protein is also obtained through cereals and dry fruits. The daily value (DV%) of proteins obtained from 100 g dry mushrooms is higher as compared to commonly consumed cereals and equivalent or higher than pulses but lower to meat (Fig 2).

Protein quality - Amino Acids

Approximately 25-35% of the total amino acids in mushrooms occur as free amino acids, the remainder being combined in the protein. The composition of the growth substrate may also have a significant effect on the amino acid composition of mushroom without changing the apparent crude protein. The data generated at ICAR-IIHR shows that mushrooms contain all the 20 amino acids including the 9 essential amino acids. Mushrooms contain all essential amino acids in higher quantity as compared to most of the common cereals, legumes & nuts. They are especially rich source of isoleucine, phenylalanine, tryptophan and Lysine which are the limiting amino acids in most of the legumes and cereals. Mushrooms are a better source of isoleucine, methionine, phenylalanine and tryptophan as compared to fruits and vegetables. They are a very good source of branched chain amino acids leucine, isoleucine and valine which are important to build muscle, decrease muscle fatigue and alleviate muscle soreness. The high isoleucine content in mushrooms is perhaps associated with its immune enhancing properties by inducing the expression of host defense peptides (i.e., β-defensins) that can regulate host innate and adaptive immunity (Fig 3a & 3b).

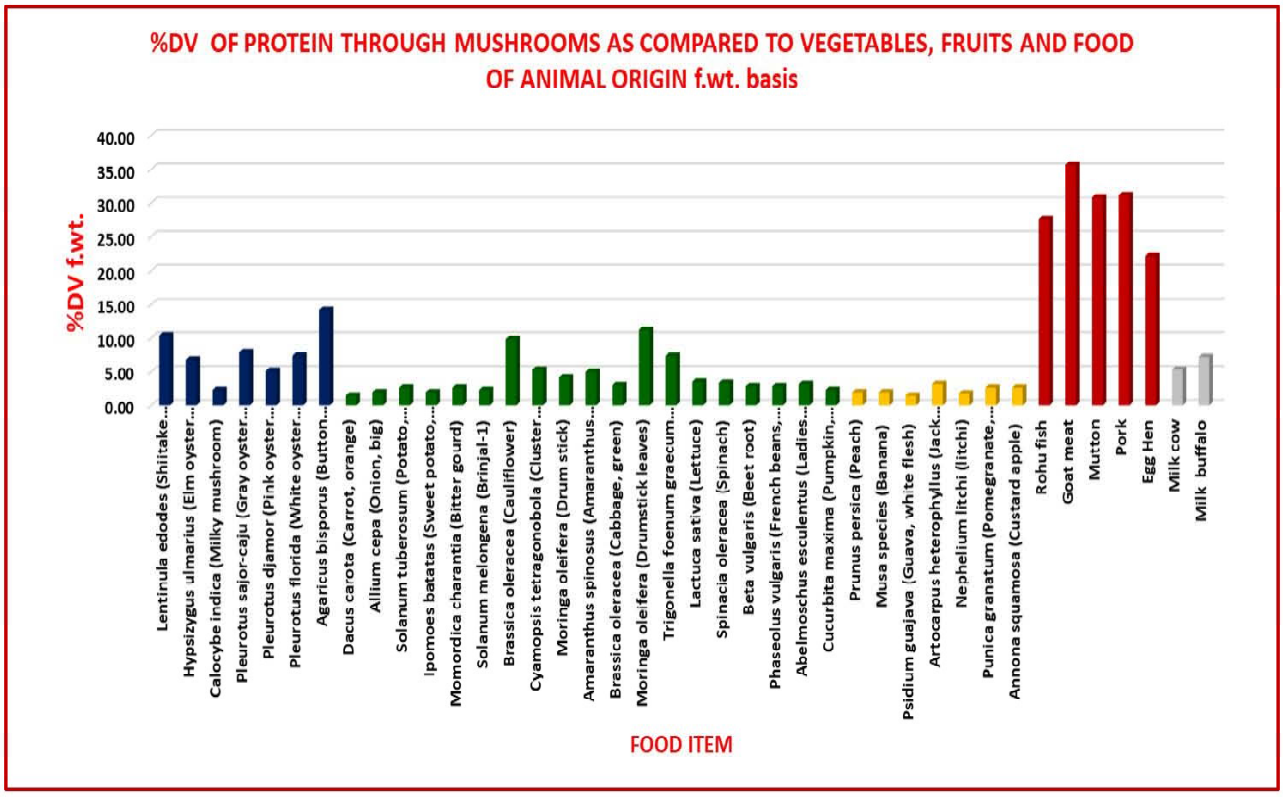
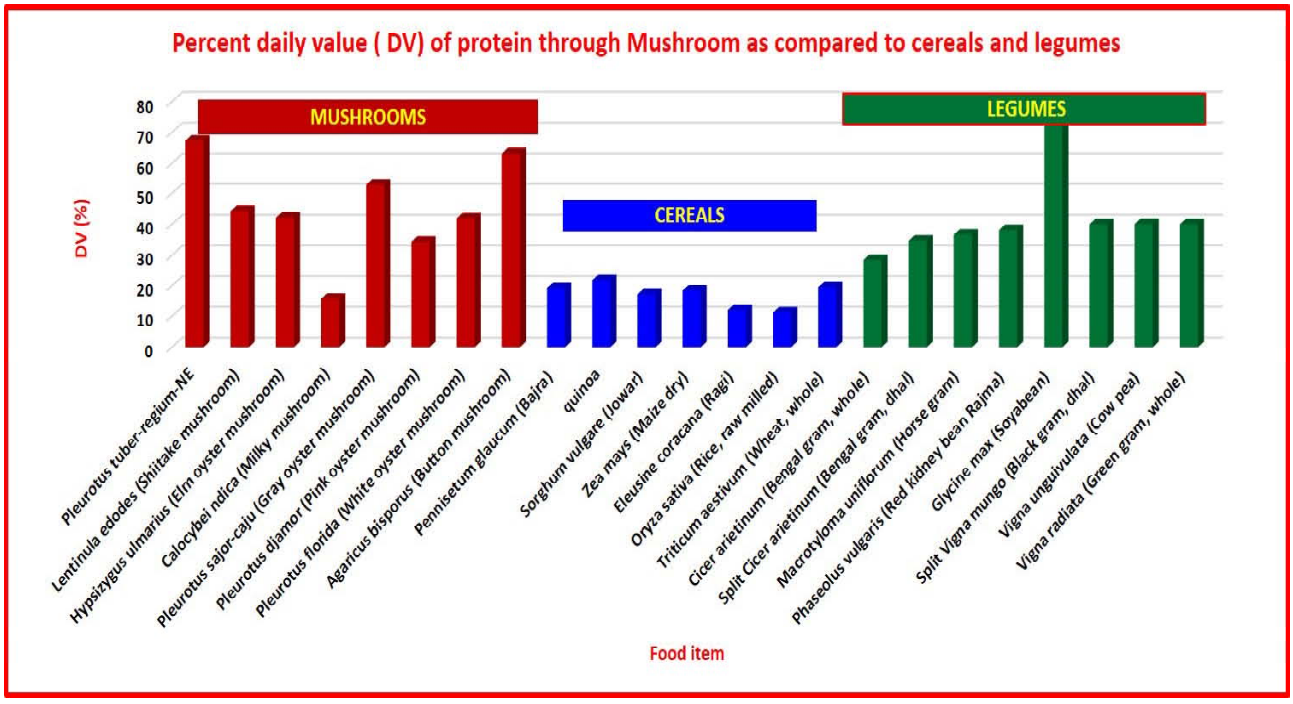


Fig 1. Comparison of daily value contribution of protein through mushroom and commonly consumed vegetables, fruits, meat and milk (Pandey et al., 2020; Longyah et al. 2017)



DV% calculated as per RDA requirement of Indians -ICMR report, 2010

Fig 2. Comparison of daily value contribution of protein through mushroom and commonly consumed cereals & legumes (Pandey et al., 2020; Longyah et al. 2017)

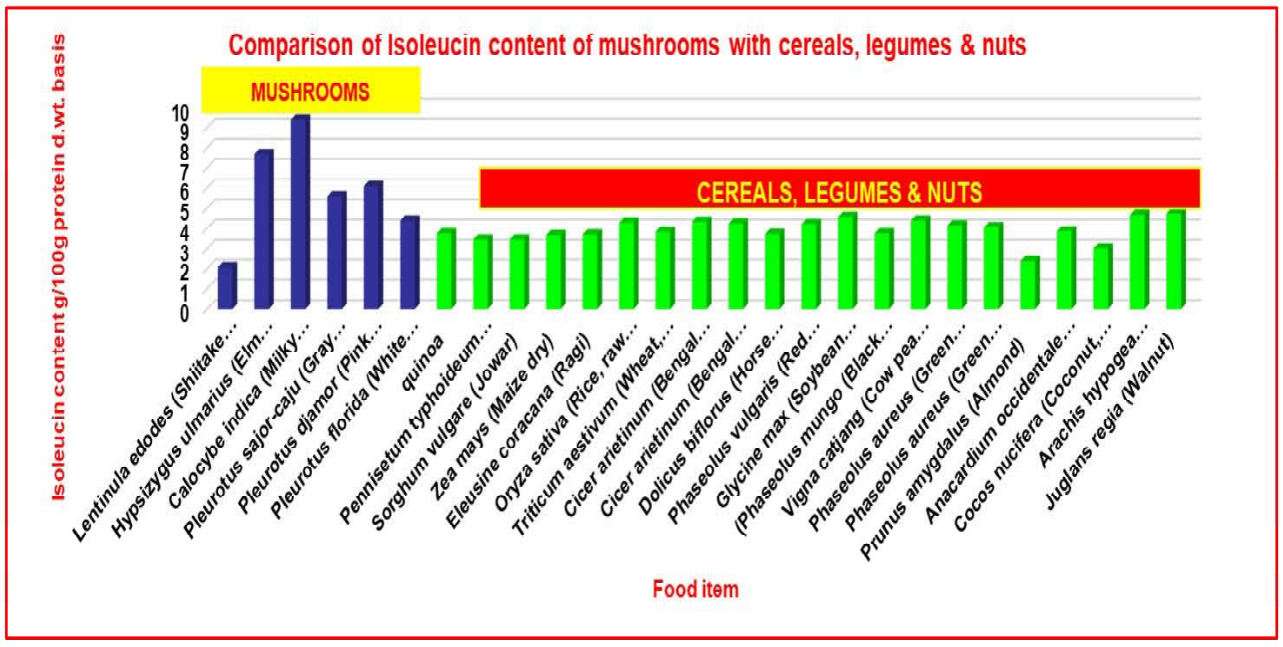


Fig 3a. Comparison of isoleucine of mushroom and commonly consumed cereals & legumes (Pandey et al., 2020; Longyah et al. 2017)

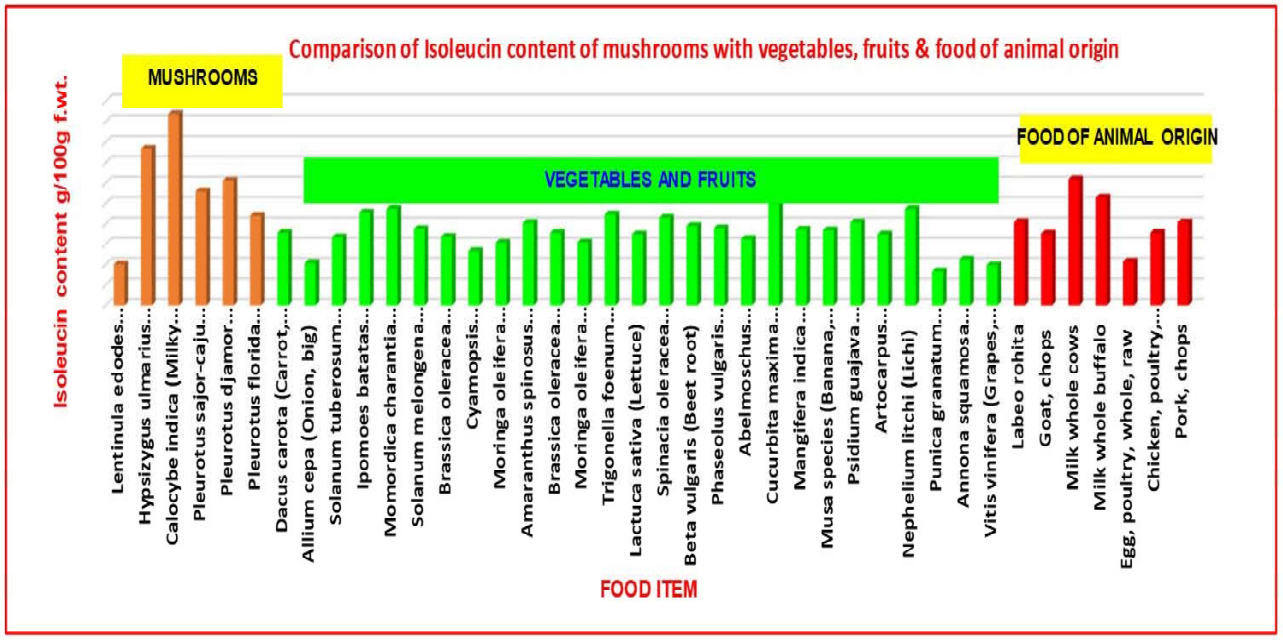


Fig 3b. Comparison of isoleucine of mushroom and commonly consumed vegetables, fruits and food of animal origin (Pandey et al., 2020; Longyah et al. 2017)

Although mushrooms are lower to meat in protein content but are devoid of many harmful ingredients like high saturated fatty acids, sodium and cholesterol found in meat. It has also been shown clinically that the consumption of same quantity of mushroom protein versus meat protein led to higher satiety factor and the feeling of fullness leading to curbing of hunger and prospective consumption (Julie *et al.,* 2017).

Carbohydrate, fat & fiber

Mushrooms are low calorie foods due to lower amount of carbohydrate, very low sugar (no glucose), high

fiber. The carbohydrate quality of mushrooms is better due to the presence of complex carbohydrates like β 1-6 glucans with high immune boosting properties. Mushrooms are low fat foods. Although mushrooms are not a choice source of lipids, they contain essential fatty acids such as linoleic, oleic, and linolenic acids. Therefore, compared to other vegetarian and animal origin food; mushrooms have the advantage of possessing high levels of polyunsaturated fatty acids (PUFA). Dietary fiber (DF) means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in humans. Mushrooms as source of DF have been underutilized as compared to other conventional sources of DF such as cereals, fruits, legumes and vegetables. In general edible mushrooms are rich in DFs with diverse beneficial health effects. Mushroom cell walls contain a mixture of fibrillar and matrix components which include chitin (a straight-chain (1'!4)-β-linked polymer of N-acetyl-glucosamine) and the polysaccharides such as (1'!3)-β-d-glucans and mannans, respectively. These mushroom cell wall components are non-digestible carbohydrates (NDCs) that are resistant to human enzymes and can be considered as source of DF as well as prebiotic medium for good gut health. Fiber content of mushrooms is lower as compared to vegetables and fruits. Foods of animal origin lack fiber totally. Hence a blend of mushrooms with meat is a very healthy and nutritious way of reducing meat intake. Mushrooms are low energy foods as compared to fresh vegetables, fruits and very low as compared to food of animal origin due to their very low carbohydrate and fat content. Hence mushrooms are recommended diets for type II diabetes and for weight reduction. Mushrooms have very low carbohydrate, fat, fiber and energy as compared to commonly consumed grains, legumes and dry fruits (Fig 4a, 4b, 4c, 4d, 4e, 4f, 4g & 4h).

Glycemic index and glycemic load of mushrooms

Mushrooms are a low Glycemic index (GI) and low Glycemic load (GL) food, meaning that they do not spike blood sugar level. Although mushrooms are technically fungi, they are considered white vegetables - like onions and garlic - with a low GI of 10–15 and a GL of less than 1 per cup (70 grams), indicating that they do not spike the blood sugar levels.

Vitamins and antioxidants

Mushrooms appear to be good sources of several vitamins. Vitamin A (retinol) activity is relatively common although several mushrooms have detectable amounts of provitamin A measured as mg carotene equivalent. Similarly, although vitamin D activity is rare in mushroom but mushrooms contain the sterol called ergosterol, which is converted to vitamin D under ultraviolet radiation rendering mushrooms as the only vegetarian source of vitamin D. Mushrooms are a very good source of the water-soluble B vitamins. Mushrooms are a very good source of B vitamins such as B_1, B_2, B_3, B_6, and B_{12} which is higher as compared to plants. It is assumed that 100 g of mushrooms can satisfy 2–9, 10–34, 7–12, and 1–8% of the daily demand for vitamins B_1, B_2, B_3, and B_6, respectively. Fresh mushrooms are characterized by a higher content of B vitamins than those subjected to drying. The ability of mushrooms to convert ergosterol in vitamin D2 (ergocalciferol) under ultraviolet-B light (UVB) with a wavelength of 280-315nm exposure is of immense importance. Vitamin D2 is a strong antioxidant which prevents the peroxidation of lipids. The ability of mushrooms to convert ergosterol to vitamin D2 under UVB has become a specialized skill to produce Vitamin D rich mushrooms which is being used as supplement to mitigate Vitamin D deficiency. Among the cultivated mushrooms the highest conversion to vitamin D2 happens in oyster mushrooms in which mere 10 minutes exposure leads to the formation of 3µg/g of vitamin D2. Consumption of merely 30-50g of UVB exposed fresh elm oyster mushroom can fulfill 100% daily requirement (15-20 µg) of vitamin D in human beings.

Ergothioneine- the unique mushroom antioxidant

Ergothioneine is a unique sulfur containing antioxidant specific to mushrooms. It is a biogenic key organic cation transporting substrate, a new type 1 (OCTN1). It is a strong hydroxyl radical (˙OH) scavenger and inhibitor of ˙OH generation through hydrogen peroxide, which has been catalyzed through iron and copper ions. It can protect against the damage due to oxidative stress and reduce reactive oxygen substances' side effects. Ergothioneine protects the water-soluble proteins from oxidative damage. Mushrooms are very rich source of this antioxidant which is not found in plants or other food sources (Fig 5).

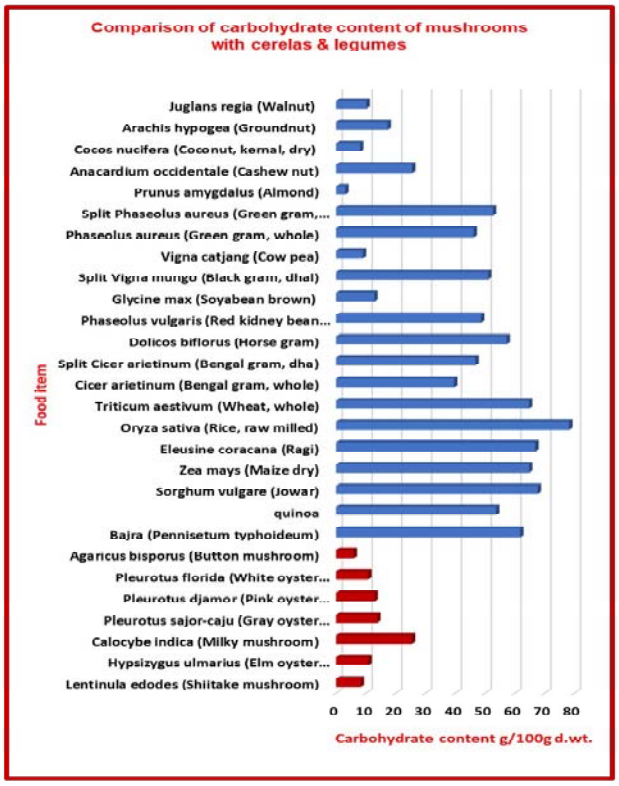


Fig 4a. Comparison of carbohydrate of mushrooms, cereals, legumes and nuts on dry weight basis

Fig 4b. Comparison of fat content of mushrooms, cereals, legumes and nuts on dry weight basis

Fig 4c. Comparison of fibre content of mushrooms, cereals, legumes, nuts and food of animal origin on dry weight basis

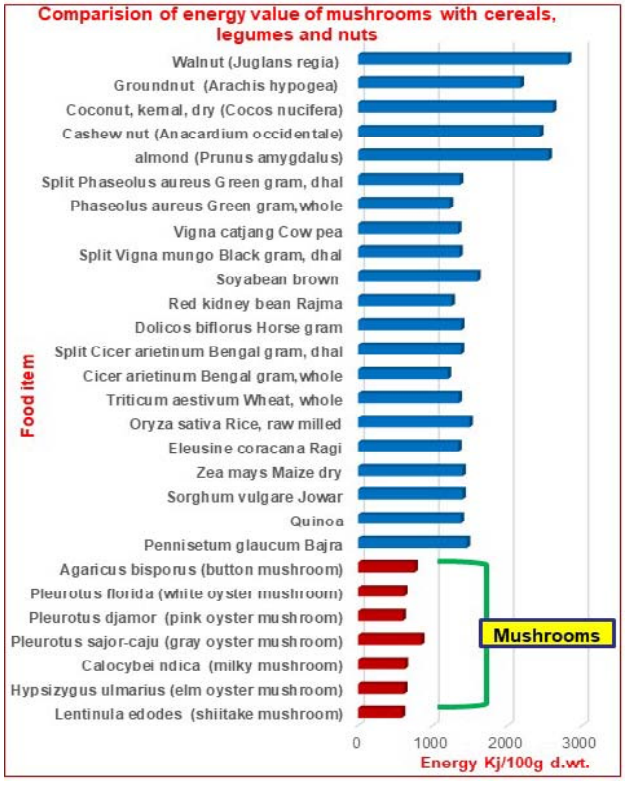


Fig 4d. Comparison of energy value of mushrooms, cereals, legumes and nuts on dry weight basis

Fig 4e. Comparison of carbohydrate of mushrooms, vegetables, fruits and food of animal origin on fresh weight basis (Pandey et al., 2020; Longyah et al. 2017)

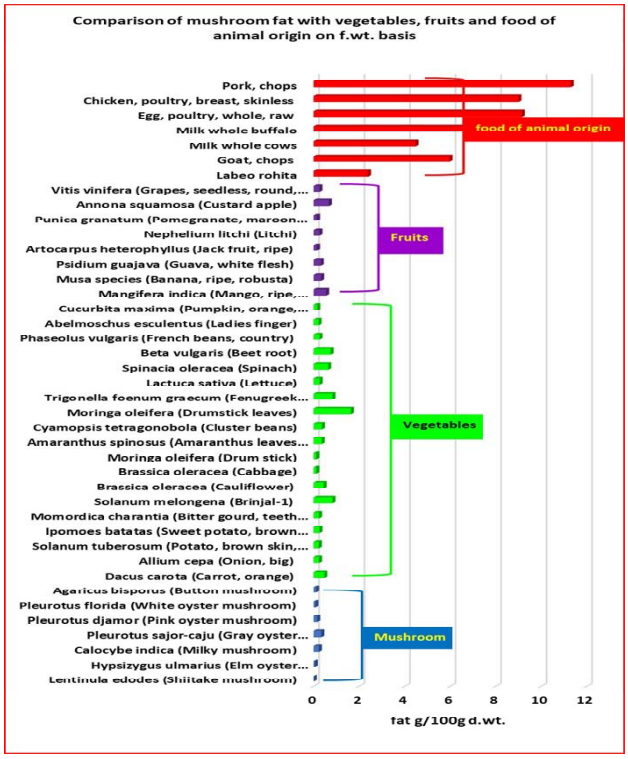


Fig 4f. Comparison of fat content of mushrooms, vegetables, fruits and food of animal origin on fresh weight basis (Pandey et al., 2020; Longyah et al. 2017)

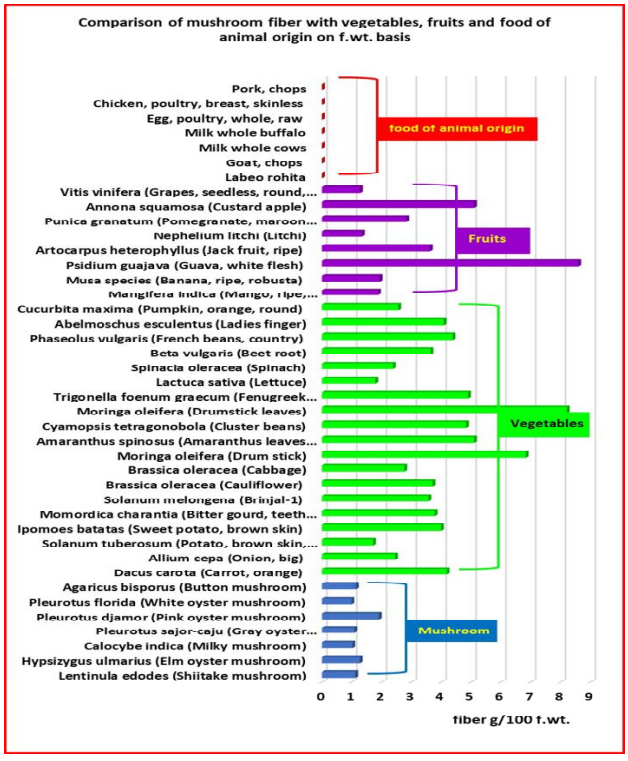


Fig 4g. Comparison of fibre content of mushrooms, vegetables, fruits and food of animal origin on fresh weight basis (Pandey et al., 2020; Longyah et al. 2017)

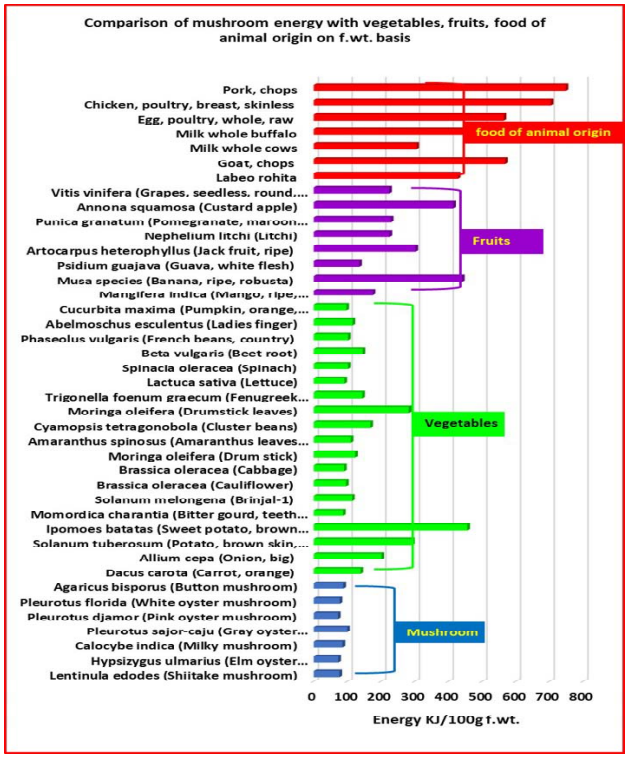


Fig 4h. Comparison of energy of mushrooms, vegetables, fruits and food of animal origin on fresh weight basis (Pandey et al., 2020; Longyah et al. 2017)

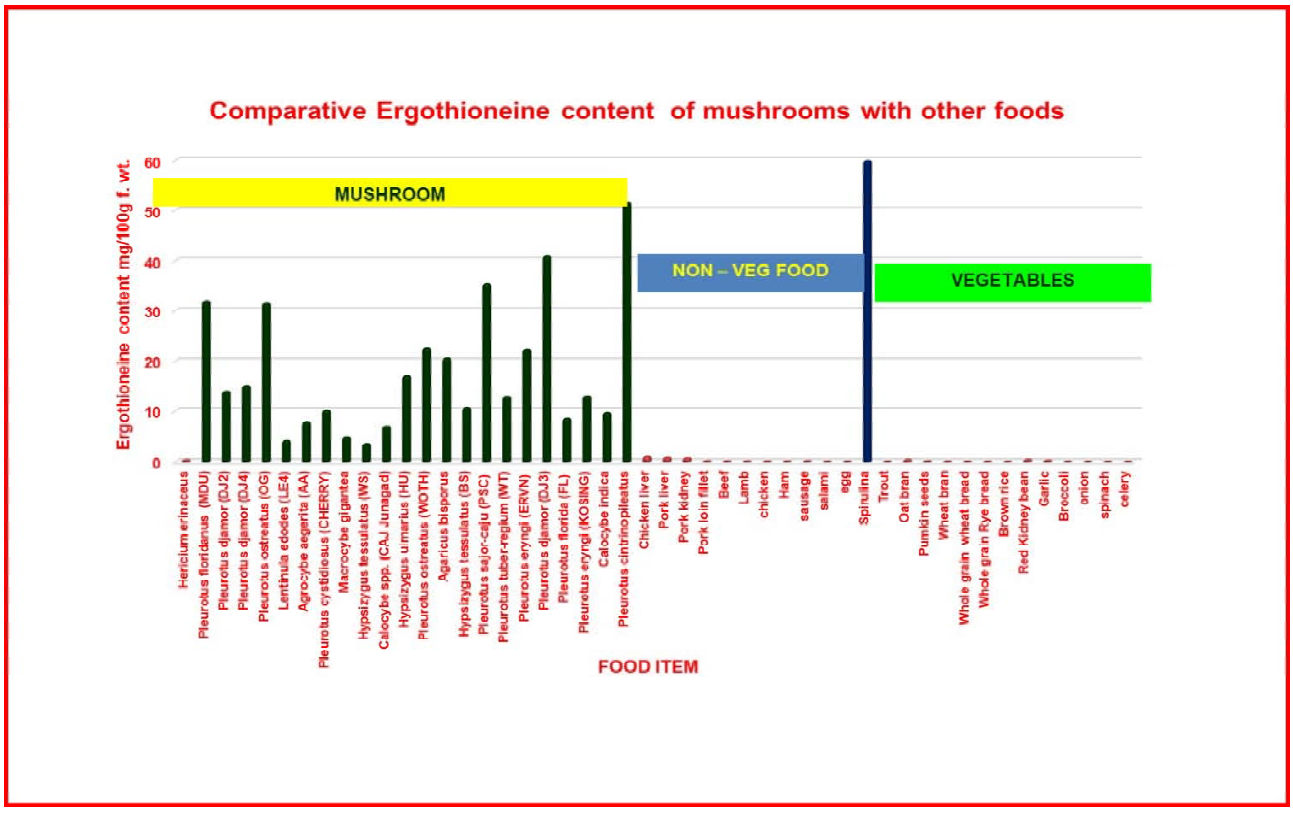


Fig 5. Comparison of ergothioneine content of mushrooms and other foods (Pandey et al., 2020; Longyah et al. 2017

Minerals in Mushrooms

Many minerals are required for better health and these are called essential minerals. Essential minerals are classified into major minerals (macronutrients) and trace minerals (microminerals or micronutrients). These two groups of minerals are equally important, but trace minerals are needed in smaller amounts than macro minerals (https://www.uofmhealth.org/health-library/ta3912). A balanced diet usually provides all of the essential minerals. The phosphorus, sodium, potassium, calcium, iron, zinc, copper and Selenium content of mushrooms is higher as compared to most of the common cereals and nuts. The phosphorus, copper, zinc and selenium content are higher as compared to most of the common vegetables and fruits. Mushrooms are good source of calcium as compared to non-leafy vegetables, fruits and common animal products. They are also a good source of iron with high iron bioavailability of 17% as compared to plant sources. Mushrooms are very low in sodium content. Shiitake, elm oyster and button mushrooms can significantly contribute towards fulfilling the daily requirements of minerals like calcium, phosphorus, potassium, iron, copper, zinc and selenium (Table 2). Further the bioavailability of various micronutrients is higher in mushrooms due to absence of anti-nutrient substances like phytates found abundantly in plant sources.

Sodium/potassium ratio important for health

Sodium is often blamed for boosting blood pressure while potassium is praised for keeping it in check. These two minerals work in tandem throughout the body. The ratio of sodium to potassium in the diet may be more important than the amount of either one alone. Our Palaeolithic hunter-gatherer ancestors consumed about 11,000 milligrams (mg) of potassium a day from fruits, vegetables, leaves, flowers, roots, and other plant sources, and well under 700 mg of sodium. That's a sodium-to-potassium ratio of 1 to 16. Today, we get more sodium (3,400 mg) than potassium (2,500 mg), for a ratio of 1.36 to 1. The higher the sodium-potassium ratio, the greater the chances of cardiovascular disease (Yang *et al.*, 2011). Mushrooms have a lower sodium to potassium ratio as compared to commonly consumed foods thereby important for cardiac well-being.

Table 2. Per cent daily value (DV %) of minerals obtained through consumption of 100 g fresh mushrooms

Mushroom variety	P	Na	K	Ca	Mg	Fe	Mn	Cu	Zn	S
Lentinula edodes (Shiitake mushroom)	33.14	3.15	21.29	25.08	5.42	4.36	21.05	15.55	10.34	14.3
Hypsizygus ulmarius (Elm oyster mushroom)	21.89	1.59	16.5	13.91	4.2	9.07	8.85	13.11	6.24	21.22
Calocybe indica (Milky mushroom)	10.72	2.81	15.34	17.4	2.59	8.16	2.95	25.77	3.66	5.14
Pleurotus sajor-caju (Grey oyster mushroom)	19.68	2.02	9.67	8.64	4.78	9.43	22.8	7.55	14.31	19.64
Pleurotus djamor (Pink oyster mushroom)	18	2.02	12.3	5.04	4.78	5.88	20.1	12.66	9	11.64
Pleurotus florida (White oyster mushroom)	26.32	2.02	13.47	11.2	8.12	12.32	20.45	9.85	18.5	13.87
Agaricus bisporus (Button mushroom)	41.76	5.46	39.15	10.08	7.1	7.32	11	53.99	14	199.57

Foot Note : DV% calculated as per RDA requirement of Indians (ICMR report, 2010)

Mushroom blends for synergistic nutrition

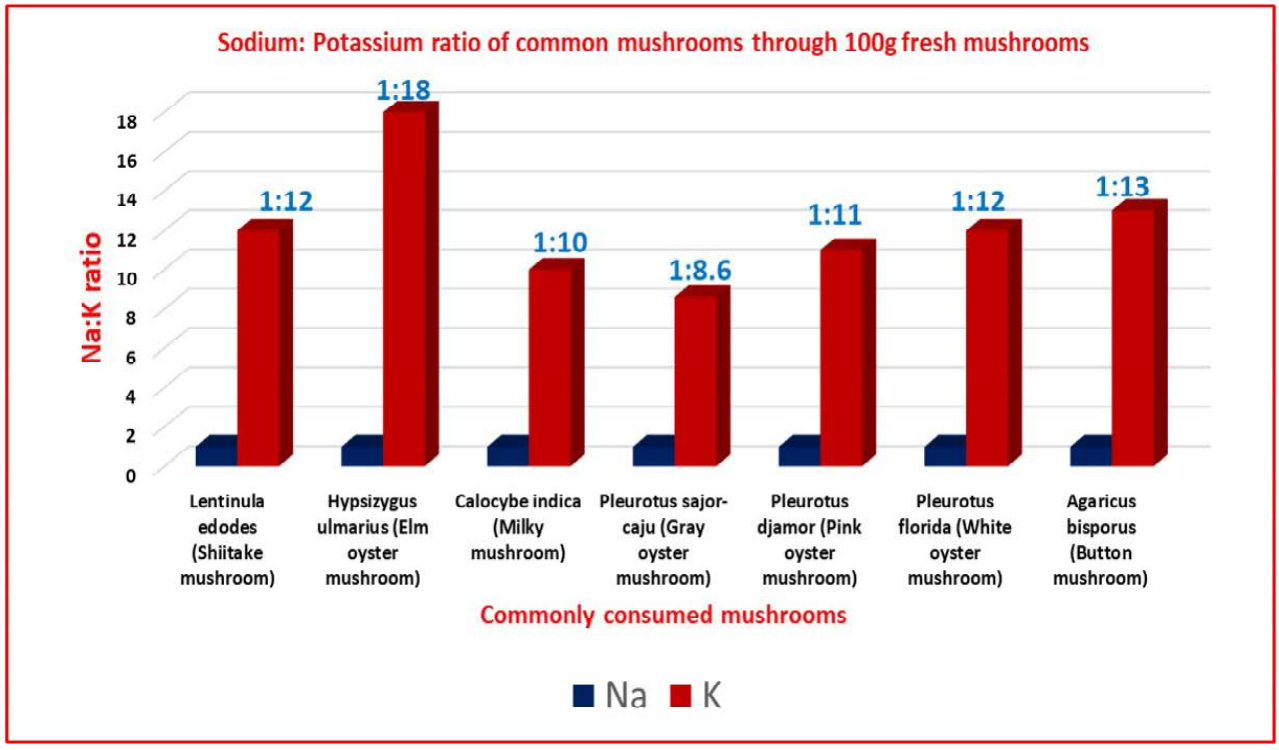
Mushrooms with unique nutrition, light aroma and neutral pH are highly amenable as blended foods with both vegetarian and non-vegetarian ingredients.

Mushrooms for vitamin nutrition

Vitamin B complex is a group of eight essential nutrients that play roles in many organs and bodily systems. Although they can work together in the body, they also carry out their own unique functions. Vitamins of B complex are water soluble vitamins. Mushrooms are an excellent source of vitamin B complex. Mushrooms however lack vitamin C and A. Hence soup blends containing mushrooms with carrots, broccoli, cauliflower and spinach can be an excellent source for vitamin A, B & C nutrition. Vitamin D also called as sunshine vitamin is a very essential vitamin associated with bone health and calcium metabolism in the human body. Mushrooms are not only an excellent source but also the only vegetarian source for vitamin D. Mushrooms can be exposed to sunlight or UV light to enhance their vitamin D content. Vitamin D is a fat-soluble vitamin. Hence its bioavailability to the body can be increased many folds by consuming sun or UV exposed mushrooms sautéed in ghee or butter.

Mushrooms - The perfect blend with non-vegetarian food to reduce carbon foot print

Livestock agriculture is a major cause of concern for climate change and rapid environmental degradation due to the production of very potent greenhouse gas methane. Globally, 14.5 percent of



Note: As per ICMR (2010), a healthy sodium to potassium ratio in the food should be 1:1.8

Fig 6. Sodium potassium ratio of commonly consumed mushrooms

all human-related greenhouse gas emissions is through livestock sector alone. It is not just the methane from cows or manure that contributes to animal agriculture's carbon footprint, it is the fossil fuels required to ship, process, package, and refrigerate the meat as well. A UN released advice states that "governments should eliminate meat industry subsidies and tax meat production" in order to reduce the global rise in consumption and the environmental damage that goes with it (riseofthevegan.com, Aug 14, 2016, accessed on 15-09-2021). In Europe, the Italian mayor expressed her wish to turn Turin into the first vegan city, encouraging the residents to stop consuming meat and turn to vegetarian and vegan diets. She stated '*The promotion of vegan (milk/egg-free) and vegetarian (meat-free) diets is a fundamental act in safeguarding our environment, the health of our citizens and the welfare of our animals.*' The German Govt. banned meat and meat related products in all its Govt. function since 2017 to set an example and its serious commitment in the fight against the "effects of the consumption of meat" and betterment of environment. (https:// www.riseofthevegan.com accessed on 15-02-2020). Burgers are possibly the most ubiquitous meal globally. It is also the most resource-intensive meal due to presence of beef filling. A simple modification of the same diet blended with mushrooms will not only lead to a healthy diet but also save the environment and its resources and lead towards a more sustainable method of nutrition. USA alone consumes 14 billion burgers annually using 71% of all beef consumption. McDonalds alone purchases 1 billion pounds of beef per year. The production of this huge amount of beef leads to production of 3.64Mt of greenhouse gasses per annum. A small modification in the burger recipe by replacing 30% of beef with mushrooms can lead to a healthier and tastier product which will reduce greenhouse gas emission, water consumption and land requirement by 29% (Reynolds, 2018). Meat consumption can be amicably reduced without losing flavor, texture or taste. Blend of mushrooms with meat not only enhances taste and nutrition but also helps in reduction of saturated fat, sodium and calorie intake and adds additional nutrients like B vitamins, vitamin D, antioxidants and potassium. This mushroom meat blend is gaining lot of importance among dieticians, professional chefs and home cooking (http://www.blenditarian.com).

Table 3. Per cent bioavailable iron from iron fortified oyster mushroom

Age / Sex requirement	*Bioavailable iron requirement (mg/day)	Percent of bioavailable iron available from 10 g Fe fortified Elm oyster mushroom powder (%)
12-16 years, girls	2.02	36
12-16 years, Boys	1.82	40
Adult males		
Pregnant women	1.14	64
Lactating women	1.31	56
Menstruating women	2.38	30
Postmenopausal women	0.96	76

As per Nazanin *et al.* (2014).

Value addition of mushrooms

Iron fortified mushrooms

Iron is one of the most important trace elements required for human health. India is very high on iron malnutrition. Both the quantity and bioavailability of iron from a food source is important towards mitigation of iron malnutrition and mushrooms score very high on both the aspects. Perhaps that is the reason that oyster mushrooms have been given the sobriquet of 'Blood builder' by the Chinese. Due to their highly porous texture, mushrooms are highly amenable for osmo- fortification techniques thereby enhancing targeted nutrition component. ICAR-IIHR is the first institution in the country to have successfully employed this technique and standardize the technology for the production of iron fortification in oyster mushrooms (Patent pending). The Iron enriched mushroom developed at ICAR-IIHR contains 33.8 mg of iron per 100 g dried iron enriched mushroom powder. Thus, consumption of merely 10 g iron enriched mushroom can give 16.09% DV for iron requirement of an adult women and 19.88% DV for adult men. Studies have also been conducted on the bioavailability of iron in animal model system and recipe has been developed for the delivery system for mass nutrition programs so that it can help in mitigating iron malnutrition.

Bioavailability of iron from iron fortified mushroom

One of the major reasons for iron deficiency is low bioavailability of iron especially from vegetarian foods of plant origin. It is reported that the non-heme iron from plant sources has a bioavailability of 5-8%. Hence bioavailability studies were conducted at ICAR-IIHR in collaboration with ICAR-NIANP in animal model to study the bioavailability of iron from iron fortified mushrooms. The study showed the bioavailability of iron from normal non-fortified mushroom was 17.7% as compared to the bioavailability of 21.68% from iron fortified mushroom. Considering the above bioavailability data and iron content of iron fortified mushroom; it can be inferred that consumption of 10 g of iron fortified mushroom powder gives 0.73 mg of bioavailable iron (Nazanin *et al*., 2014). Table 13b shows the percent daily value requirement of bioavailable iron available form iron enriched mushroom.

Delivery system for mass nutrition

Any iron fortification method is incomplete until a proper delivery system is also standardized so that the benefit of the innovation can reach the target population. This innovation of the process of production of iron fortified mushroom was taken to a logical and meaningful conclusion through the standardization of recipe for Arka mushroom fortified rasam powder which is available as licensed technology from ICAR-IIHR. This technology relates to the usage of dry oyster mushroom powder for the production of rasam powder. The same recipe can be used for the delivery of iron by substituting normal mushroom powder with iron fortified oyster mushroom powder.

Enhancing mushroom accessibility through technology and policy interventions

Mushrooms are indoor crops and require semi-permanent to permanent structures depending on the mushroom variety chosen and the local ambient climate of a place. Space and cost of mushroom growing structures are one of the constraints in rural

Fig 7. Arka mushroom rasam powder

and urban areas which makes the accessibility of fresh mushrooms difficult. ICAR-IIHR has developed models and technology which can address these issues.

Mushrooms for terrace / backyard - The Ready to Fruit (RTF) concept:

Although mushrooms are excellent nutrition source for vegetarians and a very delicious way to reduce the intake of non-vegetarian food; yet have not become a part of daily Indian diet. Mushrooms often have been projected as food for God and for the Elite. They have been sold at prices beyond the buying capacity of an average Indian. ICAR- IIHR has been consistently working towards making mushrooms as a part of daily Indian diet of every home so that mushrooms in their own humble way may contribute towards country's nutritional security and mitigation of malnutrition. The 'Ready to Fruit' (RTF) Bag technology was developed to bring mushrooms to rural homes in the villages where there is space, skill and resource constraints. Women were provided with pre-seeded and fully-grown RTF bags to grow mushrooms at home. Each one kg bag could yield 250-300g and if bag opening is done in a planned and staggered way; this technology can make available mushrooms on a daily basis. The RTF bags have not only become popular in resource crunched rural households but also in the urban homes with space constraints.

Mushrooms for Poshan Vatikas

Ministry of Women and Child Development is promoting establishment of "Poshan Vatikas" across the country. The ministry plans to set up Poshan Vatikas across all anganwadi canters with the aim to provide a fresh supply of fruits, vegetables and even medicinal plants, especially in aspirational districts. There is need to ADD MUSHROOMS to these Vatikas to bring about a healthy and diversified nutrition integration. The RTF technology developed by ICAR-IIHR very well fits into these Vatikas where mushrooms can be harvested on daily basis. ICAR-IIHR has also developed suitable low-cost outdoor structures which can be easily installed outdoors in small spaces.

Mushroom technology in MANREGA

Mushroom growing is labor intensive and for a country like India where unemployment is rampant, mushroom growing can create jobs both in semi-urban and rural areas (Martinez-carrera, D. *et al*., 1998). Some technologies can utilize family labor, thus providing employment to all members of the family. The labor of out of school youths and women can be effectively utilized. Mushroom growing can be taken up by exclusive women groups too as the activities are mostly indoors **(**Pandey and Veena, 2003). Mushroom cultivation can be integrated in many of the rural upliftment policies of the Government of India. Like Mahatma Gandhi National Rural Employment Guarantee Scheme (MGNREGA), Mid-day meals and Aanganwadi. The unemployed rural youth, landless and women groups can be engaged in a centralized mushroom growing farm at Panchayat levels. Fresh mushrooms can be sold both in rural and through linkages in the urban areas. Excess production can be easily sundried to make it enriched with vitamin D and the dried mushroom can be powdered and stored for long periods. Such powders can be utilized to produce mushroom value-added products like Arka Mushroom rasam powder, Arka mushroom chutney powders, Mushroom millet biscuits, Mushroom based health drinks etc. which can open novel avenues for rural agro-premiership especially among women. Mushrooms can be integrated in Mid-day meal schemes of rural schools. This integration will have twofold benefit. Firstly, providing nutritious vegetable to the rural poor and secondly creating employment for many who will take up the responsibility of making the mushrooms available in the villages. The agro residues produced at the village level can be utilized by women to grow mushrooms. As spin off, women and unemployed rural youth get employment, better nutrition for the village school and entrepreneurship through mushroom value-added products. The spent mushroom substrate after harvest can be composted to make quality enriched organic manure for the field in the village.

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Review

Diversified farming systems for changing climate and consumerism

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ABSTRACT

When we think about environmental degradation and climate change, the first things that come to our mind are cars and factories, but not how we grow, consume and dispose of. If we collectively put these things together, we account for nearly one-third of the human-induced climate change. That certainly put agriculture at the crossroads of system-level transformation towards healthy food, people, and the planet. Such food-system transformation requires a paradigm shift towards nature-friendly nutrition-rich diverse fruits and vegetables, and it should constitute at least 30-50% of our food plate from the current average of less than 10%. The only way left for us is to return to an ecological intensification with feedback loops that inter-links vital elements of complex agroecological transition within the planetary boundary limits. Farming in harmony with nature, carbon-neutral, enrich micro-hydrology and restore biodiversity to co-benefits the people and the planet. The context-specific regenerative agriculture practices that are ecologically sustainable and economically viable found to be best fit models for smallholder farmers and home gardeners. It is based on the sound ecological philosophy of production follows structure, composition and functions, and where ecosystem services and wellbeing become default returns on their own. The recent advances in digital augmentation with ICTs enabled citizen science to provide powerful tools to aid the integration of frontier technology with indigenous knowledge. This leads to an ideal agroecosystem integrated with diverse crops, multi-purpose tree species, animals, and peoples in collective action to restore broken food systems and combat climate change.

INTRODUCTION

The ever-increasing human population and increased demand for food and industrial needs have put tremendous pressure on natural resources and human capital. Agriculture is still a dominant sector in every aspect of sustainable development with a limited natural resource base and faces severe environmental constraints that are likely to worsen due to climate change. There is a definite need for an integrated approach for managing the world's agricultural resources to sustain productivity while safeguarding environmental flows. The ambitious conservation efforts and food system transformation are central to conserving terrestrial biodiversity (Leclere et al., 2020). A better farming system is required to bend the curve by combining production, consumption, conservation and restoration with significant co-benefits to the people, culture and nature. The farming systems data and information are critical factors to understanding current trends and status of agricultural resources and finding optimal approaches to achieve reduced vulnerability and sustainable intensification in developing countries that depend upon dryland production systems for food security and livelihoods. Therefore, an open-access policy to geospatial information, technology, and fair knowledge sharing and management mechanisms are becoming an integral part of the food security equation. Geospatial technology (remote sensing, global positioning system, and geographical information system) has progressed rapidly in the 21st century. It will keep expanding its role in almost every aspect of food security, including research, programs, policies outreach.

Enormous efforts are underway throughout the world to gather data and information on crops, rangeland, livestock, and other related agricultural resources and their production mechanisms. However, these are collected at very coarse resolution in many instances, ranging from several hundred meters to tens of kilometres. Such information or data are often used in global and regional scale models to assess status or trends at the landscape level or even larger units. However, at these scales, such data may fail to reflect ground realities that are often very different from information or data collected at larger scales and, therefore, fail to capture the agroecosystems' complex nature. This is particularly prominent in the developing world, where small landholdings and production systems are highly diverse and complex. Complexity is associated with many factors, including environmental conditions, landscape structure, functions, soil health, water availability, topography, localized weather events, poverty distribution, infrastructure, migration, local policies on land tenure, market access, and conflicts. Such an array of contextual conditions push for an integrated system approach to manage more productive, stable, and environmentally sound resilient agriculture production. They provide a science-based circular approach for addressing complex and interactive sets of increasingly regenerative practices for functional food systems (Fig. 1). Implementation and intervention of new management paradigms to ensure food security and improved livelihoods require better information in space and time. Therefore, data-driven digital augmentation is vital in transforming agri-food systems for better resilience under changing climate, diet, and demography.

Food for climate action

We often associate the root causes of climate change with factories, airplanes, traffic-congested roads, and deforestation. But equally important is the food we eat and how we grow it. The enormous challenge and the vast opportunities for action on climate change have distinguished the issue as the most pressing topic of reforms. The first step towards climate action is how we reshape our mindsets and way of living with less and healthy food habits. The environmental impact of unsustainable agricultural practices varies from how we produce food to how we consume and dispose of it. When we examine the global diet in the last three decades, we can see drastic trends and how we have become dependent on a few staple crops (primarily cereals) and industrially farmed meats. This results in a significant and negative impact on the climate and our ability to grow food now and in the future, water

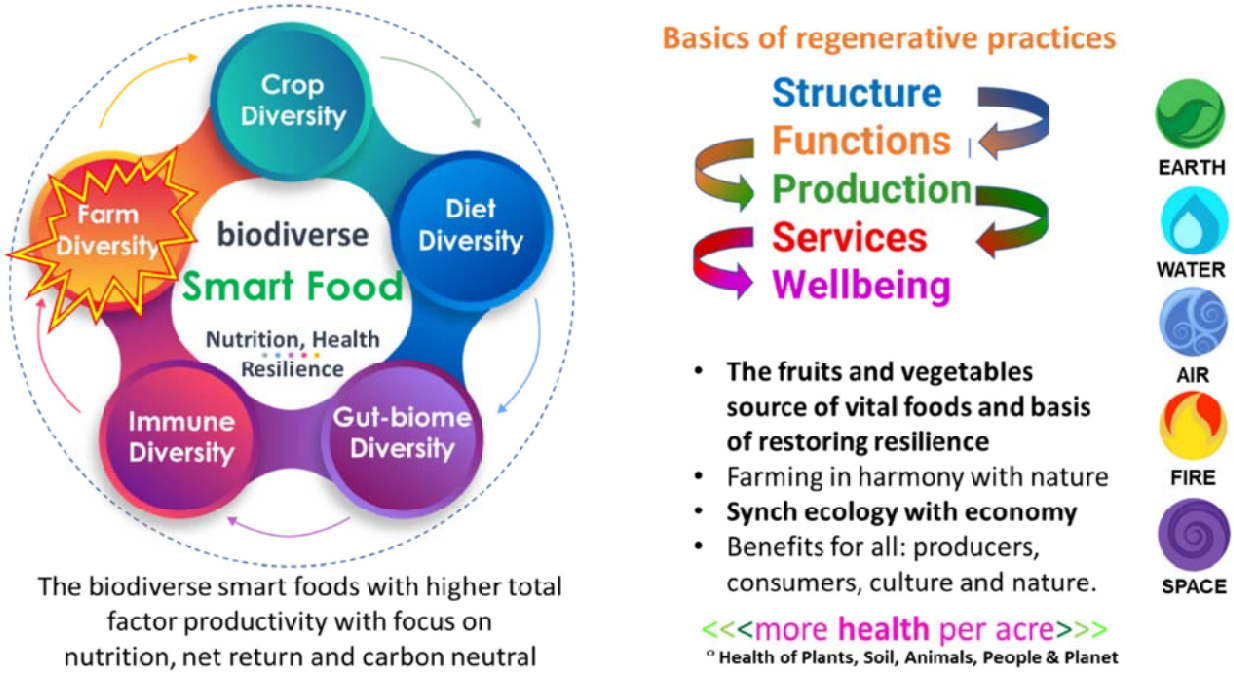

Fig. 1. Functional food systems for restoring nutrition and climate resilience.

availability, global nutrition, and overall planetary health. There is a pressing need to return our increasingly homogenous agriculture into a highly diversified agroecosystems to ensure the health of the population, our world, and its ecosystems.

An integrated agro-ecosystems

The dynamics of agricultural production is driven by environmental factors such as drastic fluctuation in temperature, erratic precipitation tends, and water supply is often the most limiting factor for profitable agricultural production. They are characterized by persistent water scarcity, extreme climatic variability, high susceptibility to land degradation, desertification, and loss of natural resources, including biodiversity, at elevated rates. However, we often fail to understand and/or hardly emphasize to understand and quantify the underlying factors and drivers responsible for building the resilience 'agroecosystems' as shock observers to withstand the changes. Refining conventional yield centric production into climate resilient agroecosystems requires systematic combinations of plant, trees and animal species and management practices to specific agroecological zones. Pursuing sustainable livelihood goals is based on several factors, including crops, climate, soils, markets, capital, trade, and tradition. It demands an integrated approach with an ecologically sound functional production system to improve livelihoods. Therefore, the international and national initiatives emphasize diversified agroecosystems through strategic research, outreach, and enabling policies to achieve sustainable development goals.

The core value of the innovative systems is their synergies to enhance the adoption rate of technologies and management practices through more significant interaction of stakeholders and researchers. While it also needs to emphasize reducing vulnerability. The conventional intensification of agriculture with a mere focus on yield alone is prone to high risk and exposure due to several associated factors such as climate, soils, lack of capital, poorly developed markets, demographic challenges, and ever-increasing pressure on the natural resources. These systems are characterized by intricate combinations and high diversity in agronomic practices, cropping patterns and intensity, water use, rangeland, trees, livestock, fish, landholding size, etc. Because of their complexity and diversity, it is necessary to characterize these systems at very high spatial resolutions to understand the risk and vulnerability factors. The contextual mapping presents emerging and future land use trends will better allow researchers and decision-makers to diagnose vulnerabilities and intervene to improve livelihoods by taking into account such factors as land cover dynamics, cropping pattern and intensities, water use and availability, changing demographics, infrastructure, poverty, markets, climate change, etc. The information generated in the preliminary analysis help to assess vulnerable areas for possible pathways to increased resilience to and mitigation of risks, whether it could be biophysical (e.g., land degradation and drought), ecological (e.g., dwindling agrobiodiversity), or socioeconomic (e.g., price shocks or policy changes in land tenure).

An ideal agroecosystem is an inclusively functioning system integrated with diverse crops, multi-purpose tree species, and bio-pulverizing livestock to produce vital food, real-forage, natural fiber, simultaneously preserving the soil health and restoring ecosystem functions. This collective action of the feedback mechanisms leads to restoring planetary health and combating climate change. To successfully implement such an approach in the climate-vulnerable agri-food systems, one must first identify suitable areas for ecologically sound agricultural production and consumerism. These domains are an essential entry point for any sustainable developmental goals, whether they are choice of crops (legumes in cereals), varieties (short duration), diversification (ration and intercropping), or efficient feedback loops (use of residuals)

Increasing system research with evolving digital technology has opened the room to reverse the global trend in homogenous cereal cropping. Incorporating agroecosystem research with in-the-field citizen science and new technology creates an ideal environment for integrating local knowledge with innovative solutions and testing the formula. Once proven, the science makes sustainable production and consumption more viable and scalable than before because it considers modern variations in settings due to prevailing conditions. The data-driven analytics created tremendous opportunities to address the gaps at multiple levels such as data, yield, nutrition, ecology, economy, and resilience for demand-driven ecological interventions across scales such as space, time, and the package of sustainable land management

practices (Figure 2). Our ongoing efforts in demand-driven interventions aim at accelerating these innovative and sustainable agroecosystems (Figure2) and food gardens (Figure 3) by modelling them for possible areas and then targeting specific sites with the appropriate interventions. They have proven an argument for scaling these interventions into mainstream production and consumption by successfully testing them. The prospects and potential role of inclusive agroecosystems in combating climate change, supported by paradigm shifts in diet patterns and lifestyle, are clearly defined.

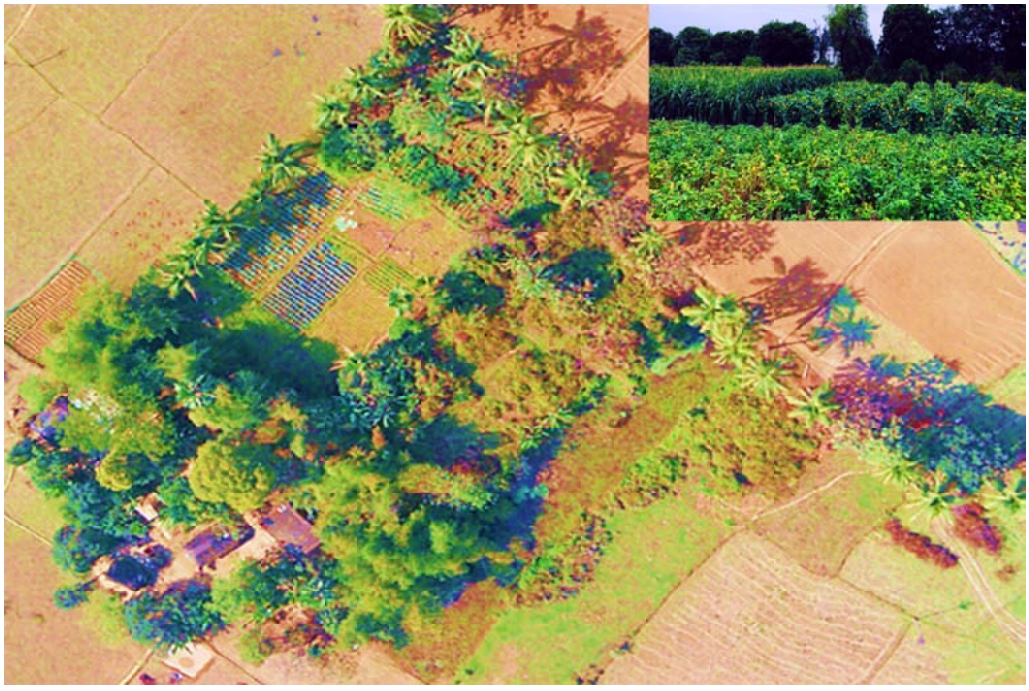

Fig. 2. An example of diversified family farming growing variety of fruits, vegetables, spices and condiments. (Aerial and inlet photos: C. Biradar)

Fig. 3. Green Gold Food Forest model for home gardens with rich in diversity of the fruits, vegetables and herbs growing in 40 x 60 feet space. (Photo. C. Biradar)

Transforming food systems

The current food systems are upsetting the ecological balances, and myriad externalities expose the system's vulnerabilities. Be it the considerable nutrition gap between our present diet consumption and a scientifically balanced diet that is planet-friendly falls within the planetary boundary as proposed by an EAT-Lancet report. However, the unfortunate erosion of tens of thousands of species culminated in the hegemony of a handful of staple food crops that dominate our calories. This calls for going back to farming with stacking functions and multi-later agriculture with trees, crops and livestock.

Food well preserved with chemicals, clean looking, travelled over thousands of miles, often does not fit into the category of healthy and sustainable food. Most modern food travels over a mile, often harvested much before its maturity, sprayed with toxic chemicals to increase its self-life in the supermarket shelves or factory bins. Over 80% of its vital nutrition is lost in several ways and processing starting from its grown, harvested, transported, preceded, cocked, served, freezes, heated, reheated. Most vital nutrition lost in the processing to consumption chain end up in eating junk food. Little vital nutrition left in the food, which demands more consumption than needed, leads to 'belly total hungry cells. We eat more food but feel hungry always because body cells not getting the healthy nutrition they need. Ideal food for a healthy body and cells must constitute freshly harvested food grown in healthy soils. Many people don't know what actual organic food tastes are. It just tastes the vegetables we used to eat 20-30 years ago to what we get now. Ask village elders they will tell no taste and flavour in the Vegetables these days because all these vegetables are grown in dead soil with tons of chemicals. We need eight carrots to have the same nutrition we obtained from one carrot 30 years ago. This applies to almost all food we grow these days. 80 population in chronic health issues which is mainly attributed to the food we eat and the lifestyle. Our culture is agriculture; indeed, the culture of humanity is agriculture. Still, it has the most vital lever to fix most of the problems humanity, the wild and the environment facing now.

An approach aimed at integrating an array of species diversity in reviving lost agrobiodiversity, integrated cultivation practices, and diverse dietary habits as building blocks of sustainable, resilient, and resource-efficient food systems. It puts forth the need for a crucial paradigm shift from mono-cropping to integrated resource-efficient agri-food systems and from more calories per acre to more nutrients (health) per acre (Fig. 4). It is only possible to build resilience and incorporate sustainability by restoring healthy food systems and rebuilding the living soil via a diversified cropping system with various plant species in the smart family agriculture. The production follows functions and needs to leverage technology and local intelligence to rebuild functional agri-food systems for a sustainable future and planetary health.

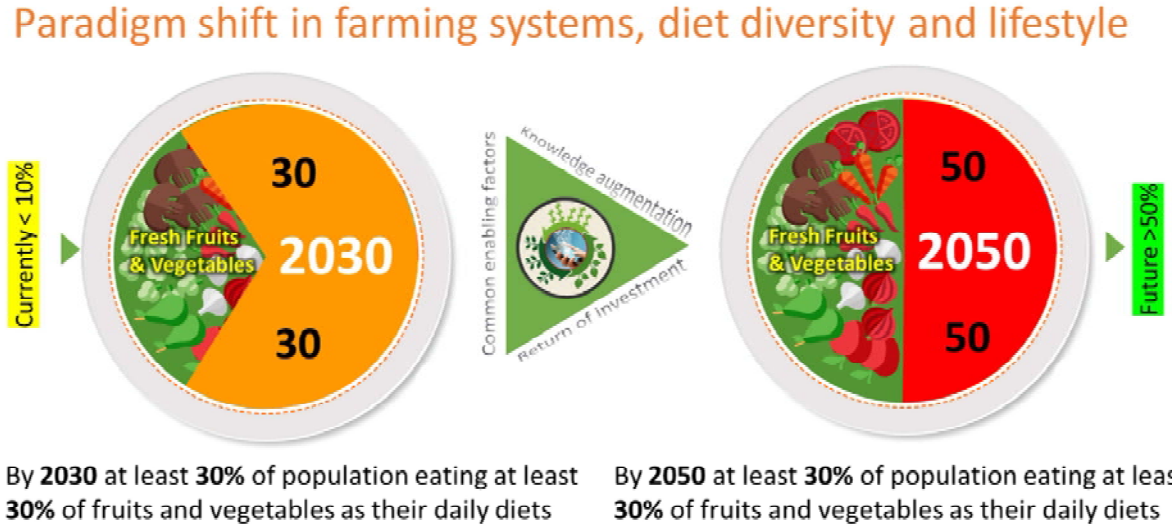

Fig. 4. The vision 30:30:30 and 50:50:50 is all about increasing the production and consumption of fruits and vegetables from mere less than 10% at present to 30% and 50% by the year 2030 and 2050, respectively.

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Original Research Paper

Characterization, inheritance of male sterility and development of male sterile and maintainer lines in ridge gourd (*Luffa acutangula* (Roxb.) L.)

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ABSTRACT

Two male sterile mutants IIHRRG-12MS (long fruited) and IIHRRG-28MS (medium long fruited) were identified from the ridge gourd germplasm IIHR-12 and IIHR-28 respectively at ICAR-IIHR, Bengaluru. These two male-sterile (*ms*) sources were characterized by the production of rudimentary male flowers in the racemes in contrast to the bright yellow flowers with fertile pollen and healthy anthers in male fertile, monoecious plants. Using these *ms* lines the inheritance of male sterility was worked out, which is cytoplasmic genic male sterility (CGMS) type, with single dominant gene either in homozygous or heterozygous condition restoring male fertility in the presence of sterile cytoplasm. In order to develop F_1 hybrids using male sterility, several male sterile and maintainer lines were developed in different genetic back grounds such as green/dark green fruit colour and short/medium long/long fruit length.

Keywords: CGMS, gene action, inheritance, maintainer lines, male sterility and ridge gourd

INTRODUCTION

Ridge gourd (*Luffa acutangular* (Roxb.)L.) is an important cucurbitaceous vegetable crop grown in tropical and subtropical countries, especially in Asia and India (Jansen *et al*., 1993). It is a crop grown for immature fruit rich in dietary fibre and minerals (Sheshadri, 1990). In addition to culinary properties, it has numerous medicinal properties which traditionally used for the treatment of stomach ailments and fever (Burkill, 1985; Chakravarty,1990).

Though cultivars of ridge gourd are monoecious, diverse sex forms were reported *viz.,* androecious, gynoecious, gynomonoecious, andromonoecious and hermaphrodite types (Choudhary and Thakur, 1965). The female flowers are solitary whereas male flowers are in racemes. Principally 2 genes are involved in production of various sex forms (Richaria, 1948). Male sterility is of practical importance in vegetable breeding as it facilitates F_1 hybrid seed production without hand pollination. Male sterility in ridge gourd was first reported from India by Deshpande *et al*. (1979) and then by Pradeepkumar *et al*. (2007). Male sterility is governed by single recessive nuclear gene in water melon (Hexun *et al*., 1998; Ping *et al*., 2010); musk melon (Dhatt and Gill, 2000; Park *et al*., 2009), cucumber (Zhang *et al*., 1994) and for the first time, cytoplasmic male sterility (CMS) with two dominant restorer genes has been reported in ridge gourd by Pradeepkumar *et al*. (2012). At ICAR-IIHR, Bengaluru also male sterile mutants were identified in ridge gourd germplasm (Varalakshmi and Deepak, 2017).

Present study was conducted to characterize that male sterility observed, to work out the genetics of its inheritance and to develop male sterile and maintainer lines in different genetic backgrounds of ridge gourd.

MATERIALS AND METHODS

The work was undertaken in the experimental field of Division of Vegetable crops, ICAR-IIHR, Bengaluru. Initially two male sterile mutant plants viz.,IIHRRG-12MS and IIHRRG-28MS in different genetic backgrounds have been identified during kharif, 2015-16 and maintained in the division ever since. Morphological characters of these *male sterile* mutants were recorded *viz*., days for emergence of first fertile male flower, days for emergence of ûrst female flower, node at which first fertile male flower appeared, node at which first female flower appeared, male bud length

and pollen fertility (%). Pollen fertility percentage was assessed from ten randomly selected male ûower buds in each line at anthesis on the basis of stainability in acetocarmine and the counts were taken from ten fields under microscope for each flower bud. Well filled, uniformly and darkly stained pollen grains were considered as fertile and the rest as sterile. Simultaneously, these *ms* plants were crossed with 22 monoecious lines *viz*.,IIHR-1, IIHR-7, IIHR-10-2, IIHR-11, IIHR-12, IIHR-17-2-1-6, IIHR -19, IIHR-23, IIHR-26, IIHR-27, IIHR-29, IIHR-31, IIHR-34, IIHR-35, IIHR-39, IIHR-40, IIHR-41, IIHR-43, IIHR-46, IIHR-47, IIHR-49 and IIHR-72-2 to study the inheritance of male sterility and fertility restoration in ridge gourd during kharif season of 2015-16. All the 22 F_1 hybrids and parental lines were grown with recommended package of practices during Rabi-summer season of 2016-17. Observations pertaining to male and female fertility were recorded from 15 plants in each line/hybrid. Among the 22 hybrids, 10 fertile hybrids (IIHRRG-28MS x IIHR-10-2, IIHRRG-28MS × IIHR-72-2, IIHRRG-12MS × IIHR -17-2-1-6, IIHRRG-12MS x IIHR-1, IIHRRG-12MS x IIHR-12, IIHRRG-12MS x IIHR-40, IIHRRG-12MSx IIHR-41, IIHRRG-12MS x IIHR-43, IIHRRG-12MS x IIHR-47 and IIHRRG-12MS x IIHR-49) were selfed to generate F_2 population as well as back crossed with respective male parent to produce BC_1 generation. Five hybrids were male sterile *viz*.,IIHRRG-12MSxIIHR-19, IIHRRG-12MSxIIHR-27, IIHRRG-12MSxIIHR-31, IIHRRG-12MSx IIHR-34 and IIHRRG-12MSxIIHR-39. Remaining seven hybrids were not uniform with respect to fertility (IIHRRG-12MSxIIHR-7, IIHRRG-12MSxIIHR-11, IIHRRG-12MSxIIHR-23, IIHRRG-12MSxIIHR-26, IIHRRG-12MSxIIHR-29, IIHRRG-12MSxIIHR-35 and IIHRRG-12MSxIIHR-46) and were not considered further in the study. F_2 population (200 plants), BC_1 generation (50 plants) were raised during the kharif season, 2017-18 and evaluated for male sterility and restoration of fertility. Chi-square (χ^2) goodness-of-fit analysis (Russell, 1996) was conducted for segregation of male fertility and sterility in F_2 populations of two crosses viz., IIHRRG-12*ms*x IIHR-17-2-1-6 and IIHRRG-28*ms*x IIHR-72-2.

In order to transfer the male sterility in to different genetic backgrounds, crosses were made between male sterile lines and ten different advanced breeding lines with different genetic backgrounds to convert them into *ms* lines as well as maintainer lines viz., IIHR-6-2(long, green), IIHR-5-1-2 (Medium long, green), IIHR-37-4-1, IIHR-23-5-4, IIHR-34-2-2, IIHR-49-3-1, IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 and IIHR-11-1-2. Male sterile progeny was repeatedly backcrossed with the male parents (maintainer lines) for six generations to develop the male sterile (A line) and maintainer lines (B line).

RESULTS AND DISCUSSION

Characterization of male sterility in ridge gourd

Male sterility is defined as failure of plant to produce the functional anthers, pollen or male gametes. At ICAR-IIHR, two male sterile mutants were identified in IIHRRG-12 (long fruited) and IIHRRG-28 (medium long fruited) germplasm lines. These two *ms* sources viz., IIHRRG-12MS and IIHRRG-28MS were characterized by the production of rudimentary male flowers in the racemes in contrast to the bright yellow flowers with fertile pollen and healthy anthers in male fertile, monoecious plants (Fig.1 and Fig. 2). Rudimentary male buds remained unopened and fell down 12–16 days after the emergence. Similar

Rudimentary male flowers | Fertile male flowers | Sterile and Fertile pollen

Fig. 1. Characterization of male sterility in Ridge gourd

Fig. 2. Male flower production in monoecious line (left) and absence of male flowers in male sterile line (right)

characteristics of the male sterile line were reported by Pradeepkumar *et al.* (2010) in ridge gourd.

Expression of male sterility and restoration of fertility in F_1 hybrids

Hybrids have expressed different fertility status, viz., complete sterile, complete fertile and some hybrids with both fertile and sterile plants. If male sterility was controlled by dominant gene, which was a rare phenomenon in cucurbits, all the hybrids should have expressed complete sterility in F_1 generation, then as all the individuals carrying *Ms* allele are sterile and do not produce progenies as pollen parents. If it is controlled by recessive nuclear gene as in musk melon (Park *et al*., 2009), water melon (Ping *et al*., 2010), squash (Carle, 1997) and cucumber (Zhang *et al*., 1994), then F_1 should have segregated into 1:1 fertile and sterile plants based on the homozygosity/ heterozygosity of the locus controlling the sterility. But here in this case, male sterility expression of F_1 hybrids indicates the role of CMS genes. CMS is maternally inherited and is associated with a specific (mitochondrial) gene whose expression impairs the production of viable pollen without otherwise affecting the plant (Kempken and Pring, 1999; Budar and Pelletier, 2001). Premature degeneration of the tapetum at the early to mid uni-nucleate microspore stage leads to the development of non-viable pollen (Roberts *et al*., 1995). General theory about the phenotype of CMS plants which usually appear normal, vigorous, and undistinguishable from the fertile analogue (Hanson and Conde, 1985) proved true in the present study also.

There are nuclear genes that can restore fertility, termed nuclear restorer (Rf) or fertility restorer (Fr) genes, which are specific for each studied CMS system (Popova *et al*., 2007). The restorer of fertility (Rf) genes in the nucleus function to suppress the CMS phenotype and restore the male fertility. Dominant nuclear fertility restorer gene in 'IIHR-1, IIHR-10-2, IIHR-12, IIHR -17-2-1-6, IIHR-40, IIHR-41, IIHR-43, IIHR-47, IIHR-49, IIHR-72-2' out of 22 genotypes is responsible for regaining male fertility of hybrids with *ms* mutant line. All these ten lines could be possible restorer lines.

Seven other crosses (IIHRRG-12MSxIIHR-7, IIHRRG-12MSxIIHR-11, IIHRRG-12MSxIIHR-23, IIHRRG-12MSxIIHR-26, IIHRRG-12MSxIIHR-29, IIHRRG-12MSxIIHR-35 and IIHRRG-12MSxIIHR-46) had both male sterile and male fertile plants in different ratios indicating that the fertility restorer genes might be in heterozygous condition in these inbred lines which can be used to develop either maintainer lines or restorer lines after progeny evaluation and back crossing.

Five hybrids viz.,IIHRRG-12MSxIIHR-19, IIHRRG-12MSxIIHR-27,IIHRRG-12MSxIIHR-31, IIHRRG-12MSxIIHR-34 and IIHRRG-12MSxIIHR-39 were male sterile indicating the maintenance of sterility and these advanced breeding lines could be possible maintainer lines. Though the five male parents exhibited high pollen fertility (52-83%), they failed to transmit this character to F_1 hybrids indicating the cytoplasmic inheritance of male sterility in ridge gourd. The average bud length of male buds of male sterile hybrids at full development stage was found to be 0.6±0.01cm which was significantly different from the average bud length of male fertile parents (1.7± 0.05cm) (Supplementary Data Table S1). These rudimentary male buds in racemes of male sterile hybrids remained unopened and fell down 12–16 days after the emergence. The anther lobes were undeveloped and pollen grains were small, shrunken and poorly stained in these hybrids throughout the crop growth indicating a stable sterility mechanism. Male fertile hybrids had high mean pollen fertility (47±6.57%) throughout the crop growth.

In the male sterile hybrids node for the first female flower was earlier (9.6th node) compared to the male fertile hybrids (10.2nd node) and also the days taken for the emergence of first female flower is less in male

sterile hybrids (41.2 days) compared to male fertile hybrids (43.4days) (Supplementary data Table S2). Similarly mean female bud length was more (94.8 cm) in male sterile hybrids than male fertile hybrids (4.6cm) and also the fruit length was more in sterile hybrids (24.8cm) than in fertile hybrids (20.2cm)

Analysis of F_2 population from the crosses, IIHRRG-12MSxIIHR-17-2-1-6 and IIHRRG - 28MSx IIHR-72-2 for male sterility and restoration of fertility:

Out of the 239 F_2 plants of the cross IIHRRG-12MS x IIHR-17-2-1-6, 182 were male fertile and 57 were male sterile till the end of the season. There were observable differences between the male sterile and male fertile plants with respect to male flower production though female flowers in both types were similar. Node for the first fertile male flower ranged from 2-14th node with the mean of 4.92 and the days taken for the first male flower ranged from 29-51 days with a mean of 42.08 days. Average male flower bud length was less in male sterile plants (0.61cm) compared to the male fertile plants (1.89 cm) (Supplementary data able S3). Mean pollen fertility of these male fertile plants was 24.95% as against zero fertility of male sterile plants. With respect to female flower traits, there were slight differences between male sterile and male fertile plants. Node for first female flower was earlier in sterile plants (9.4) compared to male fertile plants (10.18), similarly even the number of days taken for first female flower appearance was less in male sterile plants (43.3 days) compared to male fertile plants (45.99). However, the average female flower bud length and fruit length were almost same in both male sterile and male fertile plants.

In another F_2 population of the cross, IIHRRG-28MSx IIHR-72-2, out of 235 F_2 plants, 175 were male fertile and 60 were male sterile. In this cross also there were differences between male sterile as well as male fertile plants with respect to male flower production. Node for the first fertile male flower ranged from 2-8th node with the mean of 4.21 and the days taken for the first male flower ranged from 39-55 days with a mean of 42.84 days. Average male flower bud length was less in male sterile plants (0.63cm) compared to the male fertile plants (1.85 cm). Mean pollen fertility of these male fertile plants was 7.56% as against zero fertility of male sterile plants. With respect to female flower traits, there were slight differences between male sterile and male fertile plants. Node for first female flower was earlier in sterile plants (8.52) compared to male fertile plants (9.82), similarly even the number of days taken for first female flower appearance was less in male sterile plants (42.8 days) compared to male fertile plants (44.38)(Supplementary data Table S3). However, the average female flower bud length and fruit length were almost same in both male sterile and male fertile plants.

All the F_1 plants of these two *ms* x *mf* crosses and their corresponding back cross populations were male fertile. As the F_2 population segregated into two classes in both the crosses, monohybrid ratio, 3:1 was tested for significance using chi-*square* test. The chi-square value for the 3:1 (fertile: sterile) single dominant gene action exhibited a good fit to the expected ratio (80-90% probability) (Table 1 and 2). The F_2 data indicated the presence of cytoplasmic genic male sterility (CGMS) in ridge gourd with single dominant gene restoring male fertility in the presence of sterile cytoplasm. However, Pradeepkumar *et al*. (2012) earlier reported that two dominant fertility restorer genes are responsible for restoration of fertility in the presence of sterile cytoplasm in ridge gourd using Arka Sumeet variety as restorer line. This could be due to different genetic makeup of different male sterile and restorer lines used in these studies.

Assuming that MS line is having genotype, rf1rf1 and sterile cytoplasm (S) and male parent, IIHR-17-2-1-6/IIHR-72-2 possesses a genotype Rf1Rf1 carrying a fertility restorer gene in homozygous dominant state and normal fertile cytoplasm (N), F_1 will be male fertile as the genotype of F_1 is SRf1rf1. Though F_1 is inheriting a sterile cytoplasm from male sterile female parent, presence of a dominant fertility restorer gene, viz., Rf1 restores the fertility of F_1 (Table 3). In F_2 presence of single dominant fertility restorer gene in either homozygous or heterozygous condition ensures male fertility. The gene action governing male sterility can be explained with the following model.

Evaluation of back crosses made between fertile hybrids with restorers during summer

Three male fertile hybrids were back crossed with restorer lines and all these back cross progenies were male fertile indicating the restoration of male fertility in these lines (restorer lines) (Table 4).

Table 1. Segregation of male sterile and male fertile plants in F_1, Back cross and F_2 generation of the crosses, IIHRRG-28MSx IIHR-72-2 and IIHRRG-12MSxIIHR-17-2-1-6

Cross	F_1's		Back cross		F_2's	
	Fertile	Sterile	Fertile	Sterile	Fertile	Sterile
(IIHRRG-12MSxIIHR-17-2-1-6)	15	0	44	0	182	57
(IIHRRG-28MSxIIHR-72-2)	15	0	37	0	175	60

Table 2. Chi-square test for F_2 population segregating for male sterility and male fertility in ridge gourd

Cross	Genotype	F_2's (3:1)	
		Fertile	Sterile
(IIHRRG-12MSxIIHR-17-2-1-6) F_2 population	Expected	179	60
	Observed	182	57
	Difference	**3**	**-3**
	Chi Square value	**0.169**	
	Probability	**50-70%**	
		F2's (3:1)	
		Fertile	**Sterile**
(IIHRRG-28MSxIIHR-72-2) F_2 population	Expected	176	59
	Observed	175	60
	Difference	**-1**	**1**
	Chi Square value	**0.035**	
	Probability	**80-90%**	

Table 3. Proposed genetic model for Single dominant gene action in ridge gourd

Parents	Male sterile line IIHRRG-12MS/ IIHRRG-28MS	Male fertile line IIHR-17-2-1-6/ IIHR-72-2
	S(rfrf)	**N(RfRf)**
Gametes	S(rf)	N(Rf)
F_1	Male fertile S(Rfrf)	
Gametes	Rf, rf	
Eggs/pollen	Rf	Rf
Rf	SRfRf Male fertile	SRfrfMale fertile
rf	SRfrf Male fertile	SrfrfMale Sterile

BC_1 generation of the cross (IIHRRG-28MS × IIHR-72-2) x IIHR-72-2 exhibited increased male fertility compared to F_1 (IIHRRG-28MS × IIHR-72-2). All three BC_1-populations took little more days to male flower production (45-46) and wide variation was observed among the back cross populations with respect to the node for the first female flower appearance (4-26th node) and days taken for the emergence of first female flower (34-65 days) (Table 5). BC populations exhibited pollen fertility in the range of 40-78%. Wide variation was observed for average female bud length (4-6 cm) and fruit length (20-25cm) among the three back cross populations.

Table 4. Evaluation of back crosses made between fertile hybrids and fertility restorers - male flower characters

Male fertile back cross	Node at first fertile male flower		Days for the emergence of first fertile male flower		Average male bud length		Pollen fertility %	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
(IIHRRG-28MS × IIHR-10-2) × IIHR-10-2	2-7	4	39-48	42	1.0-2.6	1.85	19-63	40
(IIHRRG-28MS × IIHR-72-2) × IIHR-72-2	3-12	5	37-45	42	1.1-2.6	1.91	47-100	74
(IIHRRG-12MS × IIHR -17-2-1-6) × IIHR -17-2-1-6	3-16	5	40-48	43	1.0-2.6	1.80	19-88	58
Mean		1.0		0.8		0.1		16.8
SEm±		0.6		0.5		0.0		9.7

Development of *ms* lines (A lines) and maintainer lines (B lines) in different genetic back grounds

The identified cytoplasmic male sterility (*cms* trait) has been transferred to different genetic backgrounds, by crossing ten different advanced breeding lines with different genetic backgrounds viz., IIHR-6-2 (long, green), IIHR-5-1-2 (Medium long, green), IIHR-37-4-1 (short, green) IIHR-23-5-4 (medium, green), IIHR-34-2-2, IIHR-49-3-1(medium, green), IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 (long, dark green) and IIHR-11-1-2 with male sterile line (IIHRRG-28MS/ IIHRRG-12MS maintained through sib mating with maintainer line, IIHRRG-28/IIHRRG-12) to convert them into *ms* lines. All these F_1 populations were male sterile due to cytoplasmic inheritance of male sterility in the identified source. These F_1's were repeatedly back crossed with their respective male parents/ maintainer lines for six generations continuously. The back cross population plants which were having similar fruit attributes of maintainer lines in each generation were selected and back crossed with the maintainer line. In each generation the back cross populations were checked for maintenance of sterility and found that all were maintaining sterility in 100% population. Thus, by BC_6 generation, all these ten populations viz.,IIHR-6-2MS, IIHR-5-1-2MS, IIHR-37-4-1MS, IIHR-23-5-4MS, IIHR-34-2-2MS, IIHR-

Table 5. Evaluation of back crosses made between fertile hybrids and fertility restorers - female flower characters

Male fertile back cross	Node at first fertile flower		Days for the emergence of first female flower		Average female bud length (cm)		Average fruit length (cm)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
(IIHRRG-28MS x IIHR-10-2) × IIHR-10-2	4-25	12.1	34-62	46	5-7.5	6	16.5-30	25
(IIHRRG-28MS × IIHR-72-2) x IIHR-72-2	5-15	9.5	34-62	45	5-7.5	6	12.5-30	20
(IIHRRG-12MS × IIHR -17-2-1-6) × IIHR -17-2-1-6	4-26	24.0	34-65	45	4-5.5	4	12-28	20
Mean		15.2		45.1		5.7		21.8
SEm±		4.5		0.3		0.6		1.7

49-3-1MS, IIHR-22-4-2MS, IIHR-26-4-2MS, IIHR-70-1MS and IIHR-11-1-2MS were perfectly male sterile resembling the respective maintainer lines morphologically in different genetic back grounds such as green, dark green, long, medium long, short fruit back grounds (Fig. 3). Thus, these ten maintainer lines IIHR-6-2, IIHR-5-1-2, IIHR-37-4-1, IIHR-23-5-4, IIHR-34-2-2, IIHR-49-3-1, IIHR-22-4-2, IIHR-26-4-2, IIHR-70-1 and IIHR-11-1-2 proved to possess fertility restorer gene (Rf) in homozygous recessive condition making them as ideal maintainer lines (Pradeepkumar *et al.*, 2018). These 10 sets of male sterile (A lines) as well as maintainer lines (B lines) in different genetic backgrounds (Fig 3) are now ready for the development of hybrids using fertility restorer lines (C lines). This study confirms the presence of CGMS system in ridge gourd paving way for commercial hybrid seed production in this crop as reported by Pradeepkumar *et al.*, (2018), who for the first time developed CGMS system in ridge gourd by developing MS LA 101 and LA 101, male sterile (A line) and maintainer line (B line) respectively.

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IIHR-70-1 (long, dark green)

IIHR-6-2 (long, green)

IIHR-49-3-1(medium, green)

IIHR-37-4-1 (short, green)

Fig. 3. Fruits of male sterile and maintainer lines in different genetic backgrounds (long/medium/short fruit length and dark green/green fruit color) in ridge gourd

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Original Research Paper

Ploidy analysis among Citrus mutants using leaf meristematic tissue

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ABSTRACT

A promising method for preparing metaphase spread for counting the number of chromosomes from the emerging shoot tissue is described in this report. In the present study, we adopted enzymatic digestion of shoot tips to analyse the chromosome number. The chromosomes in metaphase stage of cell division are highly condensed and easy to count in routine cytological technique. Even the morphological features like position of centromere can be seen in metaphase. In prophase it may not be clear as the chromosomes are getting ready for cell division. In enzymatic digestion even the prophase chromosomes are visible, which can be counted. Hence enzymatic digestion technique is more efficient in citrus as compared to acid digestion method as the citrus crop is a perennial crop with small-sized chromosomes. Furthermore, the sample collection in the field was easy and actively growing vegetative flush was available throughout the year. This technique was attempted in the tissue culture lab of ICAR- CCRI in various *in vitro* and *in vivo* ploidy induction experiments in *Citrus sinensis Osbeck* (Sweet orange cv. mosambi), *C. reticulata Blanco* (Nagpur mandarin) and *C. jambhiri Lush* (Rough lemon), for confirmation of diploidy (2n=2x=18), triploidy (2n=3x=27), tetraploid (2n=4x=36), hexaploid (2n=6x=54).

Keywords: Citrus, chromosome, enzymatic digestion, mutants and ploidy

INTRODUCTION

Citrus is one of the most important fruit crops of the world grown in more than 114 countries. More than 70 per cent of the world total citrus production is from northern hemisphere particularly in China, Brazil, India, USA and countries around the Mediterranean. In India, the area under citrus is 1.05 million ha with a production of 13.97 million tonnes and average productivity of 10.30 tonnes/ ha (NHB 2019-20). India is at 3rd position in the production of citrus (FAO 2018). Previous investigation shows that modern *Citrus* species originated in north eastern India and adjacent northern Burma (Gmitter and Xulan Hu 1990). The *Citrus* genus belongs to the family Rutaceae that includes 162 species (Tanaka 1993) and is grown in tropical and subtropical region of the world. In India, 30 *Citrus* species have been reported (Singh and Chadha 1993) in which, nine species are available throughout India. North-Eastern region is a hot spot for biodiversity of citrus and having germplasm of 23 taxa including 68 varieties reported by Sharma *et al*. (2004). ICAR CCRI, Nagpur is officially holding largest collection of valuable citrus germplasm. There are 614 accessions of citrus including 23 rootstocks from exotic sources (from U.S.A. and Australia), 552 from indigenous sources and 39 scion cultivars (mandarin, sweet orange, grapefruit and pummelo from U.S.A., France, Japan and Niger). Besides, 55 superior clones of Nagpur mandarin, 12 of acid lime, 5 of 'Mosambi' sweet orange and 6 of pummelo have been identified (www.ccri.org.in).

ICAR-CCRI had been identified as National Active Citrus collection sites by NBPGR, New Delhi and cytogenetic study of entire citrus germplasm could help in identification of minute genetic variants, in detection of true hybrid in hybridization propagation. This study has enabled to understand chromosome number in evaluation of citrus group (Guerra *et al.* 1997). Further, there is dire need to understand the genetic variation at ploidy level and

morphology level, due to the existence of huge genetic biodiversity and economic importance of several Citrus species (Hynniewta *et al.* 2011). The available published literature reports suggested the prevalence of different chromosome number in different species such as 2n=18 or 2n=27 in *C. aurantifolia* (Longley 1925; Krug and Bacchi 2003) and 2n=18, 27, 36 in *C. limonia Osbeck*, (Frost 1925a, b) are some examples. Therefore, there is a need to undertake investigations on cytogenetical approaches to define the existing genetic variation in the *Citrus* genus. The present investigations were an attempt to analyse sample which were developed as a part of polyploidy breeding programs at ICAR-CCRI, and samples were triploids, tetraploids and hexaploids, in the citrus scion and rootstock, cultivars as observed in flow cytometric studies.

Citrus chromosomes are small with 2-4µm length (Krug 1943). The mitotic index is mostly low in root tips. High-grade metaphase preparation requires a proper high-resolution metaphase spread. Cytogenetic studies such as chromosome counting in various *in-vitro* and *in-vivo* ploidy experiments, *in-situ* hybridization of higher ploidy species help in cultivar improvement. Currently available and routinely used squash preparation methodology is unable to obtain good quality chromosome spreads.

The enzymatic digestion method for leaf chromosome preparation is a reliable technique to solve drawbacks in conventional squash preparation methodology (Kesara, 2003). The method was reproducible initially for *Citrus sinensis Osbeck* (Sweet orange cv. mosambi), *C. reticulate Blanco* (Nagpur mandarin), *C. jambhiri Lush* (Rough lemon), in woody tree species. Root-tips were not easily available for analysis from the field because growing roots are small and are fibrous in nature. Roots from seedlings are too small and hence only one slide can be prepared from approximately ten roots. Emerging shoot tissue is reliable and most dependable source of active metaphase chromosomes in any plant species.

In the present investigation, ploidy samples with higher number of chromosomal counts such as triploid (2n=3x=27), tetraploid (2n=4x=36) and hexaploid (2n=6x=54) and smaller size of citrus chromosome were hindrance in getting a proper metaphase spread and subsequent chromosome counting. Hence research efforts were directed to develop a technique, based on enzyme digestion hypotonic protoplast dropping methodology, which enables getting a high-quality metaphase spread and counting of chromosomes by using actively dividing meristematic tissue of shoot tips.

MATERIALS AND METHODS

Enzyme digestion and Protoplast dropping methods were used for studying chromosome numbers of mitotically active leaf meristem cells from regenerated ploidy plants following the protocols of Kesara, (2003) with some changes in the protocol, which helped in resolving the problem of spreading and visualization of chromosome in citrus crop. Citrus chromosomes are small in size 2-4µm (Krug 1943) and mitotic index is mostly low. In HCl digestion and squash preparation methodologies, the frequency of getting good quality chromosome spreads especially in citrus crop was low. For tissue collection, the diploid, triploid and tetraploid plants of different citrus species obtained from various sources *i.e.,* in-vitro, greenhouse and natural open field conditions in the experimental block of ICAR-CCRI were collected. The fresh emerging shoots of approximately 2-3 mm in size, harvested from the plants were used as the source of the mitotic cells for leaf chromosome preparation and were placed in ice water (0-4^{0}c) for 24 h to retain in metaphases. The excess water was drained with the help of filter paper and the samples were placed in 1 ml of cold fixative in 2 to 3 micro tubes that were then kept at room temp for 2 h. Carnoys fixative was changed once during this process of fixation. After completing the fixation procedure, the samples were removed from fixative and were rinsed with double distilled water and kept immersed in water for the next 30 minutes. The digestion period gets extended with the size of bud. The small buds measuring 1 to 2 mm were used. Two to four shoot buds were placed in 100µl of Cellulase / pectinase enzyme mixture incubated at 37-38°C temp for 4hrs in water bath. For Protoplast isolation, post 4-6 hrs of digestion treatment, the bud tissues were crushed into Cellulase /pectinase enzyme mixtures with help of needle and filtered with the help of the tissue filter made of nylon mesh with pore size of 30 µm. The suspension must run down inside the wall of the micro tube 2ml. The filtered suspension was kept at 4° c for about 15 to 30 min.

The tissues were then subjected to hypotonic treatment of protoplasts where, the filter suspensions in vials/ microtubes were added with 1.5 mL of cold 75mM KCL solution. The protoplast suspensions were mixed by gently inversing the tubes for 15 to 20 min and were left in stand still position for 5 to 7 min. Cleaning and fixation of the protoplasts were carried out by centrifuging the protoplast suspensions at 7000 rpm for 5min. The supernatants were discarded and added with 1 mL of ice-cold fixative to the protoplast pellet. This fixative + protoplast mixtures were kept at room temperature for the next 5 min or at 4°C for a longer time. Again, the protoplasts were spinned down at 7000 rpm for another 5 min. The fixative supernatants were discarded and this fixation procedure was repeated 2-3 times as above. A 50µl of fresh cold fixative (A mixture of 1part glacial acetic acid and 3 parts absolute ethanol) was added to each of the protoplast pellets and gently mixed into suspension. Glass slides were kept wet and chilled for further use at 4°C. The protoplast suspension was dropped on the ice-cold and wet slide from 15 cm height. The slide was immersed in the absolute ethanol for few seconds, after drying the protoplast drop followed by air drying again and adding a drop of carmine solution. The coverslip was placed and sealed with the help of transparent nail paint (Kesara, 2003).

Ploidy analysis was carried out using a flow cytometer (Partec Gmbh, Munster, Germany). Flow cytometry works by estimating the volume and florescence of isolated nuclei. The ploidy was presented in the form of a histogram of integral fluorescence with the peaks depicting the ploidy level of the respective sample. The protocol includes a series of steps starting with excision of a 0.3-cm^2 piece of emerging leaf tissue and placing in a Petri dish. The sample was prepared for analysis using a High-Resolution Staining Kit (Partec GmbH). The samples were chopped with a sharp blade in the presence of 500-800µl of Nuclei Extraction Buffer and the nuclei were filtered through a nylon screen 30-µm filter into a 3.5-mL tube and stained with 0.5- 1ml of Nuclei staining buffer (4^2, 6-diamidino-2-phenylindole dihydrochloride). After that, samples were run in the flow cytometer. When the cells with labelled with fluorescent colouring due to the staining buffer passed through the measuring area one after the other, the individual cells or particles got illuminated by the excitation light and the fluorescent light intensity was proportional to DNA content. The samples were analysed in a UV-LED Partec flow cytometer with light emission at 365 nm, adjusted to fluorescence optical detection to gain as per the control sample of cultivar or as per species. More than 5000 nuclei were assessed in each sample. Nuclear DNA histograms were constructed using CyView software (Partec Gmbh, Germany), which determines peak position and relative ploidy level of the tested samples.

RESULTS AND DISCUSSION

Research studies on polyploidy breeding program initiated at ICAR-CCRI, since last few years. Standardized the protocols for induction of triploidy, tetraploidy in commercial citrus rootstocks and scions via endosperm rescue, micro budding coupled with colchicine treatment, and also by colchicine treatment of meristimatically active seeds. (Vijayakumari and Pooja, 2013), and generated various polyploids which were tested by flow Cytometry method but the results of flow Cytometry analysis were found varying due to the shifting of histogram inconsistent peaks. At this juncture to reconfirm the results, alternate chromosome counting technique was employed to ascertain the chromosome number. The flow cytometry methods sometimes yield fluctuating results depending on genome size, age of the sample and also with parents and progeny due to prevalence of introgressive hybridization in Citrus species. The estimated counts of seed derived plants differ with that of parent trees.

Citrus is mostly propagated by vegetative/asexual means, but in this study, we generated polyploidy plants by innovative *in vitro* and *in vivo* propagation techniques. Seed is the product of natural hybridization or sexual reproduction which leads to variation in the genetic makeup of both in parent and progeny trees. Chance of getting the obvious/clear convincing results /chromosome numbers of control and test plants through flow cytometry was observed to be tough. Polyploidy occurs naturally in citrus, mostly through spontaneous mutations, and polyploids are generally slow in growth less vigorous than the diploid counterparts (Gmitter *et al.,* 1991). In our experiments we have successfully regenerated large no of polyploidy plantlets via somatic embryogenesis from hybrid endosperm rescue and also by colchicine treatment of meristematically active seeds, but while assessing the ploidy level of all polyploidy plants with conventional method of using the root tips by squash spread techniques is quite difficult due to small size

of chromosomes in citrus, mitotic index is mostly low in root tips (Hynniewta, 2011). Further the slow growth rate of polyploids creates a difference in age group of test plant sample and control plant sample. The chromosome preparation method described in this paper is technically possible and simple for implementation, for cytogenetic confirmation of ploidy of large population of citrus both in the field and at nursery level and also plants obtained by different propagation methods. In Citrus cultivars chromosomes are small in size so sometimes accurate chromosome counting is generally difficult to achieve in mutants where ploidy is high. This improved methodology of chromosome counting helped in revalidation of flow cytometry analysed samples. In this investigation, polyploids generated in various experiments were confirmed by chromosome counts and also flow cytometry (Table 1, Fig. 1-3).

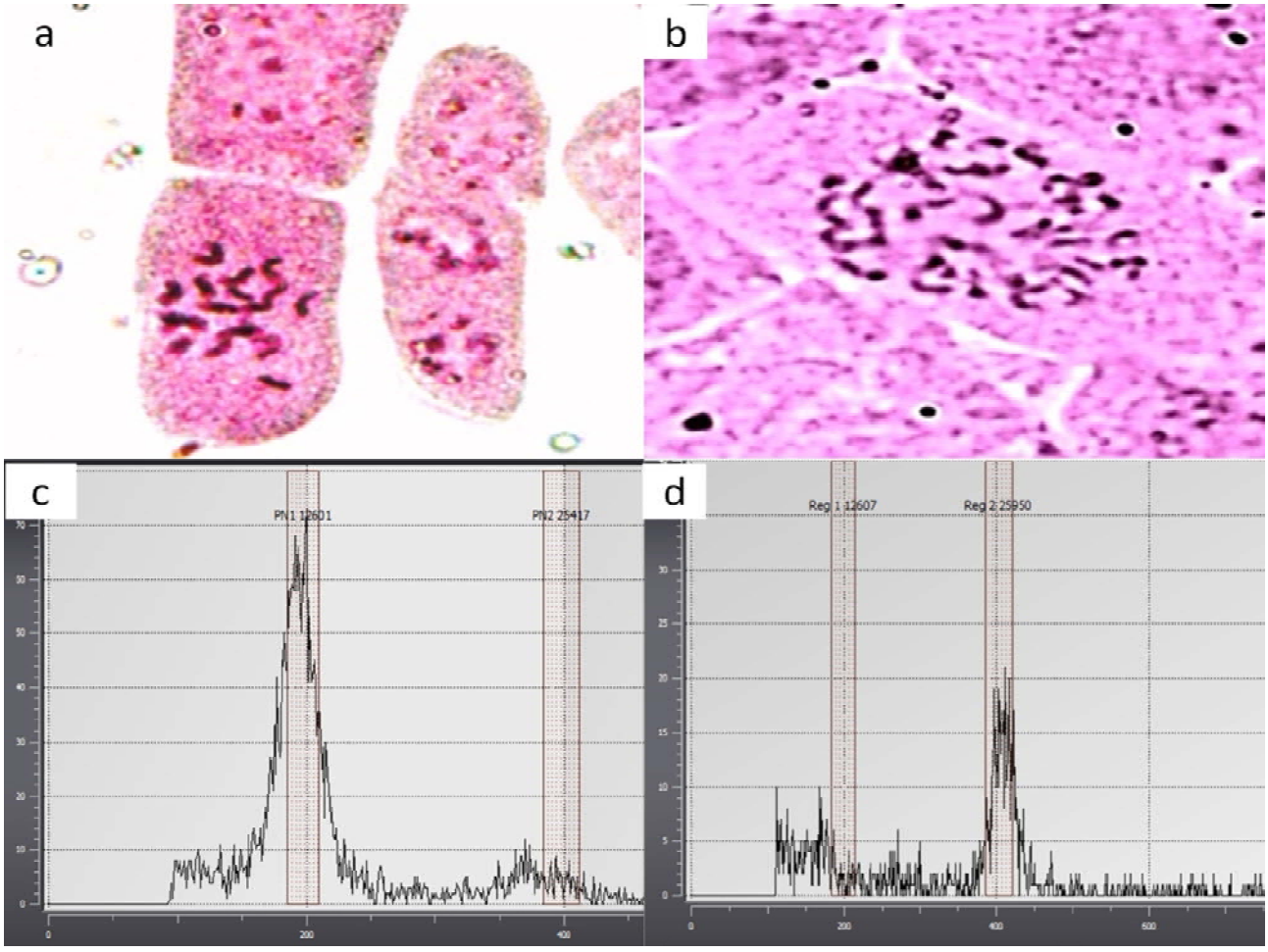

Fig. 1. Processed metaphase cells of *Citrus jambhiri Lush*. (Rough lemon) sample

(a) diploid (2n=18), (b) tetraploid(2n=4x=36).Result of Chromosomes count is correlated with flow cytometry analysis (histogram peaks) in control sample of Citrus jambhiri Lush. (Rough lemon). (d) with tetraploid sample (2n=4x = 36)

Table 1. Chromosome count of diploid mother plant and developed Polyploidy plants

Sl.No.	Species name	Chromosome count diploid mother plant	Developed Polyploidy plants chromosome counts
1	*Citrus jambhiri Lush.*	18	4x-36
2	*Citrus sinensis Osbeck*	18	(3x-27), (4x-36),(6x-54)
3	*Citrus reticulata Blanco*	18	(3x-27), (4x-36)

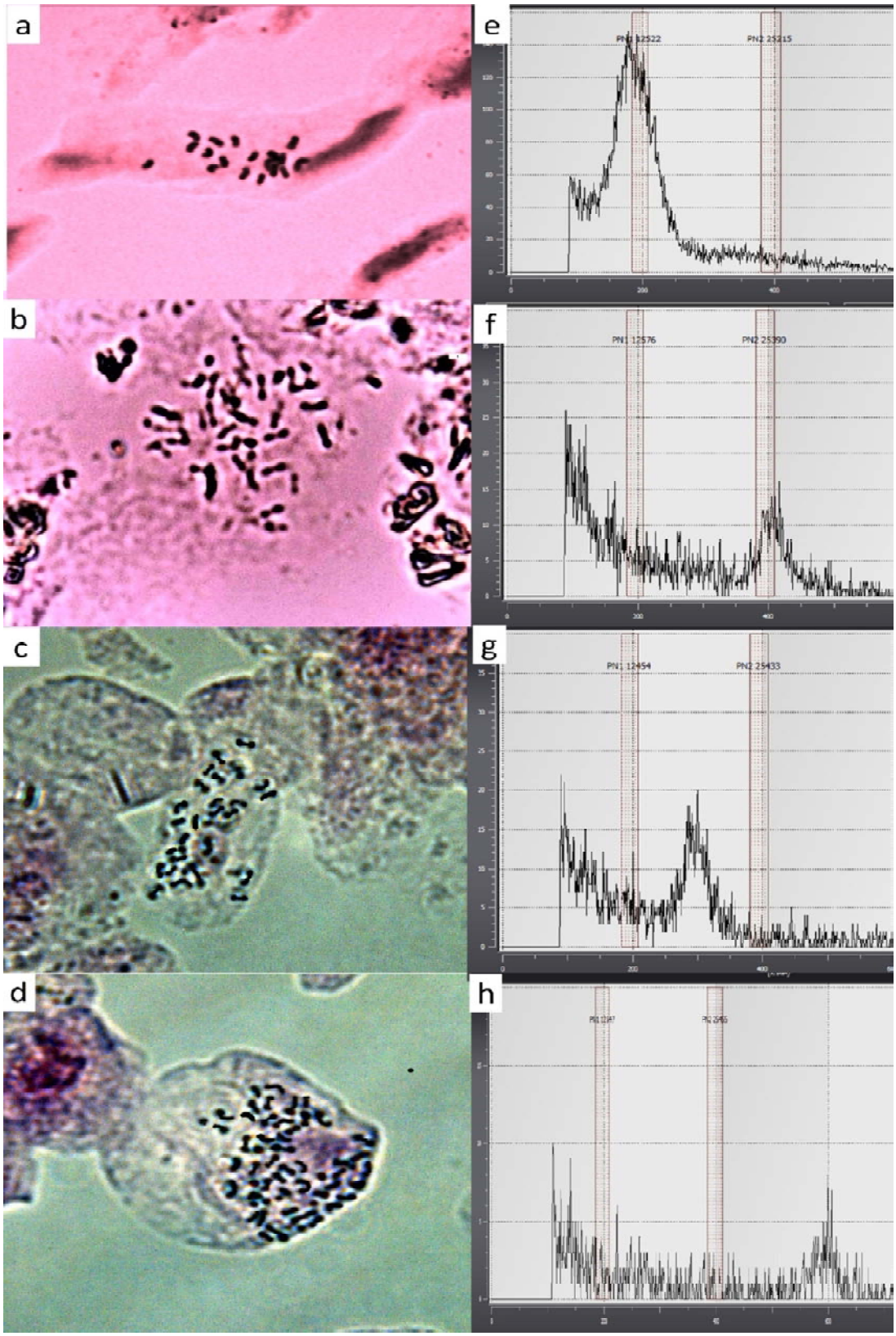

Fig. 2. Processed metaphase cells of *Citrus sinensis Osbeck* (Sweet orange cv. mosambi)

(a) Diploid (2n = 18), (b) tetraploid (2n = 4x = 27), (c) triploid (3n = 27), (d) Hexaploid (6n =54) in comparison with the flow Cytometry analysis (by histogram peaks - e to h).

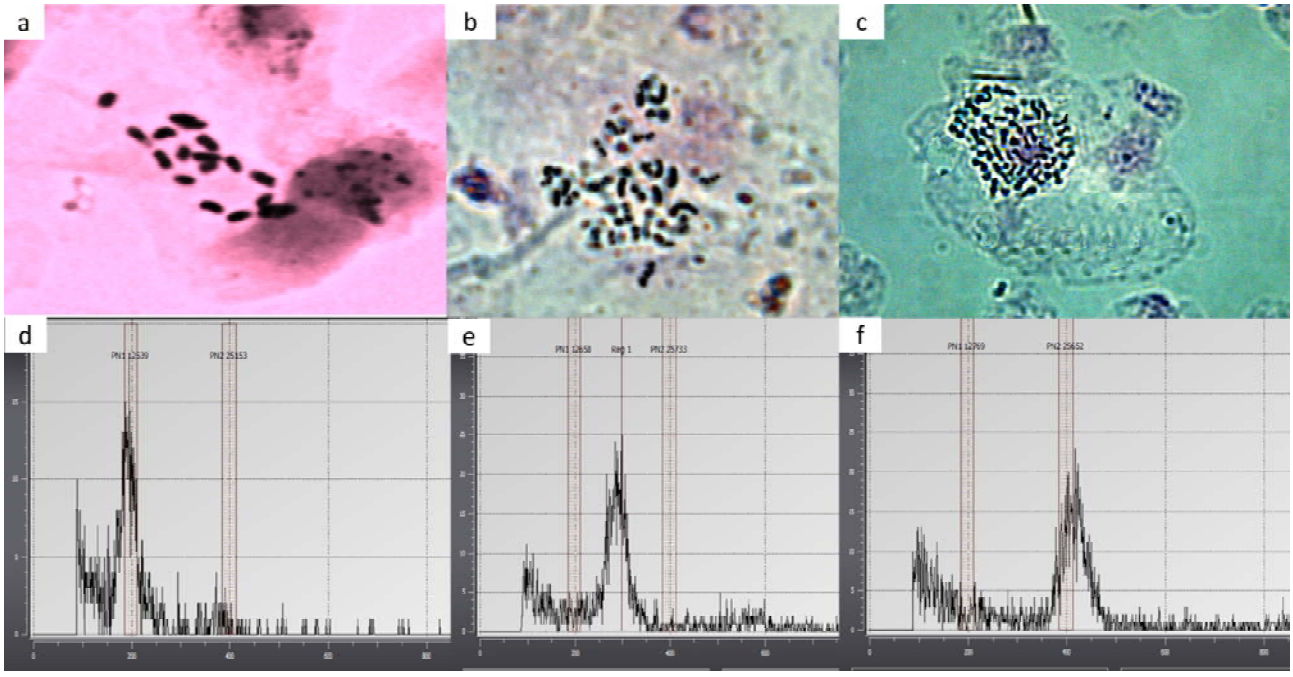

Fig. 3. Processed metaphase cells of *Citrus reticulata Blanco* (Nagpur mandarin) sample

(a) diploid (2x=18), (b) triploid (3x= 27), (c) tetraploid (4x=36), in comparison with the flow Cytometry analysis (by histogram peaks – d to f).

Shoot tip is a very convenient source of sample for chromosome preparations. Collection of shoot sample is the most simple and reliable, and it is mitotically active material from established plants. Approximately 2 to 3 mm of a healthy, vigorous growing shoot collected during active growth stage. The mitotic active phase is observed highest when the bud size is 2-3mm.Ideal time to collect such sample is in the morning (9 AM) where condition of light should be approximately 2000 lux and temperature around 25-27^{0}C. Before attaining above mentioned stage, the plant should undergo for a break of 12 hrs dark phase. After collection, samples were kept in ice cold water in the intermediate duration of carrying it from field to Lab. Collected samples of shoot tips were carefully defoliated and transferred into the ice-chilled water(0-4^{0}C) for 24hr. and treated with0.1% colchicine for 45min in the dark condition to arrest metaphase. This treatment enhances chromosome condensation and more importantly, improved the spreading of chromosome within a cell. The fixation preserves the tissue morphology and minimizes endogenous nuclease activity and other degradation processes. Fixation time affects the tissue sample quality. Higher the duration of fixation approximately up to 10hrs, results into tissue hardening. But here we have observed during research methodology optimum time for this technique in citrus 2 hrs duration is sufficient to generate a quality sample. The Enzyme digestion treatment at 37^{0}C for 6 hr treatment gave best result in citrus crop. Cellulase and pectinase enzyme with 2.5% concentration improves chromosome spreading and better sighting of chromosome. With this methodology the chromosomes are well spread at metaphase enabling well distributed chromosomes for getting clear countable chromosomes, these results are in confirmation of results obtained by (Kesara, 2003). It helps to reconfirm result of flow cytometry. Root chromosomes preparation by traditional squashed technique makes poor quality spreads, either chromosome fused together or some are lost between cells during tapping and squashings, as it was observed in preliminary experiments.

CONCLUSION

In this research paper we have used technique based on enzyme digestion treatment, protoplast dropping method and metaphase spread count which enabled better display of accurate chromosomal count. This study facilitated in double check or repetitive validation of ploidy level of samples generated in various *in-vitro* and *in-vivo* ploidy experiments, which were already observed in flow Cytometry. The results which we achieved during research is highly helpful for further karyotype analysis and *in-situ* hybridization application, as chromosomal count can be very much accurate because, of clear well spread, elongated, chromosome morphology on metaphase plates.

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Original Research Paper

Genetics of growth and yield attributing traits of brinjal (*Solanum melongena* L.) through six generation mean analysis

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ABSTRACT

Understanding gene action of different traits is of utmost importance for formulating successful breeding programs. The population was developed involving Arka Neelachal Shyama and CARI-1 to inquire the gene actions controlling the inheritance of several growth as well as yield attributing parameters through six-generation mean analysis. Three parameter model revealed the insufficiency of the simpler additive dominance model for the evaluated traits, referring to the existence of inter-allelic interactions. Six parameter model was implemented to better understand gene actions. Most of the yield and attributing traits under study except number of branches showed a high estimate of dominance as well as environmental variance, disclosing a lower extent of heritability. The number of branches was observed to be controlled by duplicate epistasis. Hence, for the fixation of this trait, the best strategy is to exercise minimal selection during advance generations, followed by intense selection during later generations (F_{4-} population onwards). The preponderance of the narrow sense type of heritability revealed that dominant effects were predominantly accountable for the existing genetic variation. Hence, recurrent selection followed by bi-parental mating and selection during the later stage of generations is advised to increase the occurrence of favorable alleles and accumulation of desirable genes.

Keywords: Brinjal, gene action, genetics, six-generation mean analysis and yield.

INTRODUCTION

Brinjal (*Solanum melongena* L.) is among the foremost important and popular vegetables consumed globally as well as in India. It consists of a significant amount of anthocyanin pigments (purple types), phenols, amide proteins as well as free reducing sugars. Brinjal is widely utilized for its medicinal properties and has also been prescribed for diabetic patients and liver complaints (Sabolu *et al*., 2014). Although brinjal is one of the prime members of the Solanaceae family, minimal breeding attempts have been undertaken for the development of potential hybrids/hybrid as well as for crop improvement by the exploitation of local germplasm in comparison to the remaining Solanaceous crops. Yield being a highly complex trait gets fluctuated by genetic as well as environmental factors, (Foolad and Lin, 2001) and applied agro technical methods (Kaşkavalci, 2007). The selection of suitable parental material is of utmost importance during crop improvement program to achieve the inherent yield potential of a crop (Koutsika-Sotiriou *et al*., 2008). In brinjal, the number of marketable fruits per plant, the number of branches, and the average fruit weight are the major yield attributing traits. In brinjal, yield per plant directly correlates with average fruit weight and number of fruits (Kaffytullah *et al*., 2011; Angadi *et al*., 2017); and these traits are highly influenced by several genetic and environmental components (Karki *et al*., 2020). The extent of the success achieved in a crop improvement program relies solely upon the accessibility to the information concerning nature as well as the measure of gene action governing the traits of commercial significance. Yield being a complex trait relying upon several other parameters along with their interactions, understanding the alliance of these traits with fruit yield will supplement the selection procedure

with enhanced accuracy and precision (Deb and Khaleque, 2009).

Keeping the above-mentioned points in view, the extent of hybrid vigour and inbreeding depression as well as the gene action governing various growth and yield attributing characters in brinjal was assessed though six generation mean analysis, as this is highly efficient technique providing the accurate evaluation of chief genetic components governing the manifestation of the quantitative traits (Mather and Jinks, 1982).

MATERIALS AND METHODS

Experimental site

The present research work was commenced during the period from 2018 - 2020 at CHES, IIHR-ICAR, Bhubaneswar, India. The experimental soil was red laterite soil with very low pH (4.4) containing organic carbon in a medium amount. The soil contained be 296 Kg/ha, 39.2 Kg/ha, and 157kg/ha of nitrogen, phosphorus and potassium, respectively.

Plant materials

For development of the population, two parents *viz*, Arka Neelachal Shyama (Large, round, green with purple stripes) and CARI-1 (Large, oblong, light green) were selected. Arka Neelachal Shyama was used as female parent, while CARI-1 was used as pollen parent. The two parents were artificially crossed for the production of F_1 hybrid. Subsequently, F_1 generation plants were selfed to obtain F_2 generation seeds as well as backcrossed with Arka Neelachal Shyama (P_1) and CARI-1(P_2) to obtain B_1 and B_2 generations respectively.

Evaluation of populations under field growing conditions

The seeds of six generations including two parents, their F_1 hybrid, segregating F_2 population and backcross populations with each of the parents were sown and proper nursery management practices were followed. During the transplanting of the seedlings, a spacing of 60 x 60 cm^2 was adopted. The number of plants evaluated differed according to the generation. For the assessment of the inheritance of growth and yield attributing traits, 30 plants each of female and pollen parents and their F_1's, 259 plants of F_2 population, 50 plants of B_1 population and 58 plants of B_2 population were planted in the open field. Recommended package of practices such as fertilizer application, intercultural operations, crop protection measures and irrigation were carried out for raising a successful crop.

Freshly harvested and marketable fruits of individual plants were utilized for genetic inheritance study. The mode of inheritance of various traits including plant height, number of branches, number of fruits and yield per plant, as well as average fruit length, girth, and weight were recorded.

Statistical analysis

In the current experiment, the scaling test was performed on means of each generation as per Mather and Jinks, 1982. The joint scaling test along with χ^2 test were utilized for the determination of the sufficiency of the additive-dominance model (Cavalli, 1952; Fowler *et al*., 1998; Singh and Chaudhary, 1977). Similarly, the χ^2 values for all the traits were checked to fit into the three-parameter model (*m, d, h*) and significant values implicated the epistatic gene interaction. By exercising the six-parameter model, six gene components including mean (*m*), pooled additive component (*d*), dominance component (*h*), additive × additive component (*i*), additive × dominance component (*j*), and dominance × dominance component (*l*) were calculated (Jinks and Jones, 1958).The magnitude of scales was estimated by the formulae: $A=2B_1 - P_1 - F_1=0$; $B=2B_1 - P_2 - F_1=0$; $C=4F_2 - 2F_1 - P_1 - P_2 = 0$ and $D = 2F_2 - B_1 - B_1 =0$. Significance of any of these scales indicated the involvement of epistasis in governing the respective traits (Mather and Jinks, 1982). The estimate of heterosis over parents as well as mid-parent value was calculated as % increase or decrease of F_1 mean over the parental and mid-parental means, respectively. Heritability (narrow-sense) was calculated by method suggested by Warner (1952).

Potence ratio was estimated as per Smith (1952) to analyze the extent of dominance, which is given below:

$$P\ (\text{relative potence of gene set}) = \frac{\text{mean of first filial generation} - \text{midvalue of the parents}}{0.5\ (\text{better parent mean} - \text{lower parent mean})}$$

Where, P = +1, suggest complete dominance, -1<P<+1 suggest partial dominance, except P=0, which suggests non-existence of dominance. When potence ratio exceeds ±1; it indicates over dominance. The signs (+/-) of the parents indicate the direction of dominance.

OP-STAT software was used for all the above statistical analysis (Sheoran *et al*., 1998).

RESULTS

Heterosis and inbreeding depression

Heterosis over the mid-parent *i.e.,* relative heterosis (Table 1) was found to be significantly negative for all the traits. Only vegetative traits under study exhibited positive relative heterosis *i.e.,* 72.09 % in case of plant height and 2.82 % for the number of branches. Relative heterosis (-13.41%) and heterobeltios over P_1 (-18.39 %), along with positive inbreeding depression (33.55 %) were recorded for fruits per plant in this population. The higher magnitude of heterobeltiosis/over P_2 (-31.64 %) as well as relative heterosis (-17.80 %) in the negative direction was observed for fruit yield per plant. In the current study, negative relative heterosis for both fruit length (-19.36 %) and fruit girth (-6.24 %) were observed in this population. Negative relative heterosis (-6.24 %) as well as heterobeltiosis/over P_2 (-25.76 %) was observed for fruit weight. Similarly, inbreeding depression ranged from 0.12 % (fruit length) to 40.60 % (yield per plant).

Gene action

For the assessment of inheritance pattern and gene actions governing growth and yield attributing traits, six generation mean analysis was implemented and the findings are presented in Table 2. Significant variability among the populations were recorded implicating the appropriate choice of the parents.

The significance estimates of three parameter model associated with joint scaling test for the means of generation of each parameter are shown in Table 3. Owing to the significant χ^2 value for all the traits, this test disclosed the constraints of the additive as well as dominance gene actions to describe the trait's inheritance. However, it expressed their cumulative effects on interaction. Hence, for the better interpretation the gene actions six parameter model was utilized (Table 4). Nonetheless, the inheritance of the studied traits could not be adequately elucidated through the normal additive-dominance model after obtaining the significant estimate of individual scales (A, B, C, or D) for the means of generations. The existence of epistasis was concluded upon the significant estimate of either of 'A', 'B', 'C' or 'D' components of the scaling test for all the traits under study.

Plant height

The highest and lowest plant height were recorded in F_1 (55.50 cm) and P_1 (29.10 cm), followed by F_2 (51.96), B2 (47.07 cm) and B1 (43.48 cm). Significant value of mean (*m*) was recorded, however additive (*d*) along with dominant (*h*) estimates appeared to be insignificant(Table 4). The additive x additive (*-i*) component contributed significantly towards the plant height similar to additive x dominance (*j*) component.

Table 1. Estimates of genetic parameter for plant vigor, yield and yield attributing traits in the cross between Arka Neelachal Shyama (P_1) and CARI-1 (P_2)

Traits	Heterosis (%)			Inbreeding depression (%)
	over P_1	over P_2	over mid parent	
Plant height (cm)	90.72	56.78	72.09	6.38
Number of branches per plant	10.98	-4.21	2.82	21.89
Yield per plant (g)	3.07	-31.64	-17.80	40.60
Number of fruits per plant	-18.39	-7.79	-13.41	33.55
Fruit length (cm)	-1.81	-31.59	-19.36	0.12
Fruit girth (cm)	-4.25	-8.15	-6.24	3.89
Fruit weight (g)	27.22	-25.76	-6.24	9.46

Table 2. Generation means (+/-SE) for fruit traits and yield per plant from the Arka Neelachal Shyama x CARI-1 derived population

Characters	Population					
	P_1	P_2	F_1	F_2	B_1	B_2
Plant height (cm)	29.10 ±1.36	35.40 ±1.62	55.50 ±2.22	51.95 ±0.61	43.48 ±2.10	47.06 ±1.17
Number of branches per plant	8.20 ±0.38	9.50 ±0.76	9.10 ±0.33	7.10 ±0.16	5.14 ±0.27	6.91 ±0.26
Number of fruits per plant	8.70 ±0.36	7.70 ±0.26	7.10 ±0.34	4.71 ±0.14	4.98 ±0.30	4.53 ±0.23
Yield per plant (g)	1472.00 ±61.78	2219.50 ±87.26	1517.25 ±86.31	901.28 ±30.16	1000.75 ±66.23	958.70 ±63.81
Fruit length (cm)	9.65 ±0.23	13.85 ±0.31	9.47 ±0.30	9.46 ±0.10	9.76 ±0.21	10.27 ±0.20
Fruit girth (cm)	23.55 ±0.39	24.55 ±0.39	22.55 ±0.35	21.67 ±0.14	21.40 ±0.39	21.98 ±0.34
Fruit weight (g)	169.27 ±1.61	290.08 ±11.88	215.34 ±9.49	194.97 ±3.80	200.45 ±8.80	210.23 ±7.40

Table 3. Joint scale test with three parameter model (*m, d* and *h)* for various traits in the cross of Arka Neelachal Shyama and CARI-1 population

	Plant height	Number of branches	Number of fruits per plant	Yield per plant	Fruit length	Fruit girth	Fruit weight
m	34.77 ±0.92**	6.68 ±0.28**	6.91 ±0.19**	1488.26 ±46.59*	11.17 ±0.17**	23.08 ±0.23**	188.20 ±28.17**
d	3.06 ±0.94**	0.51 ±0.27	-0.25 ±0.19	184.70 ±45.75*	1.52 ±0.16**	-0.50 ±0.24*	-60.40 ±5.99**
h	25.91 ±1.76**	0.48 ±0.50	-3.02 ±0.38**	-834.46 ±92.35*	-2.75 ±0.33**	-2.04 ±0.44**	-19.35 ±9.82**
χ2	52.13**	100.83**	155.01**	188.79*	49.63**	39.81**	26.52**

*and **significant at p - "0.05" and "0.01", respectively

The dominance x dominance (*l*) estimate showed superiority over additive × dominance (*j*), dominance (*h*) and additive × additive (*i*) estimates. The potence ratio of -7.381 was observed indicating overdominance in inheriting this trait.

Number of branches

P_2 (9.50) exhibited the highest number of branches, while B_1 exhibited the least number of branches (5.14), followed by F_1 (9.10), F_2 (7.11) and B_2 (6.91). Besides mean (*m*), a significant estimate of the additive (-*d*), dominance (-*h*), additive x additive (-*i*), and dominance x dominance (*l*) components was noticed (Table 4).The estimate of dominance x dominance (*l*) component exhibited superiority over the additive × dominance (*j*), dominance (*h*) and additive × additive (*i*) estimates.

Number of fruits per plant

Plants of P_1 produced the highest average number of fruits, while the minimum fruit count was noticed in B2 (4.53), accompanied by P_2 (7.70), F_1 (7.10) and B1 (4.98). The mean (m) was shown to be significant, however non-significant value was recorded for additive (*d*) along with dominance (*h*), additive x

additive (*i*) as well as additive x dominance (*j*) estimates (Table 4). In the current study, dominance x dominance (*l*) played key role in the governance of this trait. The estimate of dominance x dominance (*l*) was higher than dominance (*h*), additive × dominance (*j*) and additive × additive (*i*) estimates.

Yield per plant

The highest yield from individual plant was obtained from P_2 (2.22 kg), while the minimum yield per plant was procured from F_2 (0.90 kg), accompanied by F_1 (1.52 kg), P_1 (1.47 kg) and B_1 (1.00 kg). The mean (*m*), additive x dominance (*j*) in addition to dominance x dominance (*l*) components were recorded to be significant (Table 4), while non-significant values was reported for additive (*d*), dominance (*h*), additive x additive (*i*) components. The higher estimate of dominance x dominance (*l*) was observed in comparison with the additive × dominance (*j*), additive × additive (*i*), as well as dominance (*h*). The potence ratio of 0.87 was estimated suggesting partial dominance in the inheritance.

Fruit length

Fruit traits including fruit skin color varied significantly across the population (Fig 1). The mean maximum length was exhibited by the fruits of P_2 (13.85 cm) and the lowest fruit length was observed for F_2 (9.46 cm), succeeded by B_2 (10.27 cm), B1 (9.76 cm) and P_1 (9.65 cm). The mean (*m*) was found to be significant, however non-significant estimates were observed for additive (*d*), dominance x dominance (*l*) as well as dominance (*h*) components (Table 4). Besides additive x additive (*i*) estimate, a significant estimate of additive x dominance (*j*) components was also found for fruit length. The magnitude of additive × dominance (*j*), was greater as compared to additive × additive (*i*), dominance x dominance (*l*) as well as dominance (*h*). The potence ratio of 1.083 was found stipulating overdominance in the trait's inheritance.

Fruit girth

The highest and lowest fruit girth were recorded in P_2 (24.55 cm) and B_1 (21.40 cm), followed by P_1 (23.55 cm), F_1 (22.55 cm) and B_2 (21.99 cm). Except the mean (*m*) and dominance x dominance (*l*) components, the remaining estimates were estimated to be nonsignificant (Table 4). In the current study, the estimate of dominance x dominance (*l*) was greater than additive × additive (*i*), additive × dominance (*j*), and dominance (*h*). For fruit girth, the potence ratio of 3.00 was recorded referring towards overdominance in inheriting this trait

Average Fruit weight

The greatest and lowest individual fruit weight were exhibited by P_2 (290.08 g) and P_1 (169.27 g), followed by F_1 (215.3 g), B_2 (210.23 g) and B1 (200.45 g). The significant value of mean (*m*) was recorded. The additive x dominance (*j*) component was found to be significant, unlike the additive (*d*), additive x additive (*i*), dominance (*h*) as well as dominance x dominance (*l*) components(Table 4). The additive × dominance (*j*) estimate was shown to be more in comparison with the additive × additive (*i*), dominance x dominance (*l*) and dominance (*h*) components. The potence ratio of 0.237 was observed indicating partial dominance in the inheritance of this trait.

DISCUSSION

Heterosis and inbreeding depression

The parameters pertaining to growth and vigor showed significantly positive relative heterosis. The greater estimates of positive heterotic effects and minimal inbreeding depression for plant height revealed that crop improvement programs through heterosis could be successfully implemented for growth and vigor in brinjal (Roy *et al*. 2009; Mistry *et al*. 2018). Relative heterosis as well as heterobeltiosis over P_1 in the negative direction recorded for fruits per plant in this population. Plant yield being a highly complex trait, is the sum total of various basic yield constituents. The greater estimate of heterobeltiosis/over P_2 along with relative heterosis in the negative direction suggested that instead of heterosis breeding, selection at the later stage of the segregating population may be the best way to obtain higher yield per plant in the population studied (Sao and Mehta, 2011; Patel *et al*., 2013; Bagade *et al*., 2020). Fruit length as well as fruit girth are regarded as essential characters considering the diversity observed in consumer preference especially in India, where eastern region prefer large round types as opposed to south region preferring long types Hence, negative heterosis for these traits especially fruit length is desired. In the current study, desirable negative relative heterosis for both fruit length and fruit girth were observed in this population, which will have advantage in development of round type fruits.

Table 4. Estimates of gene effects for various traits in the cross between Arka Neelcahal Shyama x CARI-1 derived population using six parameter model

	Characters						
	Plant height	Number of branches	Number of fruits per plant	Yield per plant	Fruit length	Fruit girth	Fruit weight
A	-9.40±3.32**	7.02±0.74**	5.84±0.79**	987.75±169.74**	-0.40 ±0.57	3.29±0.94**	-16.28±20.06
B	2.83±4.30	4.77±0.98**	5.73±0.64**	1819.33 ±177.06**	2.78±0.59**	3.12±0.87**	84.96±21.22**
C	-32.33±4.82**	7.46±1.26**	11.72±1.00**	3120.86±236.20**	4.59±0.82**	6.50±1.06**	110.16**±27.11
D	12.88±2.76**	2.16±0.49**	-0.07 ±0.48	-156.88 ±109.99	-1.10±0.35**	-0.04 ±0.59	-20.73±13.78
Mean (*m*)	51.95±0.78**	7.10±0.16**	4.71±0.14**	901.28±30.16**	9.46±0.10**	21.67±0.14**	194.97±3.80**
Additive (*d*)	2.96 ±2.27	-1.77±0.37**	0.44 ±0.38	42.04 ±91.96	-0.50 ±0.29	-0.58 ±0.52	-9.78±11.50
Dominance (*h*)	-2.51±5.81	-4.07±1.13**	-0.94 ±1.04	-14.72 ±242.28	-0.06±0.80	-1.40±1.27	27.14±29.77
Additive × additive (*i*)	-25.76±5.52**	-4.32±0.99**	0.15 ±0.96	313.77 ±219.98	2.21±0.71**	0.09 ±1.18	41.47±27.57
Additive × dominance (*j*)	12.23±5.01*	-2.24 ±1.14	-0.10 ±0.89	831.58±212.75**	3.18±0.70**	-0.16 ±1.18	101.25±25.94**
Dominance × dominance (*l*)	19.19±10.28	16.11 ±1.97**	11.41 ±1.84**	2493.31±437.17**	0.17 ±1.43	6.31 ±2.34**	27.21±53.40
d+*h*	0.45	-5.84	-0.49	27.31	-0.57	-1.98	17.36
l+*i*+*j*	5.66	9.54	11.46	3638.67	5.56	6.24	169.93
Magnitude of Gene Effect	l>j>h>i	l>j>h>i	l>i>j>h	l>j>i>h	j>i>l>h	l>i>j>h	j>i>l>h
Epistasis	-	Duplicate	-	-	-	-	-
Potence ratio (F_1)	-7.38	-0.38	-2.20	0.87	1.08	3.00	0.23

*and **significant at p - “0.05” and “0.01”, respectively.

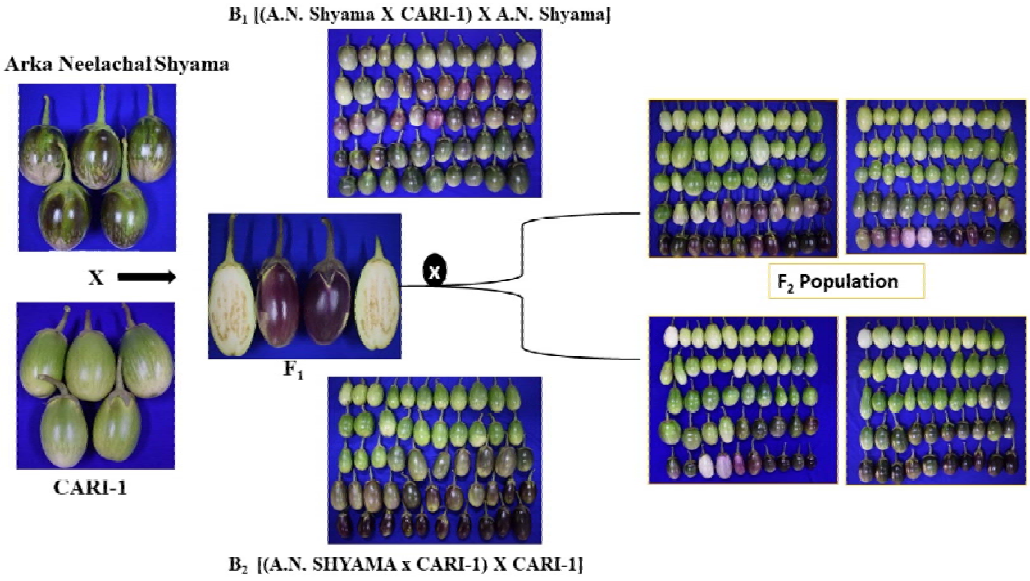

Fig 1. Segregation of fruit traits in populations derived from cross between Arka Neelchal Shyama x CARI-1

These results are consistent with the result obtained by Timmapur *et al*., (2008), Chowdhury *et al*., (2010), Sahajahan *et al*., (2016) and Sujin and Karuppaiah (2018). Negative relative heterosis as well as heterobeltiosis/over P_2 were observed for fruit weight. The pronounced negative heterosis for fruit weight revealed dominance of negative alleles contributed by the lower scoring parent (Patil *et al*., 2001; Das *et al*., 2009).

East (1908) and Shull (1909, 1910) demonstrated that a decrease in heterozygosity results in a corresponding decline in vigour due to inbreeding (Crow, 1952), which also fully satisfies the dominance hypothesis. Inbreeding depression was resulted to the extent of 40.60 % (fruit yield per plant) in our brinjal population. However, the inbreeding depression reports of this research work is also supported by the findings of recent research workers like Sao and Mehta (2010), Singh and Rai (1990) and Kumar and Pathania (2003), who reported positive inbreeding depression for a number of traits of brinjal.

Gene action

Significant variability among the means of populations were recorded suggesting that the choice of parents was appropriate.

Plant height: Additive x additive (-*i*) component contributed significantly towards the plant height similar to additive x dominance (*j*) component, which suggested the preponderance of non-additive components; hence should be considered immensely in the crop improvement programs. The magnitude of dominance x dominance (*l*) was higher than additive × dominance (*j*), dominance (*h*) and additive × additive (*i*), suggesting heterosis breeding is the best strategy to for improvement programs for enhancing plant height. Non-additive gene action governing the plant height trait in brinjal was also recorded by Singh *et al*. (2002) as well as Patel (2003).

Number of branches*:* The significant value of the additive (-*d*), dominance (-*h*) as well as additive x additive (-*i*) components pointed towards the existence of additive and non-additive gene actions controlling this trait. The contrasting signs of *h* and *l* showed the existence of duplicate epistasis interaction among the alleles. The estimate of dominance x dominance (*l*) component was superior to the additive × dominance (*j*), dominance (*h*) and additive × additive (*i*) components. Significant estimate of additive component in negative direction was observed, hence revealed that the trait could not be fixed via simple selection. The involvement of non-additive gene action in governing number of branches in brinjal was reported by Dharwad *et al*. (2011), Reddy and Patel (2014) and Sujin and Karuppaiah (2018). However, in our study, association of additive along with non-additive gene actions suggested that the population improvement through reciprocal recurrent selection should be considered as the best breeding scheme to increase the accumulation of favorable alleles (Ramalho *et al*., 2001). Again, duplicate class of inter-allelic interrelationship is operating (Patel, 2003;

Mistry *et al*., 2016) indicating the reduction in variability in segregating generations which obstruct the selection activity (Kumar and Patra, 2010). Hence, mild selection during earlier generations, followed by bi-parental mating and intense selection in the later generations should be done for improving the trait.

Number of fruits per plant: In the current study, dominance x dominance (*l*) was playing major role in the governance of this trait. For the given trait, additive gene action (Dixit *et al*., 1982; Joshi and Chadha, 1994) and non-additive gene action (Rao 2003; Aswani and Khandelwal 2005) have been reported. The estimate of dominance x dominance (*l*) was higher than additive × additive (*i*), dominance (*h*) and additive × dominance (*j*).Thus, recombination breeding and hybridization accompanied by selection during early generations could be performed for the improvement of this trait. Non-significant value was recorded for additive (*d*) along with dominance (*h*), additive x additive (*i*) as well as additive x dominance (*j*) components.

Yield per plant: Additive x dominance (*j*) in addition to dominance x dominance (*l*) components were recorded to be significant. The higher estimate of dominance x dominance (*l*) was observed in comparison with the additive × dominance (*j*), additive × additive (*i*), as well as dominance (*h*). Thus, additive along with non-additive interactions were involved in governing the trait. Hence, trait improvement strategy consisting of recurrent selection as well as bi-parental mating could be highly fruitful for the accumulation of the favorable genes and/or to remove the existing undesirable and unfavorable linkages (Mistry *et al*., 2016). Shafeeq *et al*. (2013) in their study involving genetic inheritance of yield and yield attributing traits found the similar report concluding the fruit yield per plant to be governed by both additive and non-additive gene actions. Non-significant value was found for additive (*d*), dominance (*h*), additive x additive (*i*) components.

Fruit length: Predominance of additive gene action was concluded towing to the significance of additive x additive (*i*) and additive x dominance (*j*) components. The magnitude of additive × dominance (*j*), was greater as compared to additive × additive (*i*), dominance x dominance (*l*) and dominance (*h*). Hence, selection would be the best strategy in the improvement of fruit length. This finding however was in contradiction to Mistry *et al*. (2016) who reported the fruit length trait to be governed by additive-dominance interaction. The non-significant estimates were observed for additive (*d*), dominance x dominance (*l*) and dominance (*h*) components.

Fruit girth: In the current study, only dominance x dominance (*l*) components were observed to be significant and the estimate of dominance x dominance (*l*) was higher than additive × additive (*i*), additive × dominance (*j*), and dominance (*h*). For fruit girth, both additive gene action and non-additive gene action have been reported by the researchers (Kumar and Ram, 1987; Vaghasiya *et al*., 2000; Rao, 2003; Patel, 2003).Thus, due to the involvement of the non-additive gene action, recombination breeding and hybridization accompanied by selection during later generations could be performed for the improvement of this trait (Mistry *et al*., 2016). The non-significant estimate of additive (*d*), additive x additive (*i*), dominance (*h*) and additive x dominance (*j*) were recorded.

Fruit weight: The additive x dominance (*j*) component was found to be significant. The additive × dominance (*j*) estimate was shown to be more in comparison with the additive × additive (*i*), dominance x dominance (*l*) and dominance (*h*) components. Hence, reciprocal recurrent selection can be adopted. Meanwhile, selection process needs to be delayed until required homozygosity is achieved in the inbred lines (Mistry *et al*., 2016).The additive (*d*) component in addition to additive x additive (*i*), dominance (*h*) as well as dominance x dominance (*l*) components, were recorded to be non-significant.

CONCLUSION

In the current study, the growth and yield attributing traits were identified/reported to be governed by additive and non-additive gene action along with the preponderance of combination of both the gene actions. A higher order of non-allelic interaction (involvement of more than 2 genes) was exhibited by most of the traits except number of branches in which duplicate type of epistasis was recorded. Due to the existence of a higher estimate of interactions, the frequency of the non-fixable gene effects was more as compared to the fixable gene effects. Of all the traits, only fruit length was reported to be governed by additive gene action, which can be fixed by simple selection. However, for the remaining traits combination of recurrent selection/ hybridization and bi-parental mating can be adopted followed by intense selection at later stage of segregating population.

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Original Research Paper

Assessing the genetic diversity of squash (*Cucurbita pepo* L.) genotypes based on agro-morphological traits and genetic analysis

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ABSTARCT

An experiment was conducted to estimate the genetic variability of 15 indigenous and exotic squash genotypes assessing 18 quantitative and 8 qualitative traits. Results showed that the accessions have high variability in qualitative traits like fruit size, fruit shape, fruit skin colour, lustre and fruit productivity, which allowed selection for considerable gains in these characteristics. The quantitative traits such as fruits yield per plant, fruit weight, length, diameter and total yield per hectare showed the greater phenotypic coefficient of variation (PCV) along with higher heritability which can helps to identify desirable genotypes. The obtained significant and positive correlation between fruit yield with number of leaves, nodes, fruit length, weight and number could assist in selection to improve this crop. Cluster analysis resulted in the formation of 4 groups, confirming the genetic variability among the studied genotypes. Eventually, the attained PCA analysis result revealed that the number of fruits per plant, fruit yield per plant, fruit length and days to first female flowering are the most discriminating traits which are accelerating the variability in squash genotypes. On the basis of the yield and its attributing traits, First Runner is the best genotype suited in this environment.

Keywords: Genetic analysis, genetic variability, heritability, morphological traits and squash

INTRODUCTION

Squash (*Cucurbita pepo* L.) is an economically important species of the Cucurbitaceae family that represents one of the most primitive genera (*Cucurbita)* in the plant kingdom (Tadmor *et al*., 2005). Squash is monoecious vegetable crop and familiar with its different traditional name like Zucchini (Italy); Cucuzza (Saudi Arabia), Courgette (America); Marrow (Ireland and Britain) and Baby marrow (South Africa). It is grown throughout the temperate, sub-tropical, and tropical regions, native to eastern United States and Mexico and also cultivated worldwide for its fruits (Bisognin, 2002). The major economic value of this crop is based mainly on the culinary use of immature fruits which have relatively high nutritional and medicinal value as compared to other vegetable crops. Its nutritional profile consists of various organic compounds, nutrients, vitamins and minerals, that are responsible for providing all its impressive health benefits (Kulczynski and Gramza-Michałowska, 2019). It is also a very good source of carotenoids, important anti-inflammatory and anti-oxidant compounds (Deppe, 2015) and because of its low caloric value treated as weight loss diets (Fageria *et al.*, 2012). So, keeping its importance in mind, increase the global production is one of the important ways to ensure food security.

Bangladesh is one of the most densely populated country in the world having over 160 million people and based on its current growth trends a projected population will be over 200 million by 2050 (USAID, 2017). To meet up the food demand for its uprated population, increasing the crop production in per unit areas of land is the most effective ways to ensure the food security. Squash is one of the important vegetable crops which can assure the nutritional security from its present nutritional shortage (per capita deficiency of vegetables 158 g) in Bangladesh (Anon., 2018). Topographically, Bangladesh has diverse land area

which is favorable for the crop diversification and production, however, squash can grow easily in any types of soil even in unproductive and marginal land areas. In addition, more economic growth can be achieved by producing vegetables like squash which will ultimately uplift the socio-economic condition of the farmers. Thus, there is urgent need to initiate research on squash especially for its vertical expansion and varietal improvement. Although, squash is becoming important vegetable crop in Bangladesh, there is little information available about its improvement and till date only a single variety has been recommended for winter season. In Bangladesh, few researchers have taken initiatives for studying its growth and effects of fertilizers on it (Akhter *et al.*, 2018; Baby *et al.*, 2021) but genetic variability study has not been taken up yet. Breeding for high-yielding crops require information available on the germplasm and the relationship among the agronomic traits as well as the degree of environmental influence (El-Hadi *et al.*, 2014). For its crop improvement, determining the extent of genotypic and phenotypic variability among geographical areas is important (Muralidhara and Narasegowda, 2014). Quantitative and qualitative determination (morphological characterization) of the degree of variation of traits present in genetic resources is important for vegetable breeding programs (Balkaya *et al.*, 2010; Gomes *et al.*, 2020). Morphological characterization is the first step followed by quantitative traits in the description and classification of genetic resources (Balkaya *et al.*, 2010). However, only a few studies have focused on variability analysis in relation to morphological and yield contributing quantitative traits with squash accessions. Therefore, the present research has been undertaken for the improvement of squash by assessing its genetic variability traits.

In Bangladesh, squash cultivation in summer season is challenging because of the severe attack of pests and diseases, excessive light and temperature, high rainfall and high labour cost etc. Meanwhile, a very few research works relating to its adaptability and variability have been conducted in Bangladesh especially in Sylhet region where huge amount of land has remained fallow (14% of the total land) for a long time (BBS, 2018). So, there is a great opportunity to increase squash production in this region to meet up the vegetable and nutritional requirement of the country. Considering the above points of view, the present study has been under taken to know the extent of genetic variability, heritability and genetic advance for different traits of squash genotypes in Sylhet region.

MATERIALS AND METHODS

The experiment was conducted at the Research field of the Department of Genetics and Plant Breeding, Faculty of Agriculture, Sylhet Agricultural University, Bangladesh during the period October 2019 to January 2020. Fifteen indigenous and exotic genotypes (Table 1) of squash were used in this experiment that were collected from the different parts of Bangladesh as well as from the other countries.

The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The experiment was divided into three blocks and each consisted of 15 plots. Each unit plot size was 1 x 2.3 m^2. Altogether, there were 45 unit plots in experiment and required 300 m^2 land. Both row to row and plot-to-plot distances were 0.5 m. The treatments were randomly assigned to each of the block. Each unit plot had 5 pits and in each pit 2 seeds were sown. After germination only one plant was allowed to grow. The land was prepared by ploughing and cross ploughing and different intercultural operations were accomplished according to recommended BARI Squash variety (BARI, 2018). The data were recorded based on 18 quantitative yield contributing traits i.e. plant height in cm at first harvest (PH), stem diameter in cm at first harvest (SD), number of leaves at first harvest (NL), number of nodes at first harvest (NN), days to flower bud initiation (DFBI), days to first male flowering (DFMF), days to first female flowering (DFFF), number of male flowers from flowering to last harvest (NMF), number of female flowers from flowering to last harvest (NFF), viable pollen in percentage (VP), days to first harvest (DFH), nodes at first fruit harvest (NFFH), fruit length in cm (FL), fruit diameter in cm (FD), fruit weight in g (FW), number of fruits per plant (NFPP), fruit yield per plant in Kg (FYPP), total yield in t/ha (TY) and 8 qualitative traits *i.e.* plant vigor, pubescence, stem shape, flower color, fruit size, fruit shape, fruit skin color and luster.

The recorded data on various parameters were analyzed to find out the statistical significance of the experimental results. Mean and standard deviation were calculated using Microsoft Excel software 2010.

Table 1. Name and source of the Squash genotypes used in the experiment

Genotypes	Name of the genotypes	Origin	Remarks
G1	First Runner	South Korea	Indigenous
G2	Alaska	Australia	Indigenous
G3	Blossom House	Netherlands	Indigenous
G4	Balam House	USA	Indigenous
G5	Cheonlima	South Korea	Indigenous
G6	Hungnong Squash	South Korea	Indigenous
G7	Runner	USA	Indigenous
G8	SQ-001	Australia	Exotic
G9	SQ-002	Australia	Exotic
G10	SQ-003	Australia	Exotic
G11	SQ-004	Australia	Exotic
G12	SQ-005	Australia	Exotic
G13	SQ-006	Australia	Exotic
G14	SQ-007	Australia	Exotic
G15	SQ-008	Australia	Exotic

The significance of the difference between treatment means, coefficient of variation (CV) was calculated by the Least Significance Difference (LSD) test for the interpretation of the results (Gomez and Gomez, 1984). Then the tabulated results were analyzed using one-way analysis of variance (ANOVA) and statistical differences between the means were estimated using Duncan's multiple range test (DMRT) at 1% or 5% or 0.1% probability with the help of statistical "R" software.

Estimation of Genetic parameters

Estimation of genotypic and phenotypic variances: Genotypic and phenotypic variances were estimated according to the formula given by Johnson *et al.* (1955).

Estimation of coefficient of variability (genotypic and phenotypic coefficient of variation): Both phenotypic and genotypic coefficient of variability for all characters w estimated using the formula of Burton (1952). PCV and GCV were classified into three categories *viz*., Low (< 10%), Moderate (10-20%) and High (> 20%) as suggested by Sivasubramanian and Madhavamenon (1973).

Heritability in broad sense (h^2_{bs}): The broad sense heritability (h^2_{bs}) was estimated for all characters as the ratio of genotypic variance to the total of phenotypic variance as suggested by Hanson *et al*., (1956). Heritability estimates in cultivated plants could be placed in the categories viz. as Low (0-30%), Moderate (30-60%) and High (>60%) as suggested by Robinson (1966).

Genetic advance (GA): The expected genetic gain or advance for each character was estimated by using the method suggested by Johnson *et al*., (1955). Genetic advance was classified as high (>20%), moderate (10-20%) and low (<10%). Further the Genetic advance as per cent of mean was computed by using the formula which was given by Burton (1952). Genetic advance as per cent mean was categorized into groups *viz*., Low (< 10%), Moderate (10-20%) and High (> 20%) as suggested by Johnson *et al.* (1955).

Correlation estimation

Simple correlation coefficient (r) among 12 important parameters of Squash accessions was estimated according to Singh and Chaudhury (1985). Again, cluster analysis (CA) was carried out according to Mahalanobis (1936). It divides genotypes into groups on the basis of a data set into some number of mutually exclusive groups. Furthermore, principal component analysis (PCA) was computed from

correlation matrix and genotype scores obtained for the first components with roots greater than unit (Jeger *et al.,* 1983). It provides two dimensional plots, which helps in separating different populations involved. Contribution of the different characters towards variability was discussed from the latent vectors of the first three principal components. However, Mean data for each character was subjected to multivariate analysis techniques *viz*., principal component analysis (PCA), cluster analysis (CA) and also the simple correlation coefficient analysis were done by computer using the STATA 14.0 software.

RESULTS AND DISCUSSION

Mean performance or genotypes for vegetative characters

In this experiment, fifteen indigenous and exotic squash genotypes have been characterized according to morphological traits and genetic analysis. Although morphological characteristics depends on its external factors but it is parallelly important to support these morphological variations along with their genetic studies. Results of mean performance of different squash genotypes based on different agronomic and yield contributing traits indicated that there was a significant difference in mean performance among all the genotypes. This difference could be resulted from the genetic variation among the studied squash genotypes which is also supported with the results of other previous studies on squash (Gomes *et al.*, 2020; Tsivelikas *et al.*, 2009; Villanueva-Verduzco *et al.*, 2020). A wide genetic diversity was also reported in the experimental results of Egusi-melon (Olaniyi *et al*., 2011) and cucumber (Arunkumar *et al*., 2011).

In case of vegetative characters, results showed a great significant variation for all the characters among the squash genotypes (Table 2). The highest plant height at first harvest was found in SQ-002 (36.05 cm) and the lowest was in Balam House (32.17 cm). Diameter of stem during first harvest was highest in First Runner (13 cm) and the lowest was in Balam House (9.21 cm). Number of leaves, considered as an important parameter for fruit yield, was the maximum in two genotypes *i.e*., Cheonlima (25) and SQ-001 (25). Additionally, the maximum number of nodes per plant was recorded in First Runner (14.3) and the minimum was recorded from Balam House (11.53). Different types of leaves, flowers and fruits were observed in studied squash genotypes those are presented in Figure 1. Therefore, it was observed that, the squash genotypes showed a wide range of variation in their growth-related morphological traits. Variation in morphological (Ozturk *et al.*, 2021) as well as anatomical features (Balkaya *et al.*, 2010) is a common phenomenon among different *Cucurbita* species. Additionally, Esho and Jasim (2020) found a wide range of variability for number of nodes for the first female flower in Squash.

Moreover, considering the reproductive (flowering) characters, the results showed a significant variation on days to flower bud initiation, days to first male and female flowering, number of male and female flowers and viable pollen rate for all the squash genotypes (Table 3). The genotype Runner took minimum days to first flower bud initiation (19.29 days) while the genotypes First Runner took the lowest day to first male flowering (30.54 days) and the genotype Runner was the earliest genotypes to first female flowering (35.73 days). In most of the genotypes, female flowers were emerging before male flowers with some exceptions (Table 3). Significant difference for days to female flowering was also reported by Nahar *et al.*, (2016) in sweet gourd genotypes. In addition, the male flower numbers outnumbered the female flower numbers during the experimental period for all the genotypes. Both the male and female flowers formed simultaneously right from the outset. Additionally, pollen viability was of special interest to see the degree of influence it exerts upon fruit and seed setting. The percentage of pollen viability helps us in selecting the parents for crossing in a hybridization program. The mean values of fertile (viable) pollen showed statistically almost similar results for all the 15 genotypes (Table 3). This result indicated a high possibility of cross pollination among the genotypes and this could lead a high level to of genetic variability in squash genotypes.

Some other yield contributing traits considering the fruiting characters *i.e*., days to first harvest, number of nodes at first harvest, length and diameter of fruit per plant, fruit weight, number of fruits per plant, fruit yield per plant and total yield showed a significant variation among the genotypes (Table 4). The days to first harvest ranged from 54 to 62.67 days in Runner and Balam House respectively with a mean value of 58.3 days. Low variation was observed among the genotypes with respect to number of nodes at first fruit

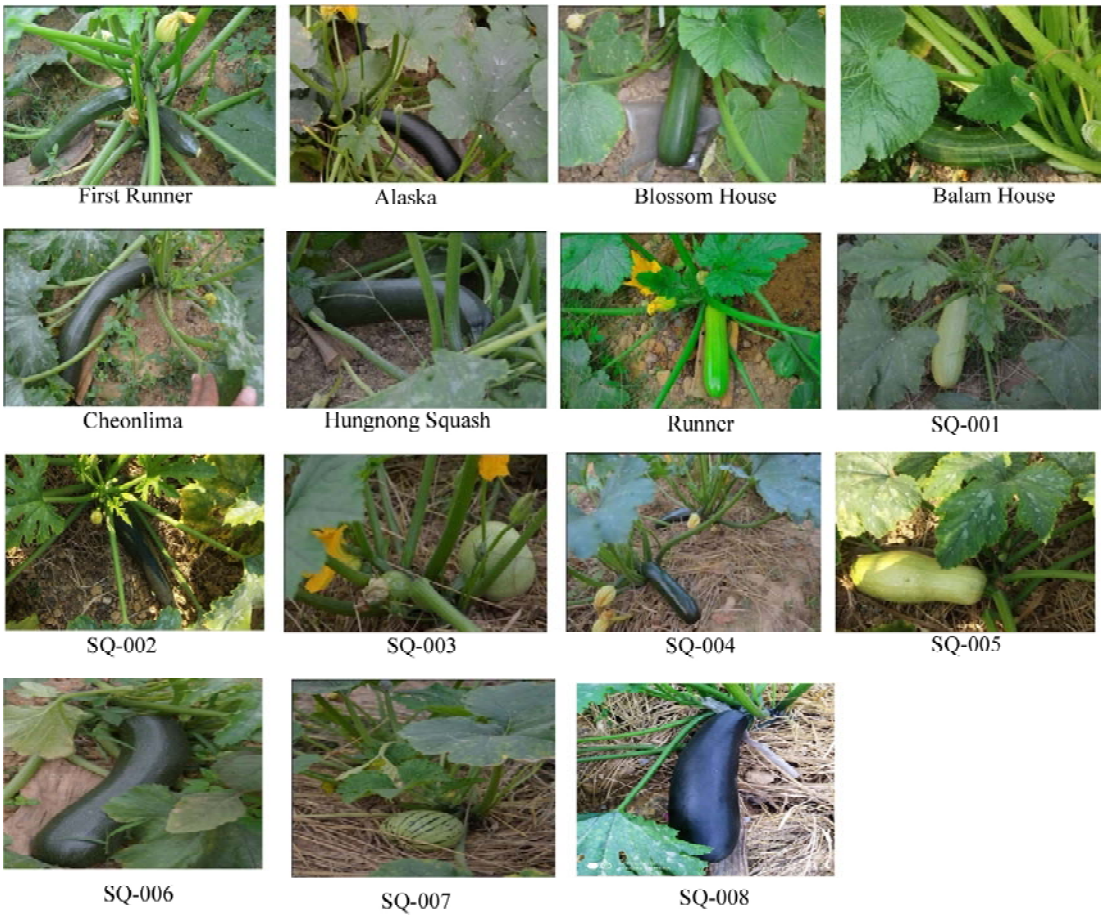

Fig. 1. Variation in leaves, flowers and fruits of fifteen squash genotypes

harvest (range: Runner 5.03 to Blossom House 6.5; mean: 5.78). The maximum fruit length was seen in the genotypes First Runner (45.58 cm), Hungnong Squash (45.42 cm) and Cheonlima (44.1 cm) while the minimum length of fruit was observed from SQ-007 (27.62 cm). Similar findings of significant variation on days to first harvest, number of nodes at first harvest and fruit length were also reported by Esho and Jasim (2020) in squash, Mohsin *et al.* (2017) in pumpkin and Nahar *et al.* (2016) in sweet gourd. Significant variation in fruit diameter (range: Alaska 17.47 cm to SQ-003 38.11 cm) was found among squash genotypes. Significant variation was also observed by Balkaya *et al.* (2010) in winter squash from the black sea region of Turkey. The individual fruit of First Runner (1165.5 g) had the highest weight followed by SQ-001 (1013.03 g) and Hungnong Squash (1002.08 g). The lowest single fruit weight was recorded in Runner (742.24 g). The variation of fruit weight could be due to the genetical, physiological and environmental influence. Abdein *et al.* (2021) reported similar results in respect of single fruit weight in summer squash. Number of fruits per plant was the maximum in case of Runner (10.2) proceeded to First Runner (10) and SQ-001 (9.53). Accession Balam House produced the minimum number (6.2) of fruits per plant. Rana *et al*. (2016) also observed significant variation in number of fruits per plant among cucumber genotypes. The yield of fruits per plant eventually contributes the total yield of fruit for each genotype. Among the studied squash genotypes, total fruit yield was varied significantly. The maximum total yield of fruit was obtained in First Runner (89.43 t/ha) preceded to SQ-001 (74 t/ha) and Cheonlima (63.48 t/ha) which was statistically different from other accessions, whereas the minimum total yield of fruit was obtained in case of Balam House (35.78 t/ha). These results corroborated with the findings of Akhter *et al.* (2018) in squash and Abdein *et al.* (2017) in sweet gourd. Uddain *et al.* (2019) also observed significant variation among the different genotypes of Zucchini squash in respect of weight of fruits per plant.

Table 2. Mean performance of squash genotypes for vegetative characters at first harvest

Genotypes	Plant height (cm)	Stem diameter (cm)	Number of leaves	Number of nodes
G1=First Runner	34.13^{bcde}	13^{a}	24.6^{ab}	14.3^{a}
G2=Alaska	33.33^{def}	12.13^{b}	22^{cde}	12.93^{bcd}
G3=Blossom House	33.68^{cdef}	11.45^{cde}	22.2^{cde}	11.73^{ef}
G4=Balam House	32.17^{f}	9.21^{j}	20.27^{f}	11.53^{ef}
G5=Cheonlima	33.61^{cdef}	11.6^{bcd}	25^{a}	14.27^{a}
G6=Hungnong Squash	32.5^{1ef}	11.63^{bcd}	23.27^{bcd}	12.07^{def}
G7=Runner	34.69^{abcd}	11.89^{bc}	23.87^{ab}	13.53^{abc}
G8=SQ-001	35.11^{abcd}	10.9^{efg}	25^{a}	14^{ab}
G9=SQ-002	36.05^{a}	11.29^{cde}	21.2^{ef}	13.93^{ab}
G10=SQ-003	34.31^{abcde}	11.36^{cde}	21.67^{ef}	11.87^{def}
G11=SQ-004	35.92^{ab}	10.03^{hi}	23.47^{abc}	11.47^{f}
G12=SQ-005	34.59^{abcd}	11.05^{def}	21.47^{ef}	11.87^{def}
G13=SQ-006	35.23^{abc}	10.48^{fgh}	21.8^{def}	12.6^{cde}
G14=SQ-007	34.03^{cde}	9.75^{ij}	21.07^{ef}	13.6^{abc}
G15=SQ-008	34.61^{abcd}	10.38j^{hi}	21^{ef}	12.93^{bcd}
Mean	34.27	11.08	22.52	12.84
SD	0.99	0.32	0.75	0.54
LSD	1.81	0.66	1.57	1.09

Means followed by the same letter (s) in a column do not differ significantly

Table 3. Mean performance of squash genotypes for various flowering characters

Genotypes	Days to flower bud initiation	Days to first male flowering	Days to first femal	Number of male flowers	Number of female flowers	Viable pollen (%)
G1=First Runner	20.86^{def}	30.53^{f}	35.80^{h}	18.73^{defg}	13.13^{b}	91.1^{abc}
G2=Alaska	22.20^{bcd}	32.47^{ef}	37.73^{g}	18.4^{fg}	12.87^{bc}	89.75^{bc}
G3=Blossom House	23.69^{ab}	31.73^{ef}	40.20^{cde}	18.53^{efg}	10.67^{ef}	87.13^{d}
G4=Balam House	24.58^{a}	33.87^{e}	41.80^{b}	18.93^{defg}	10.67^{ef}	84.47^{e}
G5=Cheonlima	22.70^{bc}	32.26^{ef}	40.33^{cde}	19.33^{bcdef}	12.8^{bc}	89.89^{abc}
G6=Hungnong Squash	22.89^{abc}	33.13^{e}	41^{bcd}	20.6^{a}	10.6^{ef}	89.11^{cd}
G7=Runner	19.29^{f}	37.73^{cd}	35.73^{h}	20.13^{ab}	14.13^{a}	90.28^{abc}
G8=SQ-001	21.49^{cde}	36.53^{d}	39.93^{def}	18.40^{fg}	12.93^{bc}	90.42^{abc}
G9=SQ-002	20.05^{ef}	42.87^{a}	43.06^{a}	20.0^{6abc}	12^{cd}	89.88^{abc}
G10=SQ-003	22.67^{bc}	43.47^{a}	36^{h}	19.73^{abcd}	11^{e}	92.36^{a}
G11=SQ-004	22.08^{bcd}	42.33^{a}	41.2^{bc}	19.6^{abcd}	12.8^{bc}	89.99^{abc}
G12=SQ-005	21.99^{bcd}	42.40^{a}	39.06^{f}	19.06^{cdef}	12.53^{bc}	91.02^{abc}
G13=SQ-006	22.59^{bc}	39.27^{c}	41.27^{bc}	19.46^{bcde}	12.8^{bc}	91.79^{ab}
G14=SQ-007	21.67^{cde}	39.53^{bc}	36.27^{h}	18.73^{defg}	9.87^{f}	89.91^{abc}
G15=SQ-008	22.48^{bcd}	41.80^{ab}	39.53^{f}	17.93^{g}	11.27^{de}	89.86^{abc}
Mean	22.08	37.33	39.26	19.18	12	89.80
SD	0.92	0.52	0.53	0.53	0.48	1.28
LSD	1.71	2.33	1.07	1.06	0.99	2.54

Means followed by the same letter (s) in a column do not differ significantly

Table 4. Mean performance of squash genotypes for various fruit characters

Genotypes	Days to first harvest	Nodes at first fruit harvest	Fruit length (cm)	Fruit diameter (cm)	Fruit weight (g)	No of fruits per plant	Fruit yield per plant (Kg)	Total yield (t/ha)
First Runner	58.47^{cde}	5.53^{cde}	45.58^{a}	19.67^{ef}	1165.50^{a}	10.2^{a}	11.92^{a}	89.43^{a}
Alaska	58.33^{cde}	5.58^{bcd}	34.43^{b}	17.47^{f}	797.60^{gh}	8.90^{de}	7.14^{efg}	53.53^{ef}
Blossom House	61.80^{a}	6.50^{a}	31.30^{de}	20.06^{ef}	816.70^{efg}	7.00^{h}	5.73^{h}	42.98^{h}
Balam House	62.67^{a}	6.34^{ab}	31.10^{de}	19.39^{ef}	810.10^{fgh}	6.20^{i}	4.77^{i}	35.78^{i}
Cheonlima	58.87^{cd}	5.77^{abc}	44.10^{a}	19.72^{ef}	948.90^{bc}	8.90^{def}	8.46^{c}	63.48^{c}
Hungnong Squash	59.8^{bc}	5.90^{abcd}	45.42^{a}	19.19^{ef}	1002.80^{b}	7.93^{g}	7.99^{cde}	59.98^{cd}
Runner	54.00^{h}	5.03^{e}	27.62^{f}	21.19^{e}	742.24^{h}	10.00^{ab}	7.22^{efg}	54.13^{ef}
SQ-001	55.80^{g}	5.19^{de}	32.2^{cd}	25.48^{cd}	1013.00^{b}	9.50^{bc}	9.95^{b}	74.00^{b}
SQ-002	61.07^{ab}	5.8a^{bcd}	34.4^{b}	21.02^{e}	909.30^{cd}	9.10^{cd}	8.31^{cd}	62.33^{cd}
SQ-003	55.93^{fg}	6.06^{abc}	11.84^{g}	38.11^{a}	921.10^{bcd}	8.13^{g}	7.47^{def}	56.05^{de}
SQ-004	57.73^{de}	6.28^{abc}	34.48^{b}	24.59^{d}	896.90^{cde}	8.30^{fg}	7.47^{def}	56.05^{de}
SQ-005	56.87^{efg}	6.16^{abc}	30.06^{e}	20.99^{e}	822.30^{efgh}	8.27^{g}	6.80^{fg}	51.0^{fg}
SQ-006	60.00^{bc}	5.92^{abc}	34.10^{bc}	18.85^{ef}	863.90^{defg}	8.93^{d}	7.90^{cde}	58.88^{cd}
SQ-007	55.60^{gh}	5.06^{e}	11.43^{g}	23.77^{b}	873.50^{defg}	7.00^{h}	6.34^{gh}	47.55^{gh}
SQ-008	57.53^{def}	5.55^{bcd}	31.7^{de}	27.42^{c}	987.18^{bc}	8.50^{ef}	8.21^{cd}	61.55^{cd}
Mean	58.29	5.78	31.97	22.93	904.74	8.46	7.71	57.78
SD	0.84	0.43	0.97	1.18	49.66	0.28	0.55	4.18
LSD	1.69	0.79	1.92	2.65	93.10	0.55	0.92	6.88

Means followed by the same letter (s) in a column do not differ significantly

Variability of yield contributing characters

The identification and utilization of an extensive germplasm is the prerequisite for improvement of a specific crop by adapting an appropriate plant breeding program. Regarding these, precise and exhaustive descriptions of the genotypes with the patterns of their genetic diversity can promote the introgression of current squash genetic base. In variability studies, high value of coefficient of variation (%CV) was found in number of nodes per plant at first harvest (5.11%), fruit yield per plant (7.13%), fruit diameter (6.89%), and fruit weight (6.14%). On the other hand, the lowest CV value was recorded in days to first female flowering (1.63%). The estimated genotypic variance (σ^2g) was higher than their corresponding environmental variances (σ^2e) for all the traits, except for plant height and number of nodes at first harvest that was very negligible (Table 5). Among the 15 accessions, the high magnitude of genotypic coefficient of variation (GCV) along with phenotypic coefficient of variation (PCV) were recorded for fruit diameter followed by the fruit yield per plant, total yield/ha and number of female flowers per plant. Very low level of GCV along with PCV was found in case of viable pollen percentage along with plant height at first harvest. Most of the characters had low GCV values than PCV values indicated considerable influence of environment in the expression of all the traits (Table 6). High GCV indicates the presence of exploitable genetic variability for the traits, which can facilitate selection (Muralidhara and Narasegowda, 2014; Yadav *et al*., 2009).

Heritability estimation gives an insight into the extent of genetic control to express a particular trait and phenotypic reliability in predicting its breeding value (Ndukauba *et al.*, 2015, Nahar *et al.*, 2016). The heritability in combination with genetic advance (GA)

Table 5. Estimates of genetic parameters for various characteristics in squash genotypes

Parameters	Mean	MSS	CV %	σ^2_g	σ^2_{ph}	σ^2_e
Plant height (cm) at first harvest	34.27	3.67**	3.16	0.83	2.01	1.17
Stem diameter (cm) at first harvest	11.08	2.90***	3.55	0.91	1.07	0.16
Number of leaves at first harvest	22.52	7.26***	4.17	2.13	3.01	0.88
Number of nodes at first harvest	12.84	3.19***	5.11	0.92	1.35	0.43
Days to flower bud initiation	22.08	5.26***	4.64	1.40	2.45	1.05
Days to first male flowering	37.33	65.33***	3.73	21.13	23.07	1.94
Days to first female flowering	39.26	17.29***	1.63	5.63	6.04	0.41
Number of male flowers	19.18	1.71***	3.29	0.44	0.84	0.40
Number of female aflowers	12.00	4.56***	4.95	1.40	1.76	0.35
Viable pollen (%)	89.80	10.80***	1.69	2.83	5.14	2.31
Days to first harvest	58.30	18.23***	1.74	5.73	6.76	1.03
Nodes at first fruit harvest	5.78	0.63*	8.24	0.14	0.36	0.23
Fruit length (cm)	31.97	96.72***	3.59	31.8	33.12	1.32
Fruit diameter (cm)	22.93	92.99***	6.89	30.17	32.67	2.50
Fruit weight (g)	904.74	34814***	6.14	10573	13668	3095
Number of fruits per plant	8.46	3.74***	3.87	1.21	1.32	0.11
Fruit yield per plant (Kg)	7.71	8.57***	7.13	2.76	3.06	0.30
Total yield (t/ha)	57.78	477.72***	7.12	153.6	170.5	16.93

* Significant at 5% level of probability; ** Significant at 1% level of probability and; *** Significant at 0.1% level of probability.

increases the intensity of selection in a breeding program. High heritability indicates less environmental influence in the observed variation (Abdein *et al.,* 2017). Thus, genetic advance measures the difference between the mean genotypic values of the original population from which these are selected. Almost all the attributes showed high heritability except nodes at first harvest (38.89%) and plant height (41.49%). The highest estimates of genetic advance (in percent of mean) were determined for total yield/ha, fruit yield per plant, fruit weight, fruit length, fruit diameter and number of fruits per plant (Table 6).

Correlation analysis (Table S1), the trait plant height had significant positive correlation with days to first male flowering, number of female flowers, number of fruits per plant, and fruits yield per plant. Other attributes such as number of leaves at first harvest was negatively and significantly correlated with the days to first male flowering. The number of nodes at first harvest showed positive and significant correlation with number of female flowers, single fruit weight, number of fruits per plant and yield of fruits ton per hectare. The number of female flowers per plant had significant and positive correlation with number of

Table 6. Estimation of heritability and genetic advance (GA) in squash genotypes

Parameters	GCV	PCV	ECV	Herita-bility	GA (5%)	GA (% mean)
Plant height (cm) at first harvest	2.67	4.14	1.47	41.49	1.21	3.53
Stem diameter (cm) at first harvest	8.61	9.34	0.73	85.05	1.81	16.34
Number of leaves at first harvest	6.48	7.71	1.22	70.76	2.53	11.23
Number of nodes at first harvest	7.47	9.05	1.58	68.15	1.63	12.70
Days to flower bud initiation	5.36	7.10	1.73	57.14	1.84	8.33
Days to first male flowering	12.31	12.87	0.56	91.58	9.07	24.28
Days to first female flowering	6.04	6.26	0.22	93.21	4.72	12.02
Number of male flowers	3.46	4.78	1.32	52.38	0.99	5.16
Number of female flowers	9.86	11.04	1.18	79.73	2.18	18.14
Viable pollen (%)	1.87	2.53	0.65	55.05	2.57	2.86
Days to first harvest	4.11	4.46	0.35	84.76	4.54	7.79
Nodes at first fruit harvest	6.47	10.38	3.91	38.89	0.48	8.31
Fruit length (cm)	17.64	18.00	0.36	96.01	11.38	35.61
Fruit diameter (cm)	23.95	24.93	0.98	92.35	10.87	47.42
Fruit weight (g)	11.37	12.92	1.55	77.36	186.31	20.59
Number of fruits per plant	13.00	13.58	0.58	91.67	2.17	25.64
Fruit yield per plant (Kg)	21.55	22.69	1.14	90.2	3.25	42.16
Total yield (t/ha)	21.45	22.60	1.15	90.07	24.23	41.93

fruits per plant. Fruit length had significant and positive correlation with fruit weight, number of fruits per plant and fruit yield per plant and also significantly and negatively correlated with fruit diameter. One of the most important traits of fruit weight was significantly and positively correlated with fruit yield per plant. Highly significant and positive association of fruit yield per plant was recorded with the plant height, number of leaves per plant, number of nodes at first harvest, fruit length, fruit weight and number of fruits per plant. Similar findings were noticed by Gomes *et al.* (2020) in Brazilian germplasm of winter squash and Mohsin *et al.* (2017) in pumpkin.

In cluster analysis (CA), the cluster means of 15 accessions of squash showed that the mean values of the clusters varied in magnitude for maximum characters (Table S2. The cluster II showed the highest total yield value along with the second highest fruit length and fruit diameter value, the highest number of fruits per plant value and the highest yield per plant value, which could contribute to total yield. From the clustering comparison of the means, it was found that cluster II expressed the best agronomic quantitative yield contributing traits and yield potentials. Comparing the means of all clusters it was showed that First Runner from cluster I, Cheonlima and SQ-001 from cluster II, SQ-008 from cluster IV and SQ-006 from cluster III expressed the best quantitative and qualitative traits and yield potentials which could be effective for the improvement of yield of squash (Fig. S1). Gomes *et al.* (2020) reported similar results in Brazilian germplasm of winter squash. Ene *et al.* (2016) also reported similar findings in cucumber genotypes. This suggests that the genotypes of squash of the same origin have diverse and broad genetic basis.

Principal component analysis (PCA) is an important multivariate technique used to examine associations between characters and measures the genetic variability of genotypes (Balkaya *et al.*, 2010; Ene *et al.*, 2016). The three principal components (PC1, PC2 and PC3) can be retained to describe the variability among the squash genotypes (Table S3). The first three components explain 63% of the total genetic variation

while the first two principal components accounted for 53% and first component accounted for 32.4% of the total genetic variation among the 18 attributes describing 15 different genotypes. The first component (PC1) described 32.4% of the total variation, second component (PC2) explained 20.6% of the total variability and the third component (PC3) evaluated only 10.02% of the total variation. The PC1 was positively and strongly associated with the plant height (0.18), stem diameter (0.29), number of leaves (0.29), number of nodes (0.28), number of female flower (0.30), viable pollen percentage (0.25), fruit length (0.13), fruit weight (0.21), number of fruits per plant (0.39) and fruit yield per plant (0.36). The PC2 was positively and highly associated to days to first harvest (0.36) and fruit length (0.47). In case of PC3, it was strongly associated with plant height (0.47), days to first male flowering (0.39), days to first female flowering (0.49), number of male flower (0.31), number of female flowers (0.24) and fruit length (0.16). Whereas, morphological (qualitative) characterization showed that limited variability present in the genotypes in respect of some characters viz., plant vigor, stem and leaf pubescence, and flower colour. Significant variability was observed in case of fruit shape, fruit size, fruit skin color and luster respectively. This finding corroborated with the findings of El-Hadi *et al.* (2014) in squash and partly agrees with the results by Khawla *et al.* (2019) in Tunisian squash and Nahar *et al.* (2016) in sweet gourd.

Qualitative characterization

Selection of qualitative traits is also very important in successful crop breeding program. Significant variation was found under this research in relation to different qualitative traits of squash accessions. Most significant variation was found in fruit size, fruit shape and fruit skin color followed by lustre (Table S4). The fruit colour, size, shape was morphologically different because of the genetic makeup present in the studied genotypes (Fig. 1). A similar morphological variation of qualitative traits was reported by Uddain *et al.* (2019) in Zucchini squash, Muralidhara and Narasegowda (2014) in pumpkin, Nahar *et al.* (2016) in sweet gourd and Ene *et al*. (2016) in cucumber.

CONCLUSION

High heritability coupled with high genetic advance observed for total yield/ha, fruit yield per plant, fruit weight, fruit length and fruit diameter in this set of germplasm indicated that, these traits will be the main contributing factors for further crop improvement programme. Significant and positive association of fruit yield per plant with number of leaves per plant, number of nodes at first harvest, fruit length, fruit weight and number of fruits per plant suggested that, these traits were inter-related and collectively contributed to the final yield of squash. The principal component analysis showed that number of fruits per plant, fruit yield per plant, fruit length and days to first female flowering were the most discriminating factors that accounted for the genetic diversity of squash and would be considered for squash improvement program. Considering yield performance, it can be recommended that First Runner is the highest yielding genotype in similar environment which might be used as a parent in developing high yielding variety of squash. The genotype Runner can be used as a parent for developing early fruiting variety whereas Alaska would be preferable for improving the appearance of fruits.

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Original Research Paper

Diversity analysis of phenotypic traits in okra (*Abelmoschus esculentus* L. Moench)

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ABSTRACT

It is necessary to obtain cultivars which provide high yield by exploiting desirable traits from wild genotypes of okra (*Abelmoschus esculentus* L. Moench). Okra genotypes were evaluated for phenotypic traits during 2018. High genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) occurred for nine traits and narrow differences between GCV and PCV indicated the influence of environment was negligible. High estimates of heritability, coupled with moderate to high genetic advance as a percent over mean, were recorded for nine traits. Thousand seed weight had a positive, significant, correlation with yield per hectare. Plant height and number of fruits per plant had direct and positive effects towards the yield per hectare The principal component analysis indicated the first 3 principal components contributed 80.517% of total variation among traits describing genotypes. Cluster analysis indicated hybridization of genotypes among inter-cluster I and II could be used to develop stable, uniform varieties in diverse climatic conditions. EC359637 and IARI Selection 2 are distantly placed and can be used for overall improvement in further crop breeding.

Keywords: Cluster analysis, GCV, heritability, okra, PCV, principal component analysis and yield.

INTRODUCTION

Okra or ladies' finger (*Abelmoschus esculentus* L.) belongs to the mallow family i.e., Malvaceae. It is a flowering, hairy, herbaceous annual plant grown for its edible pods. Its origin is considered to be at western regions of Africa because of the presence of diverse wild species (De Candolle, 1886). The development of better-quality vegetables, has a lot higher export potential not only in India but has recently discovered a way to the African and South-Eastern Nations as compared to other field crops (Ankita *et al.,* 2021).

Okra is one of the major vegetable crops grown for its high nutritive content, high export potential and antioxidant value. Okra fruits are edible constituting a high source of protein and minerals with about 88% moisture, 7.7% carbohydrate, 2.2% protein, 1.5% iron, 1.1% fibre, 0.7% mineral matter, 0.09% calcium, 0.2% fat, 0.08% phosphorous and 41 (kcal) calorific values (Bhat and Bisht, 2006). The vitamin content is 58 IU of vitamin A, 0.06 mg vitamin B, 0.06 mg nicotinic acid, 0.06 mg riboflavin and 16 mg vitamin C per 100 grams of raw okra fruits (USDA, 2019).

The yield potential is a limiting factor because of poor yielding varieties and the incidence of different pests and diseases (Tripathi *et al.,* 2011). Crop improvement in okra focuses on plant height, higher yield, early flowering, fruit length and biotic and abiotic stress resistance (Ranga *et al.,* 2019). Assessment of genotypes for estimating genetic diversity for yield and yield contributing attributes is very essential and the information about the variation present in accessible breeding materials helps in successful selection of parents for further use in crop improvement. The current investigation was undertaken to assess the nature and magnitude of genetic divergence and to identify the potential okra genotypes towards yield and its association with other morphological traits.

MATERIALS AND METHODS

Planting material

The field experiment was at the Experimental Farm of the Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, during February – May 2018. The accessions were sourced from the Indian Council of Agricultural Research – National Bureau of Plant Genetic Resources, New Delhi and their details are represented in Table 1.

Field evaluation and data collection

The soil of the experimental site was loamy in origin and was ploughed before sowing. The climate of the area represents a tropical condition with semi-arid, hot and subtropical monsoon types. Before sowing, farmyard manure and urea were applied as basal doses. The recommended package of practices and plant protection measures to raise a good crop were timely and uniformly applied.

The experiment was carried out in a completely randomized block design with three replications. Each accession was soaked in water for 8 hours and sown at a spacing of 45×30 cm of 5 m length. The following observations were recorded during plant growth and development stages viz., days to 50% flowering [DF], days to 80% maturity [DM], plant height [PH (cm)], first flowering node [FFN], fruit diameter [FD (cm)], fruit length [FL (cm)], number of fruit per plant [FP], number of seed per fruit [SF], 1000 seed weight [TSW (g)], yield per plant [YP (g)] and yield per hectare [YH (t/ha)] from 5 representative plants of each genotype.

Data analysis

Data collected were subjected to ANOVA (Analysis of Variance) to evaluate the presence of statistically significant differences among genotypes for the traits studied (Panse and Sukhatme, 1954). Genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) was calculated as per the formula suggested by Burton, 1952. Genetic advance and heritability were calculated by using the formula of Lush, (1949) and Allard (1960). Heritability of more than 80% is considered high. Genotypic correlation coefficients, path analysis, principal component analysis (PCA) and cluster analysis were calculated using OP-STAT (Sheoran *et al.,* 1998) and PAST (Hammer *et al.,* 2001). Yield per hectare was taken as a dependent variable whereas, all other traits were considered as independent variables.

Table 1: Fifteen okra (*Abelmoschus esculentus* L. Moench) genotypes repatriated from NBPGR used in the study.

Sl. No.	Genotype	Country	Acquired On	Cultivar Name
1.	EC305615	Bangladesh	28/05/90	T/B-78/-
2.	EC305740	Italy	29/05/90	ORS-773/-
3.	EC305768	Italy	29/05/90	ORS-202/-
4.	EC306696	Singapore	08/06/90	ORS-1106/ESC
5.	EC359637	-	-	-
6.	IC003769	India	-	-
7.	IC010265	Gujarat, India	09/01/63	-
8.	IC013356	India	-	-
9.	IC013664	Tamil Nadu, India	14/09/67	-
10.	IC014018	India	-	-
11.	IC014026	India	-	-
12.	IC014600	Himachal Pradesh, India	31/08/70	-
13.	Akola-Bahar	Maharashtra, India	-	-
14.	IARI Selection 2	Delhi, India	-	-
15.	AKO107	Maharashtra, India	-	-

RESULTS AND DISCUSSION

Analysis of variance and variability parameters

The genotypes showed high positive and significant variations for all the traits (Table S1). Yield per hectare obtained the highest positive and significant variation (73394588.940**) and the lowest was obtained for fruit diameter (0.280**). Gondane and Lal (1994) and Alam and Hossain (2008) also obtained similar results in okra.

Yield per hectare ranged from 5313.870-33714.430 kg/hectare with a mean of 20056.945 grams per genotype. Profitable yield i.e., production of 25000-30000 kg per hectare was achieved by EC305615, EC305740, IC013356 and IC0104018. In the genetic variability studies (Table 2), the phenotypic coefficient of variation (PCV) was higher than the comparing genotypic coefficient of variation (GCV) for every trait with the close relationship between them, therefore, the environment has low impact and subsequently, the phenotypic performance of traits ought to be utilized for selection. Moderate and high GCV values were observed for most of the traits except fruit diameter, days to 80% maturity and days to 50% flowering which exhibited the presence of a high magnitude of genetic diversity in the population examined. The previous workers also observed a similar trend of greater magnitude of PCV and GCV (Ranga *et al.,* 2021; Shanthakumar and Salimath, 2010; Prakash *et al.,* 2011). Narrow differences between the phenotypic and genotypic coefficient of variation in most of the traits indicated that they were comparatively stable to environmental variation (Majumdar *et al.,* 1969). However, fruit diameter and yield per hectare registered wider variation between PCV and GCV.

Heritability is a good index of transmission of traits from parents to their off-springs (Falconer, 1981). Among ten traits, nine traits displayed high heritability (low <30%, moderate 31% to 60% and high >60%) coupled with high genetic advance (low <10%, moderate 11% to 20% and high >20%) as percent over mean. This focuses on the predominance of additive gene effects for these traits; thus, crop improvement through selection based on these traits would be beneficial. Fruit girth showed low heritability accompanied with moderate genetic advance over mean portraying the role of non-additive effects and hence selection based on

Table 2. Estimates of variability parameters for various traits of okra genotypes.

Traits	Mean	Range	GCV	PCV	h^2	GA	GA as % of Mean
DF	37.71	28.67-50.33	17.63	18.92	86.79	12.76	33.83
DM	76.60	54.67-100.00	16.17	16.42	97.01	25.14	32.82
PH (cm)	86.39	42.27-154.50	39.10	39.16	99.70	69.49	80.43
FFN	6.01	2.70-11.13	36.88	38.55	91.56	4.37	72.70
FD (cm)	2.00	1.50-2.43	11.35	21.00	29.21	0.25	12.64
FL (g)	14.05	7.90-18.80	21.65	22.23	94.85	6.10	43.44
FP	21.23	6.40-40.60	54.41	56.56	92.56	22.90	107.84
SF	51.24	34.00-89.60	27.31	27.56	98.19	28.57	55.75
TSW (g)	212.33	61.44-422.64	40.93	52.67	60.38	139.11	65.51
YP (g)	271.31	73.37-453.10	38.72	39.01	98.54	214.84	79.19
YH (kg/ha)	20056.94	5313.87-33714.43	17.34	34.96	24.61	3556.47	17.73

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare, h^2: heritability, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, GA: Genetic advance)

this trait may not be rewarding. High estimates of heritability and genetic advance were also reported by Nwangburuka *et al,* (2012) and Hazra and Basu (2000).

Correlation coefficient analysis

The correlation is the overall or net impact of the segregating genes; few genes may increase both the traits leading to the positive correlation whereas, the others might increase the one and decrease the other causing the negative correlation (Falconer, 1981). Thus, to accumulate an optimum combination of yield contributing traits in a single genotype, it is essential to know the implication of the interrelationship of various traits (Ranga *et al.,* 2019).

In the present investigation, the genotypic and phenotypic correlation coefficient analysis is represented in Table 3 and Fig.1. Days to 80% maturity showed a highly significant and positive correlation with days to 50% flowering (0.488**, 0.427**). Plant height showed a significant and positive correlation with days to 50% flowering (0.346*, 0.318*). Fruit diameter showed a highly significant and positive genotypic correlation with days to 80% maturity (0.517**, 0.314*) and plant height (0.714**, 0.393**), and only genotypic correlation was positive and significant for day to 50% maturity (0.707**). Number of fruits per plant showed a highly

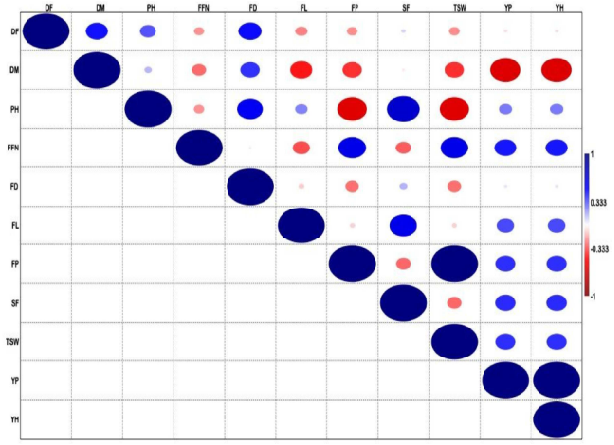

Fig. 1. Correlation coefficient studies in okra genotypes

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare)

significant and positive correlation with first flowering node (0.602**, 0.550**). Number of seeds per fruit showed a highly significant and positive correlation with plant height (0.693**, 0.684**) and fruit length (0.580**, 0.564**). 1000 seed weight showed a highly significant and positive correlation with fruit length (0.453**, 0.309*). Yield per plant showed a highly significant and positive correlation with first flowering node (0.459**, 0.442**), fruit length (0.357*, 0.355*), number of fruits per plant (0.417**, 0.395**) and number of seed per fruit (0.421**, 0.411**). Yield per hectare showed a highly significant and positive correlation with 1000 seed weight (0.391**, 0.340*). Comparable results for okra yield having a positive relationship were proposed by Ranga *et al.* (2021), Reddy *et al.* (2012), Raval *et al.* (2019) and Duggi *et al.* (2013). Yield per plant showed a highly significant and negative correlation with days to 80% maturity (-0.635**, -0.627**). Yield per hectare showed a significant and positive genotypic correlation with days to 50% flowering (-0.379*).

Path coefficient analysis

Path analysis provides information about the cause and effect in understanding the association between two variables. It allows the assessment of the direct effects of different traits on crop yield just as their indirect effects by means of other component traits. Hence, it gives a premise for the selection of predominant genotypes from diverse populations (Komolafe *et al.,* 2021). The genotypic and phenotypic path coefficient analysis is represented in Table 4 and the data revealed that plant height (3.893, 0.239) had the highest direct positive effect towards the yield per hectare and other traits such as days to 80% maturity (0.811, 0.053), number of fruits per plant (2.871, 0.174) had direct effects. Traits such as days to 50% maturity (-0.575, -0.267), and yield per plant (-1.868, -0.197) had a direct effect with a negative sign. Residual effect (0.475, 0.800) indicated the effect of other possible independent traits, which were not included in the study, on the dependent variable i.e., yield per hectare. The results are in accordance with the findings of Ranga *et al.,* (2021); Dwivedi and Sharma (2017); Das *et al.* (2012).

Principal component analysis

Principal component analysis (PCA) reflects the importance of the largest contributor to the total variations at each axis of differentiation (Sharma,

Table 3. Genotypic and phenotypic correlation coefficient studies in okra genotypes.

(Bold numbers indicated positive and significant correlation between two traits)

Traits		DF	DM	PH (cm)	FFN	FD (cm)	FL (cm)	FP	SF	TSW (g)	YP (g)	YH (kg/ha)
DF	rg	1.000										
	rp	1.000										
DM	rg	0.488**	1.000									
	rp	0.427**	1.000									
PH (cm)	rg	0.346*	0.141NS	1.000								
	rp	0.318*	0.141NS	1.000								
FFN	rg	-0.205NS	-0.294*	-0.215NS	1.000							
	rp	-0.218NS	-0.290NS	-0.205NS	1.000							
FD (cm)	rg	0.707**	0.517**	0.714**	0.009NS	1.000						
	rp	0.285NS	0.314*	0.393**	-0.085NS	1.000						
FL (cm)	rg	-0.253NS	-0.462**	0.244NS	-0.353*	-0.150NS	1.000					
	rp	-0.218NS	-0.443**	0.237NS	-0.340*	-0.064NS	1.000					
FP	rg	-0.226NS	-0.415**	-0.627**	0.602**	-0.370*	-0.093NS	1.000				
	rp	-0.210NS	-0.389**	-0.603**	0.550**	-0.208NS	-0.089NS	1.000				
SF	rg	0.080NS	-0.044NS	0.693**	-0.323*	0.183NS	0.580**	-0.295*	1.000			
	rp	0.078NS	-0.040NS	0.684**	-0.305*	0.113NS	0.564**	-0.293NS	1.000			
TSW (g)	rg	-0.171NS	-0.090NS	0.036NS	-0.244NS	-0.206NS	0.453**	0.066NS	0.110NS	1.000		
	rp	-0.112NS	-0.052NS	0.022NS	-0.249NS	0.022NS	0.309*	0.026NS	0.098NS	1.000		
YP (g)	rg	-0.064NS	-0.635**	0.262NS	0.459**	0.099NS	0.357*	0.417**	0.421**	-0.162NS	1.000	
	rp	-0.050NS	-0.627**	0.259NS	0.442**	-0.002NS	0.355*	0.395**	0.411**	-0.144NS	1.000	
YH (kg/ha)	rg	-0.379*	0.047NS	0.108NS	0.054NS	0.258NS	0.116NS	0.021NS	0.067NS	0.391**	-0.194NS	1.000
	rp	-0.201NS	0.045NS	0.047NS	-0.013NS	0.109NS	0.044NS	0.004NS	0.033NS	0.340*	-0.107NS	1.000

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare; rg = genotypic correlation coefficient, rp = phenotypic correlation coefficient)

Table 4. Genotypic and phenotypic path coefficient analysis for various okra genotypes.
(Diagonal and bold values indicate direct effect of traits on yield per plant)

Traits		DF	DM	PH (cm)	FFN	FD (cm)	FL (cm)	FP	SF	TSW (g)	YP (g)	Correlation of YH (kg/ha)
DF	gp	**-0.575**	0.395	1.347	0.011	-0.701	-0.375	-0.650	-0.120	0.169	0.119	**-0.379***
	pp	**-0.267**	0.023	0.076	-0.013	0.029	0.009	-0.037	0.002	-0.033	0.010	**-0.201**^{NS}
DM	gp	-0.281	**0.811**	0.547	0.016	-0.512	-0.683	-1.191	0.065	0.089	1.186	**0.047**^{NS}
	pp	-0.114	**0.053**	0.034	-0.018	0.032	0.018	-0.068	-0.001	-0.015	0.123	**0.045**^{NS}
PH (cm)	gp	-0.199	0.114	**3.893**	0.011	-0.708	0.361	-1.800	-1.038	-0.035	-0.490	**0.108**^{NS}
	pp	-0.085	0.008	**0.239**	-0.012	0.040	-0.010	-0.105	0.017	0.007	-0.051	**0.047**^{NS}
FFN	gp	0.118	-0.239	-0.838	**-0.053**	-0.009	-0.522	1.728	0.484	0.242	-0.858	**0.054**^{NS}
	pp	0.058	-0.016	-0.049	**0.061**	-0.009	0.014	0.096	-0.007	-0.074	-0.087	**-0.013**^{NS}
FD (cm)	gp	-0.407	0.419	2.779	0.000	**-0.991**	-0.222	-1.064	-0.274	0.203	-0.186	**0.258**^{NS}
	pp	-0.076	0.017	0.094	-0.005	**0.103**	0.003	-0.036	0.003	0.006	0.000	**0.109**^{NS}
FL (cm)	gp	0.146	-0.374	0.948	0.019	0.148	**1.480**	-0.267	-0.869	-0.448	-0.666	**0.116**^{NS}
	pp	0.058	-0.024	0.057	-0.021	-0.007	**-0.040**	-0.015	0.014	0.092	-0.070	**0.044**^{NS}
FP	gp	0.130	-0.336	-2.440	-0.032	0.367	-0.138	**2.871**	0.442	-0.065	-0.778	**0.021**^{NS}
	pp	0.056	-0.021	-0.144	0.034	-0.021	0.004	**0.174**	-0.007	0.008	-0.078	**0.004**^{NS}
SF	gp	-0.046	-0.035	2.696	0.017	-0.181	0.858	-0.847	**-1.499**	-0.109	-0.787	**0.067**^{NS}
	pp	-0.021	-0.002	0.163	-0.019	0.012	-0.023	-0.051	**0.024**	0.029	-0.081	**0.033**^{NS}
TSW (g)	gp	0.098	-0.073	0.140	0.013	0.204	0.670	0.189	-0.165	**-0.988**	0.303	**0.391****
	pp	0.030	-0.003	0.005	-0.015	0.002	-0.012	0.005	0.002	**0.297**	0.028	**0.340***
YP (g)	gp	0.037	-0.515	1.021	-0.024	-0.099	0.528	1.197	-0.631	0.160	**-1.868**	**-0.194**^{NS}
	pp	0.013	-0.034	0.062	0.027	0.000	-0.014	0.069	0.010	-0.043	**-0.197**	**-0.107**^{NS}

gp residual effect: 0.475

pp residual effect: 0.800

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare); gp = genotypic path coefficient, pp = phenotypic path coefficient)

1998). PCA (Table 5 and Table S2) was performed to provide partial visualization of the data set in a reduced dimension and first three principal components have Eigenvalues>1 and contributed to 80.517 percent variation. From the loading of the variables in PC I, it was found that days to 50% flowering, days to 80% maturity and plant height were the dominant features that contributed to 36.662 percent of the total variation. In PCA II, plant height, fruit length, number of seed per fruit, yield per plant and yield per hectare exerted a maximum influence which accounts for 27.862 percent of the total variation. Days to 50 percent flowering, days to 80% maturity, first flowering node and fruit diameter were the dominant features in PCA III which accounted for 15.993 percent of the total variation. Ranga *et al.* (2021), Ahiakpa *et al.* (2013) and Amoatey *et al.* (2015) also indicated high genetic diversity using PCA. Few traits *viz.,* fruit length, 1000 seed weight, number of seed per fruit and fruit yield per plant offered more towards variation as accounted for by different scientists in okra (Bhardwaj *et al.,* 2021; Ahiakpa *et al.,* 2013; Amoatey *et al.,* 2015; Nwangburuka *et al.,* 2012).

Table 5. Eigen value and percent variation explained by first 5 principal components and correlations between PC scores and quantitative traits.

(Bold values indicate traits which are heavy contributors in the particular principal component)

Sr. No.	Traits	PC I	PC II	PC III	PC IV	PC V	Eigen Value
1.	**DF**	**0.221**	0.061	**0.472**	**0.528**	**0.446**	**4.033**
2.	**DM**	**0.365**	-0.188	**0.265**	0.168	-0.072	**3.065**
3.	**PH (cm)**	**0.237**	**0.443**	0.173	-0.256	0.077	**1.759**
4.	**FFN**	**-0.338**	-0.076	**0.360**	**-0.506**	0.026	0.781
5.	**FD (cm)**	0.205	0.170	**0.499**	-0.013	**-0.746**	0.501
6.	**FL (cm)**	-0.056	**0.375**	**-0.430**	**0.301**	**-0.383**	0.476
7.	**FP**	**-0.436**	-0.148	0.141	**0.349**	-0.142	0.190
8.	**SF**	0.084	**0.484**	-0.102	0.187	0.114	0.131
9.	**TSW (g)**	**-0.436**	-0.148	0.141	**0.349**	-0.142	0.064
10.	**YP (g)**	**-0.328**	**0.394**	0.177	-0.030	0.127	0.000
11.	**YH (kg/ha)**	**-0.328**	**0.394**	0.177	-0.030	0.127	0.000
Percent of Total Variance Explained		**36.662**	**27.862**	**15.993**	7.100	4.552	
Cumulative Variation		**36.662**	**64.524**	**80.517**	**87.617**	**92.169**	

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare)

A biplot was drawn using the values of PC I and PC II (Figure 2). The greater the angle between the traits, the lesser the association between them. Placement of genotypes in quadrants signifies variability. Accessions are placed in quadrants using vector scores of PC I and PC II. However, no obvious grouping of genotypes was observed, and some overlapping occurred among groups the relatedness of the genotypes across the collection. In the biplot graph of PCA, quadrant I (+,+) consists of zero accessions formed the cluster 1 which were highly influenced by three traits characters viz. fruit length, yield per plant and yield per hectare through genotypes spread towards midway through X and Y-planes of quadrant-I. The cluster II corresponding to the quadrant II (-,+) contained 5 genotypes, which were influenced by number of seeds per plant, plant height, fruit diameter and days to 50% maturity. Similarly, the cluster III corresponding to quadrant III (-,-) consisted also of 3 genotypes which were influenced by days to 80% maturity only whereas the cluster IV corresponding to quadrant IV (+,-) also consisted of 7 genotypes which were influenced by first flowering node, number of fruits per plant and 1000 seed weight, respectively.

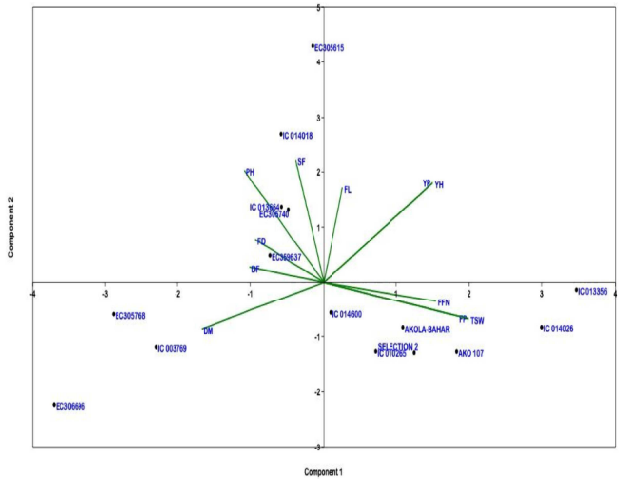

Fig. 2. Biplot between PC1 and PC2 showing contribution of various traits responsible for variability in okra.

Cluster analysis

The hierarchical cluster analysis among genotypes for yield and yield contributing traits grouped genotypes into 2 major clusters (Figure 3). Clustering was not based on a similar geographical origin. Cluster I accommodated 5 genotypes (EC359637, IC013664, EC306696, EC305768, IC003768) and cluster II comprised 10 genotypes (EC305615, EC305740, IC014018, IC010265, IC014600, IC013356,

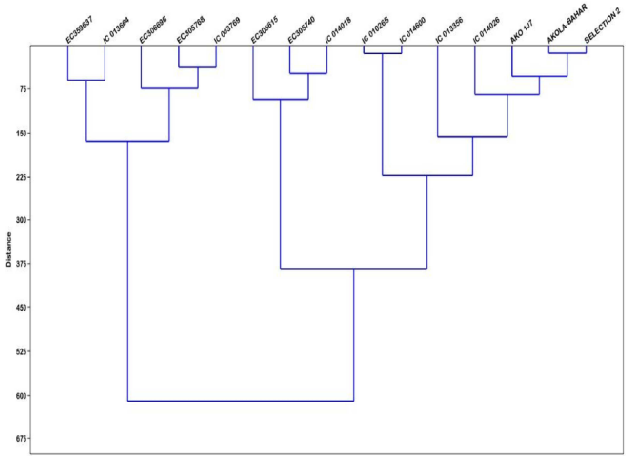

Fig. 3. Dendrogram showing the genetic relationship among fiteen orka genotypes based on quantitative traits

(DF = Days to 50% flowering, DM = Days to 80% maturity, PH = Plant height, FFN = First flowering node, FD = Fruit diameter, FL = Fruit length, FP = Number of fruit per plant, SF = Number of seed per fruit, TSW = 1000 seed weight, YP = Yield per plant and YH = Yield per hectare)

IC014026, AKO107, Akola-Bahar and IARI Selection 2). In order to determine diversity among genotypes, and verify genotypes by distance, cluster analysis placed the genotypes in a single group (Sokal and Sneath, 1973). Genotypes that are located far from each other have more variation between them and can be used to obtain improved cultivars. Genotypes which distantly placed are more diverse; those which are closer are similar morphologically. The maximum intra-cluster distance was observed for EC359637 and IC003769 in sub-cluster 1 and EC305615 and IARI Selection 2 in sub-cluster 2 while maximum inter-cluster distance was observed for EC359637 and IARI Selection 2.

CONCLUSION

Higher variations were observed for number of fruits per plant, yield per plant, yield per hectare and 1000 seed weight displaying a wide range showing the distinction of genotypes in breeding programs. Yield per hectare showed a highly significant and positive correlation with 1000 seed weight. Hence, it can be used for developing high-yielding and bold seeded cultivars resistant to biotic and biotic stress. Plant height and number of fruits per plant had direct and positive effects on the yield per hectare. The first three principal components accounted for a cumulative variance to be 80.517 % of the total variation and traits *viz*. fruit length, plant height, days to 80% maturity and days to 50% flowering assorted for more than 50 % phenotypic variations. Since EC359637 and IARI Selection 2 are distantly placed, therefore, they can be used for overall improvement in further breeding programs.

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Original Research Paper

Impact of pollination strategies on fruit set and fruit growth attributes in jasmine

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ABSTRACT

Jasmine occupies predominant position among the flower crops in India in terms of area, production and productivity. The demand for jasmine flowers is growing day by day owing to its wide range of uses and there is a pressing need for improving the crop by exploring strategies to evolve diverse genotypes. The present study focuses on the hybridization of *Jasminum* spp with the objective of introgression of desirable traits that would aid in creation of wider genetic variability. Pollination is the basis in any hybridization programme. The main aim of this research study was to determine the suitable pollination methods among self, open and cross pollination and to assess the effect of the pollination methods on the fruit set and fruit characteristics. The results of the study revealed that the overall response of *J. auriculatum* was found effective with maximum fruit set percentage. *J. auriculatum* cv Parimullai yielded the highest fruit set of 76.43% under open pollination and the least fruit set rate of 2.14% under self-pollination. Among the possible cross combination involving *J. auriculatum* and *J. grandiflorum* cultivars as seed parents with various pollen parents, *J. flexile* showed considerable results. Cross combination of *J. auriculatum* x *J. flexile* recorded maximum fruit set revealing best cross compatibility while crosses involving *J. sambac* resulted in no fruit set indicating the prevalence of fertilization barriers that hinder hybridization.

Keywords: Fruit set, fruit growth, jasmine and pollination

INTRODUCTION

Jasmine (*Jasminum* spp.) is one of the remuneratively prized and significant traditional flower crops of India. It belongs to the family Oleaceae and is one of the aromatic flowers cultivated since times immemorial and is considered as the most revered flower in our country for its attractive and fragrant flowers. Jasmine flowers are popularly used in preparation of garlands, hair adornments for women, used in religious and ceremonial occasion and also for extracting perfumery oil (Sanchita *et al.,* 2018) which is used in the cosmetic and perfumery industries. India is the largest exporter of jasmine oil in the world accounting for over 40 per cent of total world export. It has extensive application in aromatherapy as jasmine fragrance is effective in treating depression, nervous exhaustion and stress. It is also widely used in the medicinal and pharmaceutical industries (Green and Miller, 2009). Exceptional increase in the consumption of jasmine flowers by the Indian population settled in Middle East countries and the United States of America has led to the augmentative export demand for flower strings of *J. sambac* (Jawaharlal *et al.,* 2012). The genus *Jasminum* is reported to comprise of around 200 species (Bailey 1958). The commercially cultivated jasmine species in Tamil Nadu, Karnataka, Andhra Pradesh, Uttar Pradesh and some parts of Bihar and West Bengal are *J. sambac, J. grandiflorum, J. auriculatum and J. multiflorum*. Exclusive of these commercially important species, lesser-known species namely, *J. nitidum, J. calophyllum* and *J. flexile* also acquire economic importance as they produce flowers which are suitable for use as loose flower, besides being ideal garden plants (Raman, 1969; Ganga *et al*., 2015).

Hybridization leads to the development of new adaptive traits allowing the expansion of new habitats (Johnston *et al*., 2004), fitness enhancement (Burke *et al*., 1998), or the origin of new hybrid lineages

(Grant, 1981; Arnold, 1997). Hybridization can also reinforce reproductive barriers through natural selection for conspecific gene flow (Arnold, 1992), the creation of stable hybrid zones (Barton and Hewitt, 1985), or the formation of introgressive races. Hybridization is not the general outcome whenever congeneric species come into contact because there often are pre- and post-mating barriers that prevent hybridization.

Pollination is the result of pollen being transferred from the anther to the stigma of another flower. Landing of pollen on stigma is no guarantee for seed-set. Failure of fertilization after self-pollination in self-sterile or self-incompatible plants may also be due to the inability of the pollen to germinate on its own stigma. Pollination in many crops has manifested a major influence on the number of fruit set, fruit length, fruit girth and fruit shape (Nirmalaruban *et al.,* 2020). Jasmine varieties released till date are only clonal selections and mutants. Hence there is a dire necessity to evolve hybrids in jasmine using commercial and under-utilized species. Prior attempts at hybridization have failed because of the compatibility and fertilization barriers present among the species. Considering the above, the present study focuses on the method of pollination and its potency on fruit set in jasmine.

MATERIALS AND METHODS

The study was carried out at the Department of Floriculture and Landscape Architecture, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore during 2019-2021. Ten-year-old plants of *J. auriculatum* cultivars CO.1 Mullai, CO.2 Mullai, Parimullai and *J. grandiflorum* cultivars CO.1 Pitchi and CO.2 Pitchi were selected as female parent and Jasminum genotypes like *J. auriculatum* cv CO.1 Mullai, *J. grandiflorum* cv CO.1 Pitchi, *J. sambac, J. multiflorum, J. nitidum, J. calophyllum* and *J. flexile* were used as the pollen source. The experimental layout was a complete randomized design with three replications of each crossing combination. The pollen from the previously bagged flowers was collected from the male parents during 6:00 to 8:00 am in the morning of the succeeding day. Similarly in the female parent, fully opened flowers and mature buds at pre anthesis stage were emasculated between 7.00 to 10.00 am. The pollen collected from the pollen source was dusted on the stigmatic surface of the respective emasculated female parent and the flowers were bagged with butter paper cover and then labelled. For the self-pollination treatment hundred flowering shoots were randomly selected on the plants and one third of the flowering bunches were bagged without any emasculation. The remaining flowering shoots (five per plant) were tagged and the flower bunches on each shoot were thinned to five buds (approx. 24 h before anthesis) for open pollination the flowers were left untreated without any bagging. The observations on days to fruit initiation, fruit set percentage, duration of fruit retention, shape of the fruit, colour of the fruit, fruit intensity and season of fruit set were recorded. Colour was assessed for each fruit using RHS colour chart. For the analysis of embryo viability, the longitudinally dissected fruits were treated with 2, 3, 5 triphenyl tetrazolium chloride and after 3 hours of incubation the stained embryos were examined for viability. Fruit set and fruit quality characters were evaluated by variance analysis using SPSS 28.0 software.

RESULTS AND DISCUSSION

Evaluation of the best possible cross combination with the varied method of pollination is the base factor that decides the success of a hybridization programme. The method of pollination plays a significant role in the fruit set of the plant and is influenced by various factors such as morphology of the flower, pollen-pistil interaction, nutrients and environmental parameters. As observed from the data (Table 1), among the possible cross combinations of pollen parents with completely opened flowers of *J. auriculatum* cultivars, the cultivar CO.1 Mullai as female parent recorded good response with male parent *J. Flexile* by recording the earliest fruit set (38 DAP), highest number of fruits set at 60 DAP (52), fruit set at maturity (31), highest fruit set (38.75%) and maximum fruit retention in the plant (30 days). Similarly, CO.2 Mullai produced best results as female parent when crossed with *J. flexile* with maximum fruit set (46.25%) but recorded delayed fruit set (47 days). The cultivar Parimullai as female parent also responded with *J. flexile* as pollen parent showing maximum fruit set of 48.75% and the duration of fruit retention clocked up to 33 days.

The results furnished (Table 1) signify the best cross combination for the bud pollination of *J. auriculatum* cultivars with various pollen parents. CO.1 and CO.2 Mullai as female parents exhibited best results with

Table 1. Inter specific of *J. auriculatum* cultivars with various pollen parents

Hand pollination of completely open flowers							Bud pollination					
CO.1 Mullai												
Male parent	**No. of flowers pollinated**	**Initiation of fruit set (DAP)**	**No. of fruits set at 60 DAP**	**No. of fruits at maturity**	**Fruit set (%)**	**Duration of fruit retention (days)**	**No. of buds Pollinated**	**Initiation of fruit set (DAP)**	**No. of fruits set at 60 DAP**	**No. of fruits at maturity**	**Fruit set (%)**	**Duration of fruit retention (days)**
J. grandiflorum cv. CO.1Pitchi	115	45	93	28	24.34	24	125	36	114	62	49.60	32
J. sambac	60	-	-	-	-	-	100	-	-	-	-	-
J. multiflorum	100	52	79	34	34.10	30	150	32	123	52	34.67	30
J. nitidum	150	38	83	38	25.34	24	150	36	119	58	38.67	35
J. calophyllum	100	46	64	27	27.10	28	80	30	68	33	41.25	30
J. flexile	80	38	52	31	38.75	30	80	37	72	42	52.50	32
CO.2 Mullai												
J. grandiflorum cv.CO.1Pitchi	115	41	96	25	21.73	27	125	38	118	67	53.61	33
J. sambac	60	-	-	-	-	-	100	-	-	-	-	-
J. multiflorum	100	55	82	36	36.10	28	150	30	134	56	37.34	32
J. nitidum	150	49	86	40	26.67	24	150	38	129	63	42.13	35
J. calophyllum	100	41	66	28	28.10	30	80	32	74	38	47.50	30
J. flexile	80	47	54	37	46.25	30	80	39	78	45	56.25	33
Parimullai												
J. grandiflorum cv. CO.1Pitchi	115	48	102	27	23.47	29	125	34	116	73	58.40	35
J. sambac	60	-	-	-	-	-	100	-	-	-	-	-
J. multiflorum	100	55	87	38	38.10	30	150	30	127	61	40.67	34
J. nitidum	150	40	92	43	28.67	25	150	34	122	68	45.34	38
J. calophyllum	100	49	70	30	30.10	32	80	29	74	31	38.75	32
J. flexile	80	41	57	39	48.75	33	80	35	70	42	52.50	35

Table 2. Inter specific hybridization of *J. grandiflorum* cultivars with various pollen parents

Hand pollination of completely open flowers							Bud pollination					
CO.1 Pitchi												
Male parent	**No. of flowers pollinated**	**Initiation of fruit set (DAP)**	**No. of fruits set at 60 DAP**	**No. of fruits `= at maturity (malformed)**	**Fruit set (%)**	**Duration of fruit retention (days)**	**No. of buds Pollinated**	**Initiation of fruit set (DAP)**	**No. of fruits set at 60 DAP**	**No. of fruits at maturity**	**Fruit set (%)**	**Duration of fruit retention (days)**
*J.auriculatum*cv. CO.1 Mullai	135	22	106	98	72.59	48	150	25	138	124	82.67	51
J. sambac	100	-	-	-	-	-	100	-	-	-	-	-
J. multiflorum	150	34	137	118	57.34	52	150	32	133	125	83.34	57
J. nitidum	120	28	93	86	76.67	39	150	24	127	114	76.12	45
J. calophyllum	80	25	62	43	53.75	41	80	24	67	57	71.25	47
J. flexile	80	22	71	64	80.10	57	80	20	74	68	85.21	62
CO.2 Pitchi												
*J.auriculatum*cv. CO.1 Mullai	135	28	126	109	80.74	45	150	20	126	119	79.34	50
J. sambac	100	-	-	-	-	-	100	-	-	-	-	-
J. multiflorum	150	30	129	114	76.16	58	150	29	137	132	88.14	54
J. nitidum	120	30	116	93	77.50	45	150	22	131	118	78.67	49
J. calophyllum	80	24	66	45	56.25	46	80	24	72	54	67.53	53
J. flexile	80	22	75	67	83.75	45	80	21	75	66	82.54	60

DAP – Days after pollination

the combination of *J. flexile* as pollen parent while Parimullai responded well with the pollen parent *J. grandiflorum* CO.1 Pitchi. The results emphasize the fact that the pollen source and quantity influence the fruit set. Aggregated results from the Table 1 indicate that there is significant amount of fruit set failure from the fruit set at 60 DAP till maturity. The failure in fruit development or the malformation of fruits accounts to the low fertilization rate (Koubouris *et al.,* 2010) or loss of pollen viability (Deng *et al.,* 2017) or inadequate nutrient availability (Nyomora *et al.,* 1999). Competition between fruits for assimilates and growth regulators are the factors that are responsible for different fruiting behaviour of the assessed cultivars.

In crosses involving *J. grandiflorum* cultivars as female parents, CO.1 Pitchi and CO.2 Pitchi evinced best results with *J. flexile* as the pollen donor. Maximum fruit set (80.10 and 83.75% respectively in CO.1 Pitchi and CO.2 Pitchi) with the earliest fruit set initiation of 22 days were recorded for the crosses effected with hand pollination of open flowers. For the bud pollination, CO.1 Pitchi had best compatibility with *J. flexile* while CO.2 Pitchi proved the best results with the combination that entailed *J. multiflorum* as the pollen parent (Table 2). The major drawback in the crosses involving *J. grandiflorum* as seed setting parent is the abnormal fruit set. The initiation of the fruit set is expressed by the bulging of the ovary proving the development of the fruit but as time progresses the ovary fails to develop completely causing misshapen fruits further arresting the growth of the embryo resulting in the loss of fruit set. Existence of pre-fertilization barriers like low pollen viability, early senescence of pistil cells and low pistil receptivity are the possible barriers in hybrid set (Deng *et al.,* 2016). Early and rapid senescence of pistils is harmful for pollen adhesion and germination resulting in the arrest of pollen tube growth after it enters the stigma. Hybrid sterility can also be accounted due to the structural changes in the chromosomes (Sharma and Sharma, 1958).

None of the crosses involving *J. sambac* as male parent resulted in fruit set both in hand pollination and bud pollination implying that prevalence of pre-fertilization barriers hinders the fruit set. Low pollen fertility, pistil receptivity and pollen-stigma compatibility, ovule sterility (Deng *et al.,* 2010; Sua´rez *et al.,* 2012) have been enumerated as major reasons responsible for the hampered hybrid set.

With respect to open pollination *J. auriculatum* cv Parimullai recorded maximum fruit set of 76.43% with the earliest fruit initiation of 32 days and retained the fruits up to 28 days (Table 3.) while *J. grandiflorum* cv CO.2 Pitchi proved best with the highest fruit set (83.40%), earliest initiation of fruit set (38 days) and longest duration of fruit retention(55 days) although malformation of the fruits occurred during their growth stage. The favourable fruit set in *J. auriculatum* may be attributed with as the absence of embryo antagonism (Veluswamy *et al.,* 1981) and better source-sink relationship supporting the nutrient availability (Keshavarz *et al.,* 2011). Failure in the fruit development and maturity can also be caused due to the abnormalities in the endosperm. Irregularities in the endosperm result in embryo starvation leading to distorted embryo sac (Veluswamy *et al.,* 1981). Along with pre-fertilization barriers, obstructions post fertilization also poses a threat in hybridization.

Data in Table 3 are pertinent to self-pollination in *J. auriculatum* and *J. grandiflorum*. The cultivars CO.2 Mullai, CO.1 Mullai and Parimullai of *J. auriculatum* recorded fruit set rates of 20.86 %, 8.16 % and 2.14 % respectively. Thus, the results revealed that *J. grandiflorum* exhibited better self-pollination efficiency in comparison with *J. auriculatum* but the fruit malformation in *J. grandiflorum* stands as a stumbling block the hybridization attempts involving this species.

Data furnished in Table 4 demonstrated that fruits evolved from crosses involving *J. multiflorum* and *J. nitidum* exhibited oblate shape while those from the crosses involving *J. grandiflorum* cv CO.1 Pitchi, *J. calophyllum* and *J. flexile* expressed spherical shape. Fruit intensity was profuse for most of the cross combinations in bud pollination when compared to hand pollination of the open flowers. Peak season of fruit set concurred with June to November under both the pollination methods. *J. flexile* and *J. multiflorum* as pollen parents responded well with Parimullai as female parent in terms of fruit growth (Table 5). Colour of the fruit varied from light green to yellow green and medium green and turns black on maturity. Fruits of *J. auriculatum* yielded from open pollination performed better in terms of growth as well as the intensity of the fruit set while self-pollinated fruits

Table 3: Open and self-pollination of *J. auriculatum* and *J. grandiflorum* cultivars

Cultivars	No. of flowers pollinated	Initiation of fruit set (DAP)	No. of fruits set at 60 DAP	No. of fruits at maturity	Fruit set (%)	Duration of fruit retention (days)
Open pollination						
J. auriculatum CO.1 Mullai	250	46	164	138	55.20	28
J. auriculatum CO.2 Mullai	235	41	183	169	71.91	24
J. auriculatum Parimullai	250	32	217	191	76.43	28
J. grandiflorum CO.1 Pitchi	235	45	204	189	80.42	52
J. grandiflorum CO.2 Pitchi	235	38	228	196	83.40	55
Self-pollination						
J. auriculatum CO.1 Mullai	150	43	38	12	8.16	26
J. auriculatum CO.2 Mullai	115	40	76	24	20.86	24
J. auriculatum Parimullai	150	42	91	30	2.14	25
J. grandiflorum CO.1 Pitchi	115	42	97	74	64.34	57
J. grandiflorum CO.2 Pitchi	115	40	103	81	70.43	58

DAP- Days after pollination

proved better in terms of fruit growth with bolder fruits though the fruit set was poor. The efficiency of the fruit set depends upon the flowers that have pollen-laden anthers that appear to set fruit far better when cross- pollinated than when fertilized with their own pollen (Ortega *et al.,* 2006). Despite the lack of complete fruit development in *J. grandiflorum*, peak season of the fruit set was observed during February to April. The fruits were conical in shape and yellow-green in colour (Table 6). In terms of method of pollination, open pollination contributed the most for the successful fruit set followed by bud pollination (Fig 1.). Results pertaining to fruit growth and quality parameters (Fig 2.) revealed that bud pollination followed by hand pollination of open flowers ensured significantly superior fruit set.

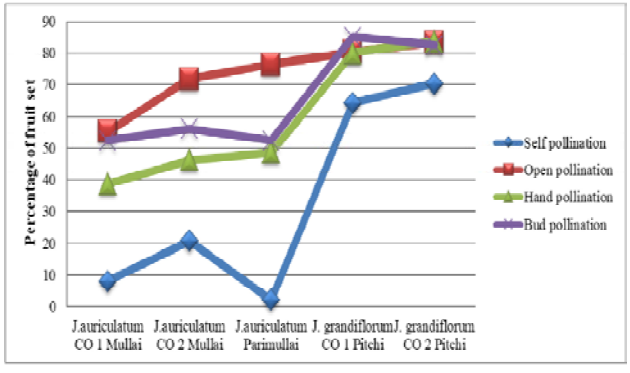

Fig. 1. Effect of various pollination methods on fruit set in *Jasminum* spp

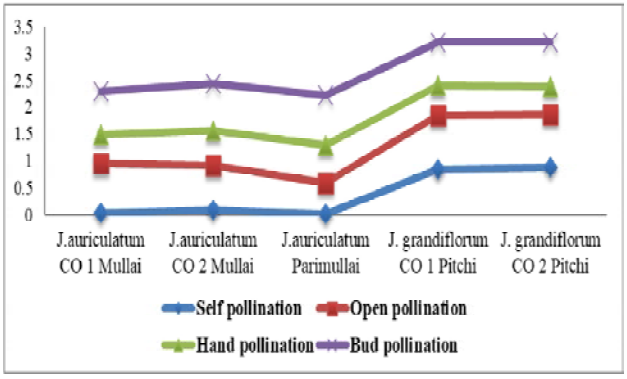

Fig. 2. Effect of various pollination methods on fruit intensity in *Jasminum* spp

Among all the possible cross combinations *J. flexile* corresponded well with all the cultivars of *J. auriculatum* and can be considered as the best pollen donor parent for the successful hybridization of the crop. *J. auriculatum* cv. Parimullai provided best results among all the cultivars in terms of fruit set and intensity , thus proving to be an elite female parent amongst the cross combinations. This study

Table 4. Analysis of fruit characteristics for cross combination of *J. auriculatum* CO.1 and CO.2 Mullai cultivars with various pollen parents

Hand pollination of completely open flowers							Bud pollination					
CO.1 Mullai												
Male parent	**Fruit Intensity**	**Season of fruit set**	**Shape of the fruit**	**Colour of the fruit**	**Fruit length (cm)**	**Fruit girth (cm)**	**Frit intensity**	**Season of fruit set**	**Shape of the fruit**	**Colour of the fruit**	**Fruit length (cm)**	**Fruit girth (cm)**
J. grandiflorum cv.CO.1Pitchi	Very sparse	Jun-Nov	Spherical	Medium green	1.06	1.45	Moderate	Jun-Nov	Spherical	Medium green	1.16	1.35
J. multiflorum	Moderate	Jun-Oct	Oblate	Light green	1.12	1.28	Moderate	Jun-Oct	Oblate	Light green	1.21	1.38
J. nitidum	Sparse	Jun-Oct	Oblate	Light green	1.09	1.31	Moderate	Jun-Oct	Oblate	Light green	1.11	1.31
J. calophyllum	Slightly sparse	Jun-Nov	Spherical	Yellow green	1.04	1.28	Moderate	Jun-Nov	Spherical	Yellow green	1.06	1.28
J. flexile	Moderate	Jun-Nov	Spherical	Yellow green	1.17	1.30	Profuse	Jun-Nov	Spherical	Yellow green	1.15	1.32
CO.2 Mullai												
J. grandiflorum cv. CO.1Pitchi	Very sparse	Jun-Nov	Spherical	Medium green	1.03	1.24	Profuse	Jun-Nov	Spherical	Medium green	1.16	1.35
J. multiflorum	Moderate	Jun-Oct	Oblate	Light green	1.16	1.28	Moderate	Jun-Oct	Oblate	Light green	1.21	1.38
J. nitidum	Sparse	Jun-Oct	Oblate	Light green	1.08	1.25	Slightly profuse	Jun-Oct	Oblate	Light green	1.11	1.31
J. calophyllum	Sparse	Jun-Nov	Spherical	Yellow green	1.12	1.28	Slightly profuse	Jun-Nov	Spherical	Yellow green	1.06	1.28
J. flexile	Moderate	Jun-Nov	Spherical	Yellow green	1.21	1.42	Profuse	Jun-Nov	Spherical	Yellow green	1.15	1.32

Table 5. Analysis of fruit characteristics for cross combination of *J. auriculatum* cv. Parimullai as female parent with various pollen parents

Hand pollination of completely open flowers							Bud pollination					
Parimullai												
Male parent	**Fruit Intensity**	**Season of fruit set**	**Shape of the fruit**	**Colour of the fruit**	**Fruit length (cm)**	**Fruit girth (cm)**	**Frit intensity**	**Season of fruit set**	**Shape of the fruit**	**Colour of the fruit**	**Fruit length (cm)**	**Fruit girth (cm)**
J. grandiflorum cv. *CO 1* Pitchi	Slightly sparse	Jun-Nov	Spherical	Medium green	1.09	1.37	Profuse	Jun-Nov	Spherical	Medium green	1.16	1.35
J. sambac	Nil	-	-	-	-	-	Nil	-	-	-	-	-
J. multiflorum	Moderate	Jun-Oct	Oblate	Light green	1.15	1.47	Moderate	Jun-Oct	Oblate	Light green	1.21	1.38
J. nitidum	Sparse	Jun-Oct	Oblate	Light green	1.06	1.42	Slightly profuse	Jun-Oct	Oblate	Light green	1.11	1.31
J. calophyllum	Sparse	Jun-Nov	Spherical	Yellow green	1.12	1.28	Slightly profuse	Jun-Nov	Spherical	Yellow green	1.06	1.28
J. flexile	Moderate	Jun-Nov	Spherical	Yellow green	1.21	1.42	Profuse	Jun-Nov	Spherical	Yellow green	1.15	1.32

Table 6: Analysis of fruit characteristics for open pollinated and self-pollinated *J. auriculatum* and *J. grandiflorum* cultivars

Cultivars	Fruit Intensity	Season of fruit set	Shape of the fruit	Colour of the fruit	Fruit length (cm)	Fruit girth (cm)
Open Pollination						
J. auriculatum CO.1 Mullai	Profuse	Jun-Nov	Spherical	Medium green	1.09	1.31
J. auriculatum CO.2 Mullai	Profuse	Jun-Nov	Spherical	Light green	1.12	1.35
J. auriculatum Parimullai	Profuse	Jun-Nov	Spherical	Medium green	1.18	1.46
J. grandiflorum CO.1 Pitchi	Highly	Feb-Apr profuse	Conical	Yellow green	0.53	0.31
J. grandiflorum CO.2 Pitchi	Highly	Feb-Apr profuse	Conical	Yellow green	0.51	0.46
Self-Pollination						
J. auriculatum CO.1 Mullai	Moderate	Jun-Oct	Oblate	Light green	1.16	1.28
J. auriculatum CO.2 Mullai	Sparse	Jun-Oct	Oblate	Light green	1.08	1.25
J. auriculatum Parimullai	Sparse	Jun-Nov	Spherical	Yellow green	1.12	1.28
J. grandiflorum CO.1 Pitchi	Moderate	Jun-Nov	Spherical	Yellow green	1.21	1.42
J. grandiflorum CO.2 Pitchi	Moderate	Jun-Oct	Oblate	Light green	1.16	1.28

indicates the failure of fruit set and fertilization barrier prevailing in jasmine upon hybridization. Understanding the type of the barriers prevailing in jasmine facilitates the integration of conventional approaches with biotechnological tools to overcome the complications and obtain interspecific hybrids with desirable traits.

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Original Research Paper

Genetic diversity study in tropical carrot (*Daucus carota* L.)

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ABSTRACT

Genetic diversity study was conducted at ICAR- Indian institute of Horticultural Research, Bengaluru during 2018-19. In this study, 80 accessions were evaluated for 16 yield and yield attributing traits. The Mahalanobis' D^2 analysis grouped these accessions into seven clusters. Cluster I was the largest with 69 genotypes followed by cluster III comprising six genotypes while, the clusters II, IV, V, VI and VII contained one genotype each. Among the traits studied, yield contributed maximum (38.04 %) towards diversity, followed by root weight (26.58%), root color (9.18%) and plant height (6.7%). As far as root weight (g) [d1], leaf weight (g), root weight (g), number of leaves, TSS(°Brix), leaf weight (g), root diameter (mm), core diameter (mm), and root cracking are concerned, they contributed 3.45, 2.09, 1.77, 1.71, 1.55, 1.52, 1.46, 1.33, 1.01 and 0.82 percent respectively. Diversity analysis has given an indication about the genetic variation among the carrot accessions which will prove useful in selection of diverse parents in crop improvement programme.

Keywords: Carrot, cluster analysis, genetic diversity, root weight and yield

INTRODUCTION

Carrot (*Daucus carota* subsp. *sativus*), an important root tuber vegetable crop of Apiaceace family is a diploid species (2n = 2x =18) grown globally for its rich nutritional contents of vitamin A and carotenes. Other members of this family include celery, dill, parsley, fennel, cumin, coriander, cilantro and many other vegetables and spices. The objective of carrot breeding programmes is to evolve high yielding and well adapted cultivar with desirable economic traits. Edible carrots are thought to have originated in Afghanistan before the ninth century, according to historical evidence. Eastern carrots, as they were known to have yellow or purple roots. Their cultivation extended throughout Central and North Asia, as well as Japan (17^{th} century). The Near East is often regarded as the second-largest source of variation for cultivated carrot variation. Western carrots differ from Eastern carrots in that they have fewer pubescent leaves and a reduced tendency to flower early. During the Middle Ages, yellow and purple carrots were widely grown in Europe, but they were gradually replaced by white and then orange-rooted varieties, which first appeared in the early seventeenth century, presumably as a result of selection from yellow carrot and hybridization of cultivated carrot and its wild relatives (Rubatzky *et al*. 1999). Carrots with orange roots expanded from Europe to other continents, eventually becoming the most common commercial crop in the world. Carrots with different root colours are more regularly grown in Asia, and they have just lately been reintroduced to specialist markets in Europe and America (Simon *et al*. 2008). A long history of carrot selection and the use of diverse parental materials in breeding programmes throughout the world have resulted in considerable variation in available cultivars. An understanding of the extent and nature of genetic variation within a crop species is required for efficient breeding effort. Better understanding of genetic diversity or genetic similarity might aid in the maintenance of long-term selection gain in plants (Chowdhury *et al*. 2002). Therefore, the present study was study in tropical carrot genotypes genetic diversity and cluster analysis.

MATERIALS AND METHODS

The present study was conducted at Vegetable Research Block of Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research,

Hesaraghatta, Bengaluru (latitude 13°58' North and longitude 78°45' East and an altitude of 890 meters above mean sea level) during Rabi, 2018. Eighty accessions were used to study the genetic diversity. The experiment was laid out in a randomized block design with three replications and observations were recorded on a single plant basis for the following characters *viz.*, plant height (cm), number of leaves, leaf length (cm), root length (cm), root diameter (mm), root weight (g), core diameter (mm), root core color, TSS (°Brix), root cracking, root color, root fresh weight (g), root dry weight (g), leaf fresh weight (g), leaf dry weight (g) and yield (t ha^{-1}). Multivariate analysis was done utilising Mahalanobis D^2 statistic (Mahalanobis, 1936) and genotypes were grouped into different clusters following Tocher's method.

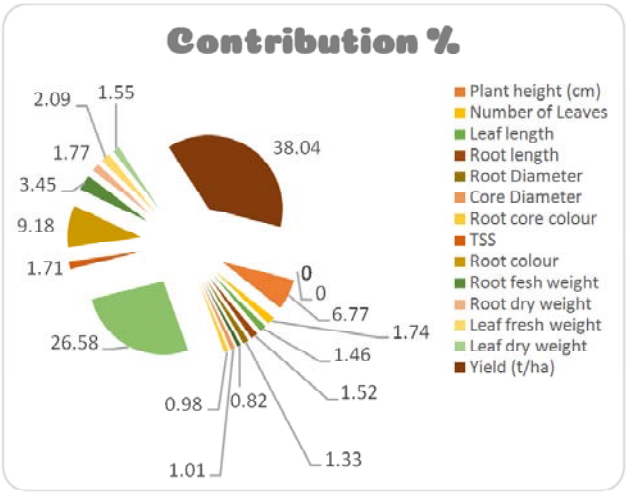

Fig 1. Per cent contribution of 16 characters towards diversity in carrot

RESULTS AND DISCUSSION

Using the pivotal condensation method, the mean values of genotypes were transformed into standardized uncorrelated mean values. The relative percent contribution of different characters included in the study towards diversity is presented in Table 1 and Figure 1. Yield contributed maximum (38.04 %) towards diversity, followed by root weight (26.58%), root colour (9.18%) and plant height (6.77%). Root fresh weight, leaf fresh weight, root dry weight, number of leaves, leaf dry weight, root length, leaf length, root diameter, core diameter and root cracking percent contribution showed 3.45,2.09,1.77,1.74,1.55, 1.52, 1.46,1.33, 1.01 and 0.82 respectively. Similar finding was reported by Jain *et al.,* (2010) Amin and Singla (2010), Nayak and Nagre (2013), Madavi *et al*., (2015), Reshmika *et al*., (2015) Tripathy *et al*., (2017) and Tirkey *et al*., (2018).

Table 1. Relative contribution of 16 characters to genetic diversity in 80 accessions of carrot

Sl. No.	Character	Contribution %	Times ranked first
1	Plant height(cm)	6.77	214
2	Number of leaves	1.74	55
3	Leaf length	1.46	46
4	Root length(cm)	1.52	48
5	Root diameter(mm)	1.33	42
6	Root weight(g)	26.82	840
7	Core diameter(mm)	1.01	32
8	Root core color	0.98	31
9	Root cracking	0.82	26
10	TSS(°Brix)	1.71	54
11	Root color	9.18	290
12	Root fresh weight(g)	3.45	109
13	Root dry weight(g)	1.77	56
14	Leaf fresh weight(g)	2.09	66
15	Leaf dry weight(g)	1.55	49
16	Yield (t/ha)	38.04	1202

Table 2. Clustering pattern of 80 accessions of carrot by D^2 analysis

Characters group	No.of Accessions	List of Accessions
1 Cluster	69	Acc-63, Acc -69, Acc -163B, Acc -52B, Acc -148, Acc -22B, Acc -52C, Acc -87, Acc -56B, Acc -77B, Acc -21A, Acc-72, Acc -76B, Acc -152B, Acc -76C, Acc -60A, Acc -155, Acc -50, Acc -22A, Acc -40, Acc -154A, Acc -140, Acc -77, Acc -777A, Acc -21C, Acc -54, Acc -113A, Acc -76A, Acc -72, Acc -76, Acc -70, Acc -84, Acc -22D, Acc -01, Acc -135, Acc -102, Acc -135, Acc -88, Acc -21, Acc -21B, Acc -60B, Acc -68, Acc -106A, Acc -153, Acc -02, Acc -77C, Acc -101, Acc -113B, Acc -144C, Acc -56, Acc -146, Acc -41, Acc -152A, Acc -145, Acc -06, Acc -105, Acc -54B, Acc -85, Acc -88, Acc -106B, Acc -144A, Acc -144B, Acc -54A, Acc -113B, Acc -105, Acc -20, Acc -80, Acc -164, Acc -156
2 Cluster	1	Acc -154B
3 Cluster	6	Acc -52A, Acc -163A, Acc -51, Acc -173, Acc -147, Acc -63
4 Cluster	1	Acc -75
5 Cluster	1	Acc -50
6 Cluster	1	Acc -150
7 Cluster	1	Acc -56A

The genetic diversity among 80 genotypes was measured by employing D^2 statistics and grouped into six clusters using Tocher's method given as by Rao (1952). Distribution of accessions in each cluster is presented in Table 2 and Figure 2. Cluster I was found largest with 69 accessions followed by cluster III comprising six accessions, cluster II and IV, V, VI and VII comprising one accessions in each cluster. Similar genetic diversity studies were carried out by many workers in this crop *viz.*, Amin *et al*., 2010, Kumar *et al*., 2021 and Meghashree *et al*., 2018.

Cluster mean of 16 yield and yield contributing characters were assessed and presented in Table 3. along with supplementary data (Table S1 and Fig. S1). The mean comparison of the different characters indicated considerable differences among the clusters for all the characters. Maximum mean for plant height was observed in cluster III (85.9cm) followed by cluster II (85.1cm), while minimum cluster means of 53.8 cm were observed in cluster V. There were maximum number of leaves observed in cluster VII which recorded 18.3, followed by cluster IV which recorded 9.6, and cluster II recorded a minimum of 6.5. The maximum mean for leaf length (72.3 cm) was observed in cluster VII followed by cluster III (69.5cm) and minimum mean (47.0cm) was observed in cluster V.

The highest mean for the root length was recorded in cluster VII (19.3cm) followed by cluster V (16.3cm) while, lowest mean of 13.0 cm was shown by cluster VI. The highest mean for root diameter was observed in cluster VII (5.3mm) followed by cluster VI (4.7mm) while the lowest mean of 2.6 mm was shown by cluster IV. Root weight recorded a maximum mean in cluster III of 123.7g followed by cluster II of 116.8g while the minimum mean of 33.3g was observed in cluster IV. The core diameter recorded a maximum mean in cluster VII of 3.7mm followed by II of 2.7mm while, the minimum mean of 1.5mm was observed in cluster IV. The root core color that is self-core color was yellow (2) in cluster V followed by orange in other VI clusters. Root cracking was either obsent or rarely observed in cluster V, I and cluster III having mean 0, 0.1 and 0.6 percent respectively while other clusters was having root cracking having mean of 1.0 percent. The cluster mean observed in TSS (°Brix) was highest for cluster VI (14.2) followed by cluster II (13.8) and it was lowest for genotypes under cluster VII (11.17). Root color was very dark in cluster VII, III. IV and II having mean of 4.3, 4.0, 4.0 and 4.0 while dark orange color observed in cluster I having a mean of 3.6 and cluster VI was having orange root color with a mean of 2.0.

Root fresh weight recorded maximum cluster mean in cluster II (96.6g) followed by cluster III (95.0g) while

cluster IV depicted minimum mean of 63.3g. Cluster IV recorded a maximum mean of 11.3g of root dry weight followed by 10.9 g observed in cluster III. Whereas, minimum mean of 8.0 g was observed in genotypes under cluster II. The maximum mean for leaf fresh weight (213.3g) was observed in cluster VII followed by cluster III (59.6g) while minimum mean of 18.5 g was observed in cluster V. with regard to leaf dry weight Cluster VII recorded a maximum mean of (leaf dry weight) 40.6g followed by 14.6g observed in cluster II while minimum mean of 2.2g was observed in genotypes under cluster V. The highest mean for yield was recorded in cluster IV (12.9t ha^{-1}) followed by cluster VI and VII (12.0 t ha^{-1}) while, the lowest mean of 9.0 t ha^{-1} was shown by cluster III. Similar reports were made by Amin *et al*., 2010, Kumar *et al*., 2021 and Meghashree *et al*., 2018.

Based on these results, Mahalanobis D^2 was found to be a useful tool in grouping genotypes phenotypically and geographically. Findings revealed that in carrot, there is a vast scope for developing new varieties with greater yield potential and to better other attributes of economic importance, using this elite germplasm. In crop improvement programmes, intercrossing among genotypes with outstanding mean performance for these characters would prove to be effective.

CONCLUSION

Genetic divergence has been considered as an important factor in selecting the genetically diverse parents for efficient and successful hybridization programme in order to get potential transgressive segregants and also provide new recombination of genes in the gene pool. It is desirable to select genotypes from clusters showing high inter-cluster distance cluster VI (Acc -150) and cluster VII (Acc -56A) for further crop improvement programme.

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Original Research Paper

Studies on genetic variability and relationship of bael (*Aegle marmelos* (L) Correa) using morphological and molecular markers

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ABSTRACT

Bael (*Aegle marmelos* (L) Correa) is an important underutilized fruit crop of India. A total of 25 bael trees were selected from 356 bael trees of Sakharayapattana in Chikkamagalur district, Karnataka, India based on the fruit morphological traits (fruit weight, pulp weight, skull thickness, seed weight per fruit, No. of seeds per fruit, No. of locules per fruit, No. of seeds per locule, pulp wt. : seed wt.). These 25 trees were evaluated for phenotypic and genotypic variations using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. RAPD and ISSR markers showed significant polymorphism among the trees. Jaccard's genetic similarity value of RAPD and ISSR was found in the range of 0.00–0.95 and 0.06–0.56, respectively suggesting a moderate level of genetic diversity. The present study revealed that molecular markers can be successfully utilized for determining genetic diversity and relationship of bael trees for further varietal improvement.

Keywords: Bael, genetic variability, morphology and molecular markers

INTRODUCTION

Bael (*Aegle marmelos*(L) Correa) belongs to the family Rutaceae and is an important underutilized indigenous fruit crop of India and has high medicinal and nutritional values. Since pre-historic times, it was found as wild in Sub-Himalayan tract and dry deciduous forests of Central and Southern Indian region. Therefore, a large number of landraces are available in different diversity regions (Pandey *et al.*, 2013) Each tree is genetically different from others as most of them are of seedling origin. Traditionally, morphological characters have been used to identify and characterize the bael. However, there is a high level of genetic variability which can sometimes be used accurately to distinguish each tree. When the morphological traits are used for determining diversity and relationships among plant species, they are not sufficient because of environmental influences. Thus, the usefulness of molecular markers has been investigated as a means of characterizing and discriminating against different species more precisely (Benharrant *et al.*, 2002). The introduction of molecular biology techniques, such as DNA-based markers, allows for direct comparison of different genetic materials independent of environmental influences. The viability and purity of accessions can be analysed by utilization molecular markers. This process can increase both the quantity and quality of plant (Mujeeb *et al.,* 2017) Molecular characterization would be more rewarding in terms of accurate identification and characterization of most closely related trees at the intra-specific level. The degree of similarity between the banding patterns provides information about genetic similarity and relationships between the samples studied. The application largely depends on the type of markers employed, distribution of markers in the genome, type of loci they amplify, level of polymorphism and reproducibility of the products (Virk *et al.,* 2001 and Fernandez *et al.,* 2002). Among the molecular markers, RAPD and ISSR markers have been extensively used to study genetic diversity and relationship. These markers can detect polymorphism in a single reaction. The main objective of the study was to characterise bael trees using morphological molecular markers, to evaluate the genetic diversity and relationship.

MATERIALS AND METHODS

Among 356 trees, 76 fruiting trees were subjected to study of variation in fruit morphological traits like fruit weight, pulp weight, skull thickness, seed weight per fruit, No. of seeds per fruit, No. of locules per fruit, No. of seeds per locule, pulp wt. : seed wt. Based on the fruit morphological traits the best 25 trees were selected for molecular marker analysis. Plant material (leaves) of 25 bael trees were collected for genomic DNA isolation using standardized cetyl trimethyl ammonium bromide (CTAB) extraction protocol (Benharrant *et al.*,2002) and thenthe DNA was quantified using a spectrophotometer and the quality of the DNA was checked on 0.7% agarose gel.

RAPD-PCR Amplification

Twelve RAPD primers were used for RAPD analysis of 25 bael trees. PCR amplification was carried out using 1X Taq buffer solution and 1 U Taq DNA polymerase (Bangalore Genie Pvt. Ltd.), 1.25 mM $MgCl_2$, 0.8 mM dNTP mix, 5 µM of a single decamer primer and 50 ng genomic DNA and the volume made up to 20 µl using sterilized double-distilled water. The amplification was performed in VWR Peqlab thermocycler with initial pre-denaturation at 94 °C for 4 min followed by 40 cycles of denaturation at 92 °C for 2 min, at annealing temperature (Table 1.) for 1 min, and extension at 72 °C for 2 min. Final extension was performed for 5 min at 72 °C. Amplification products were separated by electrophoresis on 1.5 % Agarose gel stained with ethidium bromide at 80 V. Bands were visualized and photographed in a gel documentation unit.

Table 1. List of RAPD primers and their annealing temperatures

Primer	Marker sequence (5' to 3')	Annealing temperature (°C)
OPA-02	TGCCGAGCTG	37
OPN-03	GGTACTCCCC	37
OPN-12	CACAGACACC	37
OPM-05	GGGAACGTGT	37
OPM-06	CTGGGCAACT	38
OPX-17	GACACGGACC	36
OPM-12	GGGACGTTGG	38
OPM-15	GACCTACCAC	36
OPM-20	AGGTCTTGGG	38
OPB-1	GTTTCGCTCC	36
OPA-08	GTGACGTAGG	36
OPA-1	CAGGCCCTTC	38

ISSR-PCR Amplification

Sixteen primers, which gave the best amplification results with the sample DNA, were selected for ISSR-PCR analysis. PCR-amplification was carried out using 1X Taq buffer solution and 1 U Taq DNA polymerase (Bangalore Genie Pvt. Ltd.), 1.40 mM $MgCl_2$, 0.8 mM dNTP mix, 8 µM of a single decamer primer and 50 ng genomic DNA and the volume made upto 25 µl using sterilized double-distilled water. The amplification was performed in VWR Peqlab thermocycler 2 min at 94°C, followed by 40 cycles each of 1 min at 94°C (denaturation), 1 min at 55°C (annealing for ISSR primers), 2 min at 72°C (extension) followed by one final extension of 7 min at 72°. Amplification products were separated by electrophoresis on 1.5 % Agarose gel stained with ethidium bromide at 80 V. Bands were visualized and photographed in a gel documentation unit and analyzed.

Data Analysis

Amplified bands generated from RAPD and ISSR-PCR amplification were scored based on the presence (1) or absence (0) of bands for each primer and used to calculate a genetic similarity matrix using software NTSYS-pc version 2.1. Cluster analysis was performed for molecular data using the "unweighted pair group method using arithmetic means" (UPGMA) algorithm, from which dendrograms depicting similarity among trees were drawn and plotted using NTSYS-pc software.

RESULTS AND DISCUSSION

The variations in fruit morphological traits among the trees are depicted in Table 2. Significant maximum fruit weight was observed in tree SB-353 (320.00 g) and minimum fruit weight was observed in tree SB-115 (54.30 g). Pulp weight was found significantly maximum in tree SB-353 (202.40 g) whereas, minimum pulp weight was observed in SB-71 (22.53 g) and it was on par with the tree SB-148. The difference in fruit weight might be attributed to an increase in pulp weight, seed weight, skull weight of trees. The findings are in agreement with the results of earlier researches (Pandey *et al.,* 2008, Pandey *et*

Table 2. Fruit morphological traits of 25 bael trees

Tree No.	Fruit weight (g)	Pulp weight (g)	Skull thickness (mm)	Seed weight (g)	No. of seeds / fruit	No. of locules / fruit	No. of seeds / locule	Pulp wt. : Seed wt.
SB-353	320.00	202.40	4.95	19.80	40.00	10.67	3.75	10.22
SB-351	136.00	58.37	6.82	8.80	16.00	11.00	1.46	6.64
SB-147	99.10	36.50	4.40	7.70	10.00	8.00	1.25	4.74
SB-90	110.30	45.10	6.92	0.12	1.00	9.00	0.11	392.17
SB-111	146.20	48.10	5.33	8.70	44.00	10.00	4.40	5.53
SB-33	153.50	57.10	3.96	18.00	56.00	8.00	7.06	3.18
SB-80	85.90	27.70	5.54	13.22	26.00	9.00	2.90	2.10
SB-350	91.20	39.50	3.89	5.40	40.00	9.00	4.49	7.33
SB-288	95.50	30.80	5.51	3.50	33.00	10.00	3.32	8.81
SB-161	123.30	47.30	5.47	15.30	34.00	8.00	4.28	3.09
SB-2	162.00	46.00	4.09	12.00	46.00	10.00	4.64	3.85
SB-148	65.70	22.60	4.06	2.40	15.00	7.00	2.14	9.40
SB-16	157.20	48.00	4.48	20.50	62.00	9.00	6.89	2.34
SB-91	125.10	46.07	3.87	0.15	1.00	9.00	0.11	322.12
SB-66	127.30	35.80	7.10	9.96	33.00	9.00	3.67	3.59
SB-1	190.90	49.00	5.60	25.80	84.00	10.00	8.45	1.90
SB-73	159.60	65.50	4.89	15.50	26.00	7.67	3.40	4.85
SB-9	75.70	27.70	4.82	4.71	13.00	9.00	1.46	5.90
SB-115	54.30	10.47	3.99	5.38	22.00	10.00	2.24	1.92
SB-273	96.20	34.00	6.67	7.00	17.00	11.00	1.55	4.92
SB-272	91.90	33.50	4.23	7.10	22.00	9.00	2.46	4.72
SB-146	105.50	42.30	5.40	3.60	9.00	11.00	0.83	11.86
SB-71	56.00	22.53	4.02	5.60	3.00	7.00	0.43	4.04
SB-354	150.00	73.00	4.18	9.60	34.00	12.00	2.83	7.62
SB-175	136.00	53.42	5.93	5.20	25.00	10.00	2.50	10.31
F value	**	**	-	-	*	-	-	**
S. Em±	5.31	1.14	0.06	0.29	0.71	0.43	0.20	15
CD @ 5%	15.08	3.24	0.18	0.82	2.02	1.21	0.56	42.61

** Significant @ 5% and 1%, * Significant @ 5%, - Non significant

al., 2013 and Mitra *et al.,* 2010).The maximum number of seeds per fruit was found in tree SB-1 (84.00) and minimum in SB-90 and SB-91. The difference in seed weight may be attributed to differences in the number and size of seeds among the trees. The results are in conformity with the earlier findings (Pandey *et al.,* 2008, Pandey *et al.,* 2013; Singh and Misra, 2010). Pulp weight : Seed weight was found maximum in SB- 90 (392.17) and it was minimum in SB-1 (1.90). The decrease in seed number per locule has a positive correlation with higher pulp content. Findings are in agreement with the results of earlier researches (Pandey *et al.,* 2013 and Singh and Misra, 2010). The traits like skull thickness, seed weight per fruit, no. of locules per fruit and no. seeds per locule were observed non-significant among the trees.

RAPD analysis

The simplicity of laboratory assay for RAPD markers makes them an attractive method for obtaining intraspecific distinctions. This technique is already used for cultivar identification and genetic variability analysis of several underutilized fruit crops like tamarind (Diallo *et al.,* 2007) and bael (Nayak *et al.,* 2013). In this study, a set of RAPD primers were used for distinguishing the superior trees of bael. The comparatively higher percentage of polymorphic bands detected in the present study indicated that RAPD fragments are moderately polymorphic and particularly informative in the estimation of the genetic relationship of bael trees studied. The polymerase chain reaction of bael genomic DNA using 12 selected RAPD primers generated a total of 1,399 amplified bands (Table 3.). The highest number of bands was observed with primer OPX-17. The size of amplified fragments ranged between 300 and 1800 bp and the lowest number of bands was observed with primer OPN-03. The size of amplified fragments ranged between 500-900 bp. Comparatively, moderate level of polymorphic information content (0.39 to 0.77) value was seen in selected polymorphic primers. The highest PIC value (0.77) was observed for primer OPM-12 whereas, the lowest PIC value (0.39) was observed for OPM-06. It was observed that DNA primers showed an average PIC value of >0.5, which confirms that the primers are highly informative. The maximum average number of bands across trees was found for primer OPX-17 (7.88) while minimum was in primer OPN-03 (1.68).The highest genetic similarity coefficient of 0.95 was found between the SB-147 and SB-90 may be due to their same place of origin. The trees SB-175 and SB-66, SB-9 and SB-1 showed the lowest similarity coefficient (0.00). But the molecular diversity was not in agreement with most of the morphological diversity as reported in *Colocasia esculenta* (Singh *et al.,* 2012). Comparatively high amplitude of the genetic similarity coefficient established in the present study confirms the

Table 3. List of RAPD primers, their sequence and generated bands

Primer	Marker sequence (5' to 3')	Range of amplicon size (bp)	Total No. of bands	Average no. of bands across trees	PIC value
OPA-02	TGCCGAGCTG	200-1400	102	4.08	0.74
OPN-03	GGTACTCCCC	500-900	42	1.68	0.56
OPN-12	CACAGACACC	100-1000	196	7.84	0.58
OPM-05	GGGAACGTGT	300-1000	150	6.00	0.45
OPM-06	CTGGGCAACT	300-900	154	6.16	0.39
OPX-17	GACACGGACC	300-1800	197	7.88	0.47
OPM-12	GGGACGTTGG	300-750	78	3.12	0.77
OPM-15	GACCTACCAC	300-1200	61	2.44	0.61
OPM-20	AGGTCTTGGG	600-1000	136	5.44	0.50
OPB-1	GTTTCGCTCC	500-1200	111	4.44	0.58
OPA-08	GTGACGTAGG	600-1000	55	2.20	0.65
OPA-1	CAGGCCCTTC	300-1200	117	4.68	0.50

occurrence of considerable genetic variability among bael trees. However, variation was higher than that reported for 25 cultivars of mango (range 0.69-0.89) (Rajwana *et al.,* 2008). A dendrogram (Fig 1.) was constructed from values of similarity coefficients generated from RAPD data. The trees were divided into six major genotypic groups at a 0.446 similarity coefficient, containing 6 clusters respectively, based on the unweighted pair group method using arithmetic average cluster analysis. The trees SB-2, SB-351, SB-161, SB-353 placed in a distinct cluster while other clusters subdivided into sub-clusters. Cluster 'a' consists of 19 trees, where these trees separated from each other at 0.57 similarity coefficients forming a distinct cluster for SB-175. This cluster was further divided at 0.614 forming a distinct cluster for SB-80. Cluster 'b' comprised of two trees SB-123 and SB-273. It was observed that SB-147 and SB-90 were placed very closely at a similarity co-efficient of 0.95.

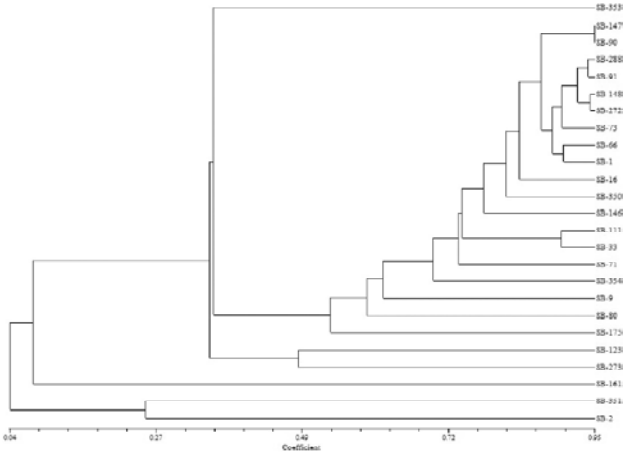

Fig. 1. Dendrogram deviding the 25 trees of bael based on Jaccard genetic similarity coefficient from analysis.

ISSR analysis

Polymerase chain reaction of bael genomic DNA using 16 selected ISSR primers generated a total of 1,496 amplified bands (Table 4.). The highest number of bands was observed with primer UBC-807 and the lowest number of bands was observed with primer UBC-890. Comparatively higher polymorphic information content (0.83 to 0.99) was shown by selected polymorphic primers. The highest PIC value (0.99) was observed in primer UBC-888 whereas, lowest PIC value (0.83) was observed in UBC-815. Average number of bands across trees were found maximum in primer UBC-807 (7.28) while minimum in primer UBC-890 (1.60). The highest genetic

Table 4. List of ISSR primers, their sequence and generated bands

Primer	Marker sequence (5' to 3')	Total No. of bands	Average No. of bands across trees	PIC value
UBC 807	AGA GAG AGA GAG AGA GT	182	7.28	0.90
UBC 810	GAG AGA GAG AGA GAG AT	125	5.00	0.88
UBC 811	GAG AGA GAG AGA GAG AC	59	2.36	0.97
UBC 815	CTC TCT CTC TCT CTC TG	63	2.52	0.83
UBC 824	TCT CTC TCT CTC TCT CG	97	3.88	0.94
UBC 825	ACA CAC ACA CAC ACA CT	137	5.48	0.94
UBC 834	AGA GAG AGA GAG AGA GYT	103	4.12	0.95
UBC 836	AGA GAG AGA GAG AGA GYA	76	3.04	0.96
UBC 840	GAG AGA GAG AGA GAG AYT	65	2.60	0.98
UBC 841	GAG AGA GAG AGA GAG AYC	108	4.32	0.92
UBC 842	GAG AGA GAG AGA GAG AYG	117	4.68	0.94
UBC 859	TGT GTG TGT GTG TGT GRC	88	3.52	0.98
UBC 888	BDB CAC ACA CAC ACA CA	56	2.24	0.99
UBC 889	DBD ACA CAC ACA CAC AC	96	3.84	0.84
UBC 890	VHV GTG TGT GTG TGT GT	40	1.60	0.97
UBC 891	HVH TGT GTG TGT GTG TG	57	2.28	0.96

similarity coefficient of 0.56 between the SB-1 and SB-73may be due to their same place of origin and occurrence of an intense gene flow between these trees. But the molecular diversity was not in agreement with most of the morphological diversity as reported in *Colocasia esculenta* (Singh *et al.,* 2012). Comparatively high amplitude of the genetic similarity coefficient established in the present study confirms the occurrence of considerable genetic variability among bael trees. A dendrogram was constructed from values of similarity coefficients generated from ISSR data. According to the dendrogram (Fig. 2.), the trees were divided into nine major genotypic groups at a 0.30 similarity coefficient, containing nine clusters respectively, based on unweighted pair group method using arithmetic average cluster analysis. The trees SB-354, SB-351, SB-175, SB-353 placed in a distinct cluster while other clusters sub divided in to sub-clusters. Cluster 'a' consists of five trees, where these trees separated from each other at 0.57 similarity coefficients forming a distinct cluster for SB-175. This cluster was further divided at 0.33 forming a distinct

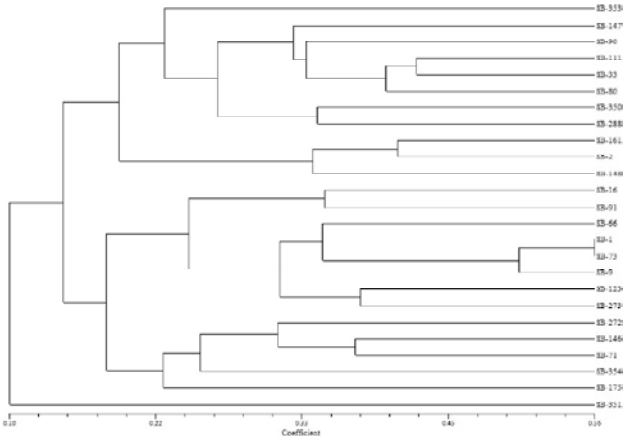

Fig. 2. Dendrogram of 25 trees of bael based on Jaccard genetic similarity coefficient ISSR markers analysis.

cluster for SB-147. Cluster b comprised of two trees SB-350 and SB-288. Cluster c comprised of three trees SB-161, SB-2 SB-148. Cluster d, e, f comprised of two, six and three trees respectively. At a similarity co-efficient of 0.56, it was observed that SB-1 and SB-73 were placed very closely.

RAPD and ISSR combined analysis

A dendrogram was constructed using values of similarity coefficients generated from RAPD and ISSR data. According to the dendrogram (Fig. 3.), the trees were divided into nine major genotypic groups at a 0.51 similarity coefficient, containing nine clusters respectively, based on unweighted pair group method using arithmetic average cluster analysis. The treesSB-353, SB-80, SB-175, SB-123, SB-273, SB-161, SB-2, SB-351 placed in a distinct cluster while other clusters sub divided in to sub-clusters. Cluster 'a' consists of four trees, where these trees separated from each other at 0.59

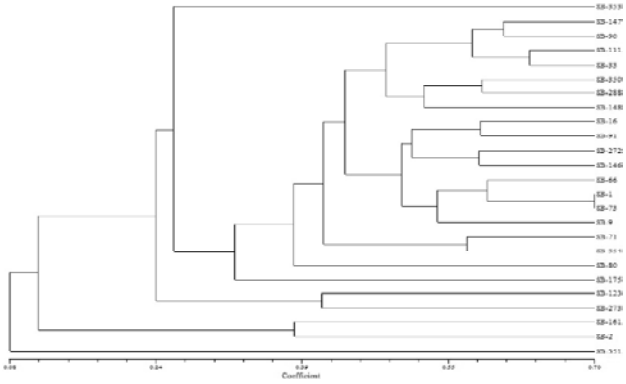

Fig. 3. Dendrogram of 25 trees of bael generated based on combined RAPD and ISSR data

similarity coefficients. Cluster 'b' comprised of three trees SB-350, SB-288 and SB-148. Cluster 'c' comprised of four trees SB-16, SB-91, SB-272 and SB-146. Cluster 'd' and 'e' comprised of four and two trees respectively. At similarity co-efficient of 0.70 it was observed that SB-1 and SB-73 were placed very closely.

CONCLUSION

Both the molecular markers analysis showed a high degree of variation among the selected bael trees. The present study revealed that both the molecular markers can be successfully utilized for inferring genetic diversity and genetic relationship of bael trees. The similarity between SB-1 and SB-73 confirmed the importance of these markers for distinguishing the bael trees based on environmental and genetic factors. Findings of this study indicate that identification of trees from various locations mainly based on morphological characteristics may have encountered the mismatches and mistakes. This indicates the importance of characterisation of trees both at morphological and molecular level for efficient maintenance and exploitation of precious germplasm and to determine groups of high genetic similarity and dissimilarity, which is the key for

establishing breeding strategies in genetic improvement programme of bael.

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Original Research Paper

Molecular characterization of ginger genotypes using RAPD and SSR markers

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ABSTRACT

Genetic diversity among ginger genotypes collected from different parts of the country was studied using molecular markers (30 RAPD and 55 SSR). Compared to RAPD primers SSR primers were efficient in distinguishing the genotypes. A total of 86 and 23 polymorphic bands were observed with RAPD and SSR primers, respectively. Percentage polymorphism observed between RAPD and SSR primers was 97.40 % and 56.54 %. Grouping of genotypes by using combined data of RAPD and SSR primers indicated that irrespective of their place of collection or geographical origin, 30 genotypes were clustered into different groups which showed that, each individual genotype is having wider variability or it might be due to the genetic similarity existing among them.

Keyword: Ginger, molecular markers, monomorphic and polymorphic

INTRODUCTION

Ginger of commerce is the underground rhizome of *Zingiber officinale* Rosc. (2n=22), belonging to the family Zingiberaceae and it is originated from South-East Asia. It is one of the oldest and most important spices, being cultivated in Tropical Asia for over 3000 years. It is one of the earliest oriental spices known to Europe and is still in large demand today. The rhizomes may be scraped or peeled before drying and are esteemed for their aroma, flavour and pungency. It may also be used in powdered form (Purseglove *et al*., 1981). Largest collection of ginger germplasm (675 accessions) is being conserved at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala which is also NAGS centre of ginger.

Most of the varieties have vernacular names and as the crop is propagated vegetatively hence the chances of mixing are very high. Generally, ginger genotypes are identified based on morphological traits, but the assessment of these traits is difficult and their evaluation can be subjective considering that most of these cultivars are related. Most of the ginger cultivars are not easily differentiated based on rhizome or aerial morphological features, further confounding the confusion to a greater extent.

The development in molecular approaches for identification of plant varieties/genotypes seems to be more effective than the traditional morphological markers because it allows direct access to the hereditary material and makes it possible to understand the relationships, between plants (Williams *et al*., 1990; Paterson *et al*., 1991). Molecular marker technology is the powerful tool for determining genetic variation in ginger genotypes as they can reveal abundant difference among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation, management and untouched by environmental influence. Although RAPD markers are suitable for genetic diversity analysis of clonal organisms (Bardakci, 2001), SSR markers are more reproducible and useful in evaluating genetic diversity and cultivar identification (Goulao and Oliveira 2001; Pomper *et al*., 2010; Nas *et al*., 2011). In view of the above, the present study used both RAPD and SSR markers to analyze the presence of diversity among different ginger genotypes.

MATERIAL AND METHODS

Plant material

Twenty-seven ginger genotypes, one *Zingiber* sp., one *Curcuma* sp. and one *Kaempferia* sp. collected from different parts of the country and maintained at NAGS centre IISR, Kozhikode were used in the study (Table S1).

Genomic DNA isolation

Young leaves from 45-60 days old plants were selected for DNA isolation. Genomic DNA was isolated using the CTAB method (Syamkumar *et al*., 2003). One gram of young, clean leaf was ground in liquid nitrogen into fine powder with the help of pestle and mortar. DNA was extracted with CTAB extraction buffer. DNA was purified and quantified by gel (0.8% agarose gel) based quantification.

RAPD and SSR analysis

Thirty randomly selected RAPD primers were used in the study (Table S2). A 25 µl reaction mixture was prepared as follows: 3 µl of dNTP (10 mM), 1 µl primer (10 mM), 3.5 µl of 10 X reaction buffer with 15mM $MgCl_2$, 0.5 µl of Taq DNA polymerase (3 U/µl) and 1.6 µl of template DNA. PCR amplification was done in a thermocycler with an initial denaturation of 94 °C for 3 minutes followed by 35 cycles of 94 °C for 45 seconds, annealing at 37 °C for 45 seconds and extension at 72 °C for 1 minute followed by a final extension at 72 °C for 15 minutes. The PCR amplified products were analysed on a 1.5 % agarose gel stained with ethidium bromide. The gels were digitally photographed by Bio-Imaging systems (Syngene GBOX-CHEMI, England).

A set of 55 SSR primers were used in the present study *viz.,* 22 EST SSR primers (Anu, 2016), eight ginger genomic SSR primers (Lee *et al*., 2007), 18 genomic SSR primers (Siju *et al*., 2010a) and 7 EST SSR primers (Siju *et al*., 2010b) from *Curcuma longa* (Table S3). A 20 µl reaction mixture was prepared as follows: 2 µl of dNTP (10 mM), 2 µl primer (10 mM), 2.5 µl of 10 X reaction buffer with 15mM $MgCl_2$, 0.2 µl of Taq DNA polymerase (3 U/µl) and 1.5 µl of template DNA. PCR amplification was done in a thermocycler with an initial denaturation of 94 °C for 5 minutes followed by 35 cycles of 94 °C for 45 seconds, 45 seconds of annealing temperature (52-65 °C) and extension at 72 °C for 1 minute followed by a final extension at 72 °C for 20 minutes. The PCR amplified products were analysed on a 3.0 % agarose gel stained with ethidium bromide. The gels were digitally photographed by Bio-Imaging systems (Syngene GBOX-CHEMI, England).

Data analysis

The independent as well as combined data generated for 30 genotypes from RAPD and SSR primers were subjected to statistical analysis. RAPD and SSR products were scored visually for presence (1) and absence (0) of bands. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) described by Rohlf (1990). A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the marker data from the ginger with unweighted pair group method (UPGMA). Parameters such as PIC and genotypic gene diversity were estimated by using the formula developed by Anderson *et al*. (1993) and Mariette *et al*. (2002), respectively.

RESULTS

Molecular variability of ginger genotypes through RAPD

Using RAPD analysis, polymorphic fragments were generated in ginger genotypes. The selection of primers was based on clear, scorable and reproducible amplified banding patterns.

Out of 30 primers used, 11 RAPD primers showed amplification and the number of amplification products obtained was specific to each primer. The size of the amplified products varied from 400 to 2800 bp. Of the 11 primers, ten primers *viz*., OPA 09, OPA 17, OPA 18, OPB 08, OPD 03, OPD 07, OPD 18, OPH 08, OPI 07 and OPL 12 were found to show 100 per cent polymorphism which is presented in Table 1. Of the 88 total alleles observed, 86 alleles were polymorphic and maximum numbers of 14 alleles were obtained with primer OPL 12, followed by primer OPA 09 and OPI 07 with 10 alleles. Minimum numbers of 3 alleles were generated with primer OPD 03. Thus, amplifications varied across the primer employed. Among the 11 RAPD primers, the Polymorphism Information Content (PIC) was high in OPD 03, OPD 07 and OPH 08 (0.998) (Table 1).

Table 1. Polymorphism among ginger genotypes detected by RAPD markers

Primers	Total allele	MB	PB	% MM	% PM	Total amplicons	Allele brange	PIC	Genotypic gene diversity
OPA 09	10	0	10	0	100	55	750-2600	0.981	0.816
OPA 17	6	0	6	0	100	80	1000-2500	0.985	0.555
OPA 18	8	0	8	0	100	98	400-1800	0.988	0.591
OPB 08	6	0	6	0	100	107	500-1500	0.996	0.448
OPD 03	3	0	3	0	100	87	1000-2000	0.998	0.275
OPD 07	7	0	7	0	100	92	1500-2300	0.998	0.561
OPD 18	9	0	9	0	100	169	500-2800	0.993	0.324
OPH 08	8	0	8	0	100	70	1200-2700	0.998	0.708
OPH 15	7	2	5	28.57	71.43	111	1000-2300	0.997	0.390
OPI 07	10	0	10	0	100	175	400-2600	0.993	0.358
OPL 12	14	0	14	0	100	253	400-2800	0.988	0.437
Total	88	2	86	28.57	1071		10.92	5.463	
Mean	8	0.18	7.82	2.59	97.40	117.90		0.99	0.50

MB – Number of Monomorphic Bands; PB – Number of Polymorphic Bands; % MM – Per cent Monomorphism; % PM – Per cent Polymorphism; PIC - Polymorphism Information Content

Each RAPD pattern was compared with other patterns and genetic similarity matrix for all the thirty genotypes was constructed from binary data of markers using Jaccard's algorithm.

The coefficient of genetic similarity ranged from 39 - 97 per cent. Maximum similarity of 95 per cent was noticed between Himachal and Zaheerabad local. Further, the information generated out of RAPD banding pattern was used for clustering through unweighted mean pair group arithmetic mean method (UPGMA) (Fig. 1).

The genotypes were divided into two main groups, I and II sharing 39 % similarity which were further subdivided into clusters. Among the genotypes, two

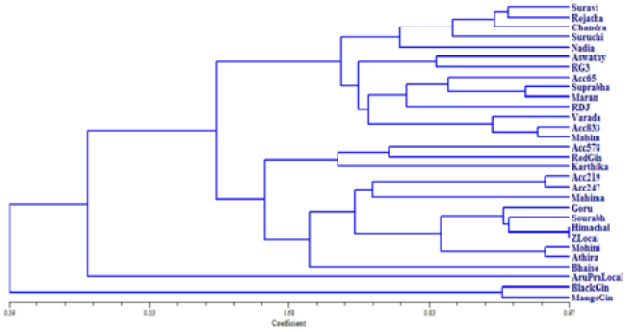

Fig. 1. UPGMA dendrogram based on RAPD markers using Jaccard's similarity coefficient

genotypes (black ginger and mango ginger) were grouped under group I with sharing similarity of 90 % and other 28 genotypes (Suravi, IISR Rejatha, KAU Chandra, Suruchi, Nadia, Aswathy, RG 3, Acc. 65, Suprabha, Maran, Rio de Janeiro, IISR Varada, Acc. 833, Mahim, Acc. 578, Red ginger, Karthika, Acc. 219, IISR Mahima, Gorubathane, Sourabh, Acc. 247, Mohini, Athira, Bhaise, Arunachal Pradesh local, Himachal and Zaheerabad local) were grouped under group II with sharing similarity of 47 %.

Group II consisted of two sub clusters namely A and B sharing similarity of 47 %. Cluster A consisted of one genotype *viz.,* Arunachal Pradesh local. Cluster B was sub divided into C and D sharing similarity of 60 %. Group C further divided into cluster E and F sharing approximately 65 % similarity. Cluster E was subdivided into G and H sharing 70 % similarity. Cluster G consisted only one genotype Bhaise. Cluster H consisted of nine genotypes (Acc. 219, Acc. 247, Mahima, Gorubathane, Sourabh, Himachal, Zaheerabad local, Mohini and Athira). Among the nine genotypes, Himachal and Zaheerabad local showed 97 % similarity followed by 94 % similarity was observed between Mohini and Athira as well as Acc. 219 and Acc. 247. Cluster F consisted of three genotypes *viz.,* Acc. 578, red ginger and Karthika showing 71 % similarity.

Molecular variability of ginger genotypes through SSR

Out of 55 SSR primers screened, sixteen primers amplified and produced 34 alleles among them 25 were polymorphic bands and 10 were monomorphic bands. SSR fragments ranged from 100 to 1200 bp in size (Table 2).

Maximum number of alleles detected was seven from ZOM 103 primer. With the average of 62.80 per cent polymorphism produced by sixteen SSR primers, cent per cent polymorphism was detected by the primers ZOC 11, ZOC 28, ZOC 156, ZOC 33, ZOM 064, ZOM 140 and CLEST 16. Polymorphism information content (PIC), a measure of gene diversity was an average of 0.92 with a range of 0.889 by ZOM 033 to 0.982 by CLEST 16 primer.

Jaccard's similarity coefficients among the thirty genotypes helped to establish genetic relationships (Fig. 2). Phylogenetic analyses of thirty genotypes, conducted on SSR banding patterns, indicated that maximum percentage of similarity (100 %) was observed between KAU Chandra, IISR Mahima and Mohini; IISR Rejatha and Nadia; Acc 65, Suprabha and Maran; Rio de Janeiro and Sourabh; Suruchi and Acc. 833; IISR Varada and Bhaise.

Thirty ginger genotypes were used to study their variability through SSR analysis using sixteen primers. The SSR pattern obtained for these genotypes with different primers were defined by the presence or absence of bands. Each SSR pattern was compared with each other and euclidean distance matrix was calculated for all the 30 ginger genotypes. The relationship among the genotypes was represented as dendrogram using UPGMA.

The genotypes were divided into two main groups, I and II sharing 59 % similarity. Group I comprised of only one genotype, mango ginger. Group II was further subdivided into cluster A and B with similarity

Table 2. Polymorphism among ginger genotypes detected by SSR markers

Primers	Total allele	MB	PB	% MM	% PM	Total amplicons	Allele brange	PIC	Genotypic gene diversity
ZOC 11	1	1	0	100	0	30	250	0.893	0
ZOC 28	3	0	3	0	100	31	150-280	0.923	0.655
ZOC 92	1	1	0	100	0	30	190	0.943	0
ZOC 98	3	1	2	33.33	66.66	88	250-280	0.952	0.022
ZOC 100	2	1	1	50	50	58	150-170	0.922	0.033
ZOC 156	3	0	3	0	100	36	150-250	0.897	0.60
ZOC 33	1	0	1	0	100	29	180	0.889	0.633
ZOM 040	2	1	1	50	50	42	190-210	0.921	0.3
ZOM 055	1	1	0	100	0	30	190	0.921	0
ZOM 064	1	0	1	0	100	28	250	0.954	0.066
ZOM 103	7	2	5	28.57	71.43	101	150-1200	0.988	0.545
ZOM 107	3	1	2	33.33	66.66	32	190-400	0.893	0.644
ZOM 111	1	1	0	100	0	30	300	0.906	0
ZOM 140	2	0	2	0	100	58	140-150	0.940	0.033
CLEST 15	1	1	0	100	0	30	150	0.948	0
CLEST 16	2	0	2	0	100	56	170-190	0.982	0.066
Total	**34**	**11**	**23**	**695.23**	**904.75**	**709**		**14.87**	**3.597**
Mean	**2.12**	**0.68**	**1.43**	**43.45**	**56.54**	**44.31**		**0.92**	**0.22**

MB – Number of Monomorphic Bands; PB – Number of Polymorphic Bands; % MM – Per cent Monomorphism; % PM – Per cent Polymorphism; PIC - Polymorphism Information Content

percentage of 74. Cluster A consisted of 2 genotypes (Acc. 578 and black ginger) sharing similarity of approximately 81 %. Cluster B was subdivided into 2 clusters C and D sharing percentage similarity of 84 %. Cluster C divided into 2 sub clusters E and F with 89 % similarity. Cluster E consisted of 4 genotypes namely Red ginger, Athira, Karthika and Zaheerabad local sharing 92 % similarity. Cluster F consisted of 8 genotypes *viz.,* Suruchi, Acc. 833, Aswathy, RG 3, IISR Varada, Bhaise, Acc. 219 and Gorubathane. Among the 8 genotypes Suruchi and Acc. 833 shared 100 % similarity; IISR Varada and Bhaise were also 100 % similar to each other.

Cluster D was subdivided into 2 clusters namely G and H with similarity percentage of approximately 88 %. Cluster G consisted of 7 genotypes sharing 91 % similarity, among 7 genotypes Acc. 65, Suprabha and Maran showed 100 % similarity and Rio de Janeiro and Sourabh were also 100 % similar. Cluster H consisted 8 genotypes sharing 91 % similarity, among them genotypes KAU Chandra, IISR Mahima and Mohini were 100 % similar. Similarly, genotypes IISR Rejatha and Nadia also showed 100 % similarity.

Molecular variability of ginger genotypes through pooled RAPD and SSR markers

The data obtained on RAPD and SSR primers were pooled to assess the polymorphism. Data obtained from pooled analysis of RAPD and SSR primers revealed that, the ginger genotypes were divided into 2 main groups I and II sharing 49 % similarity (Fig. 3). Group I consisted of only one genotype black ginger. Group II was further subdivided into 2 clusters A and B sharing approximately 50 % similarity. Cluster A consisted of only one genotype *i.e.,* Mango ginger. Cluster B further divided into cluster B and D with 53 % similarity. Cluster C consisted of two genotypes (Himachal and Zaheerabad local) sharing approximately 63 % similarity. Cluster D is subdivided into cluster E and F sharing similarity percentage of 68. Cluster E was subdivided into G and H with 72 % similarity. Cluster G consisted of eight genotypes namely Acc. 219, IISR Mahima, Gorubathane, Mohini, Athira, Acc 247, Sourabh and Bhaise. Among them, Acc. 247 and Sourabh showed maximum similarity of 90 %. Cluster H consisted of three genotypes, Acc. 578, Red ginger and Karthika sharing 77 % similarity. Cluster F was divided into 2 clusters, I and J sharing 77 % similarity. Cluster I consisted of nine genotypes namely Aswathy, RG 3, Acc. 65, Suprabha, Maran, Rio de Janeiro, IISR Varada, Acc. 833 and Mahim. Among them, genotypes Suprabha and Maran showed 100 % similarity. Cluster J consisted of 5 genotypes (Suravi, KAU Chandra, IISR Rejatha, Suruchi and Nadia) sharing approximately 83 % similarity.

Comparison of RAPD and SSR marker systems for their efficacy in assessing genetic diversity of ginger genotypes

To compare the utility of the two marker systems, thirty ginger genotypes were analyzed with eleven RAPD and sixteen SSR primers. Various parameters *viz*., total number of alleles, number of polymorphic bands per assay unit, mean percentage of polymorphism per assay, number of monomorphic bands per assay and polymorphic information content (PIC) value were recorded as criteria to differentiate their efficacy and the results are presented in Table 3.

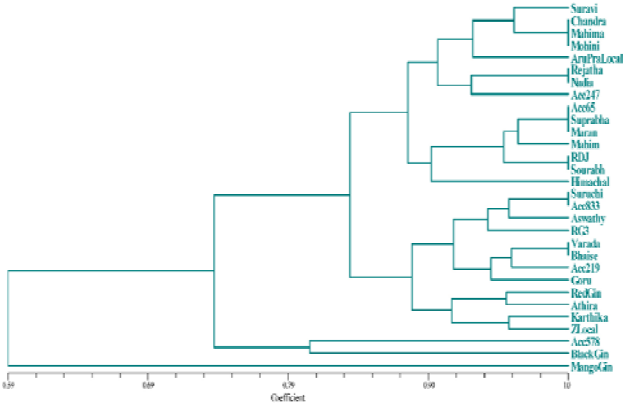

Fig. 2. UPGMA dendrogram based on SSR markers using Jaccard's similarity coefficient

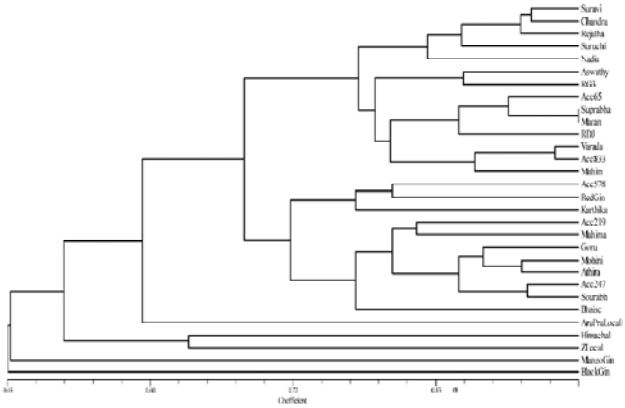

Fig. 3. UPGMA dendrogram based on RAPD and SSR markers using Jaccard's similarity coefficient

Table 3. Comparative analysis of banding patterns generated by RAPD and SSR

Components	RAPD	SSR
Number of alleles per assay unit	11	16
Total amplicons	1297	709
Total number of alleles	88	34
Mean number of alleles per assay unit	8	2.12
Number of polymorphic bands per assay unit	7.83	1.43
Mean (%) polymorphism per assay	97.40	56.54
Number of monomorphic bands per assay unit	0.18	0.68
Mean PIC per assay	0.99	0.92

The mean number of alleles per assay unit, number of polymorphic and monomorphic bands per assay unit in SSR analysis was 16.0, 1.56 and 0.62 respectively, and in case of RAPD primers it was 11.0, 7.82 and 0.18 respectively. Mean percentage of polymorphism per assay was 96.97 % in RAPD, whereas, it is 62.80 % in case of SSR primers.

DISCUSSION

Knowledge of the genetic variation within and among populations is an important component for understanding the variability in any crop. Therefore, information on population diversity may be used in selection and crop improvement process. Molecular methods are much faster, more specific, sensitive and accurate. Molecular markers are nowadays widely used to distinguish the genotypes in several horticulture crops (Li *et al.*, 2007; Karimi *et al*., 2010 and Ansari and Singh 2013 and 2014). As ginger is clonally propagated and it is difficult to distinguish between the genotypes using morphological markers, molecular approaches are highly useful for characterization of ginger genotypes. In the present study 30 RAPD and 55 SSR markers were used to study the genetic variability.

RAPD dendrogram was not associated with exact geographical localities from which the ginger genotypes were collected. The considerable polymorphism detected in this study illustrated that, it is possible to find genetic divergence among ginger cultivars of the same origin. These results are in accordance with Nayak *et al*. (2005) and Sera *et al*. (2003), who also reported similar results in ginger and coffee respectively. These results in ginger indicate that, RAPD markers were able to provide more reliable information than morphological characters to identify closely related ginger genotypes (Nayak *et al*., 2005 and Palai and Rout 2007). Diversity among the cultivars revealed the presence of genotypic diversity among the genotypes. Variability to certain extent might be due to the different environmental conditions.

SSRs are widely used as versatile tool in plant breeding programme as well as in evolutionary studies because of their ability for showing diversity among the cultivars (Adato *et al.,* 1995). Therefore, in the present investigation, out of 55 SSR primers screened, 16 primers amplified and produced 34 alleles among them 23 were polymorphic bands and 11 were monomorphic bands. Pandotra *et al*. (2013); Das *et al*. (2016); Jatoi *et al*. (2006) and Lee *et al*. (2007) also reported the use of SSR markers to study the variability and genetic diversity existing at the population level.

Dendrogram obtained revealed that, irrespective of their place of collection or geographical origin they have grouped into different clusters which showed that, each genotype selected in the study is having wide variability or it may be due to genetic similarity existing among them. SSR primers used were highly efficient in separating *Curcuma* sp. from the *Zingiber* species but those did not distinguish the ginger genotypes based on any character or place of collection. Jatoi *et al*. (2006) also reported that clustering pattern within the genus *Zingiber* did not reflect any relationship between genotypic variation and place of collection. Similar results were obtained by Jaleel and Sasikumar (2010) and they reported that, collection of the accessions based on vernacular identity irrespective of the geographical proximity may be the probable reason for this behaviour. It also implies that genes amplified by the markers need not be strictly linked with any agronomic traits.

CONCLUSION

RAPD and SSR primers were used to study the diversity among ginger genotypes collected from different agro climatic regions of the country. Among 11 RAPD primers, ten primers *viz*., OPA 09, OPA 17, OPA 18, OPB 08, OPD 03, OPD 07, OPD 18, OPH 08, OPI 07 and OPL 12 were found to show 100 per cent polymorphism. Among the sixteen SSR primers, cent per cent polymorphism was detected by the primers ZOC 11, ZOC 28, ZOC 156, ZOC 33, ZOM 064, ZOM 140 and CLEST 16. Irrespective of their place of collection or geographical origin, 30 ginger genotypes were clustered into different groups which showed that, each individual genotype is having wider variability or it may be due to the genetic similarity existing among them.

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Original Research Paper

Response of Dashehari mango to different Zn levels on yield and pulp nutrient contents grown on sandy loam soils of Lucknow

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ABSTRACT

Dashehari is the leading mango variety grown in Indo-Gangetic plain. Its yield is affected severely by the micronutrient deficiencies. Zinc and boron are the two important micronutrients which limit the yield and quality of Dashehari mango in this region. Hence a field study was taken up to understand the responses of Dashehari mango to different levels of Zn. Results indicated yield enhancement with proper Zn supplementation through foliar sprays. Highest yield of 43.50±2.00 to 50.72±2.40 kg tree^{-1} was recorded with 1.0% $ZnSO_4$ application, followed by 42.27±1.26 (1.5% $ZnSO_4$) to 47.85±1.65 (0.75% $ZnSO_4$) kg tree^{-1}. TSS (19.63±0.25 to 20.27±0.40°Brix), acidity (0.150±0.01 to 0.200±0.02%) and ascorbic acid (29.46±2.29 to 35.17±1.32 mg per 100 g) variations were noted under the influence of various Zn treated fruits. Foliar spray application also caused nutrient richness in mango fruit pulp showing improvement in Zn concentration in fruit pulp from 1.17±0.10 to 1.73±0.10 mg kg^{-1}. Highest concentration of B, Cu, Fe and Mn were observed (3.13±0.018, 4.37±0.06, 7.87±0.06, 20.10±0.15 mg kg^{-1} respectively) with P and K concentrations of 0.026±0.0002& 0.28±0.001% respectively. Significant difference in leaf and soil Zn content was also recorded. The results indicated that yield and quality of Dashehari mango can be improved with foliar spray of Zn in sandy loam soil.

Keywords: Dashehari mango, pulp nutrient concentration, soil and foliar nutrient, yield and quality attribute and Zn levels.

INTRODUCTION

The response of fruit tree to externally applied mineral nutrients needs to be quantified to provide technology innovations to fruit growers as ready to use package of practices. This process might lead to nutrient richness in the end product i.e. fruit pup. This is very significant in case of sand, loamy sand, sandy loam soils having low water holding capacity, soil organic matter, nutrient reserve and microbial activity. Significant response of the tree to nutrient application depends on several attributes like tree physiology, soil response, weather interactions and varietal ability *etc*. Adak *et al.* (2021) scientifically explained that there is an urgent need for revisiting policy issues in terms of soil nutrition *vis-à-vis* productivity and profitability for subtropical zone. Soil nutrients play significant role in responding to the signal transduction to roots and from roots to sink. The source-sink continuum often either hastens or restricted by the pools of nutrients. Lower the nutrient pool, response to end product may be low. However, foliar application may improve the positive response through xylem-phloem pathways through leaf stomata. Adak *et al.* (2019) indicated that lower soil nutrient index is responsible for lower productivity of Dashehari mango in farmers' field in Maal area of Uttar Pradesh. This certainly had contributed to yield variations within the orchards. Similarly in apple orchards Aggelopoulou *et al.* (2010) described the spatial yield and quality variability within the apple orchards. Nutrient deficiency in the foliar part is one of the top most priority for any commercial or non-commercial orchards to indentify and its possible solutions for correction of nutrient limitations. Several nutrients were recorded to be deficient on long-term basis in orchards. Raja *et al.* (2005) inferred boron deficiency in mango and also suggested for possible remediation. Tehranifar and

Tabar (2009) observed that foliar application of K and B (1.5 and 3.0 g L^{-1}) leads to nutrient richness in pomegranate. Liu *et al.* (2021) emphasized potassium fertilization during fruit development for improving quality and potassium use efficiency of tomato in deficit irrigation regime. The quality of the produce is to be authenticated for which low cost near-infra-red spectroscopy technology could be employed (Yang *et al.,* 2021). Similarly, Davarynejad *et al.* (2009) recorded positiveness of foliar nutrition technology in enhancing the yield, quality and alternate bearing as well in pistachio fruit tree. The statistical significance of such response is to be recorded and multivariate interpretation should be done in order to understand the foliar chemical composition of essential nutrients (Raghupathi and Shilpashree, 2018) for development of technologies for corrections. On the present field study, trails were laid out to record the response of Zn levels on nutrient richness and productivity level on sandy loam soil at Lucknow, Uttar Pradesh.

MATERIAL AND METHODS

The field study was conducted on 9th and 10th year old mango cv Dashehari trees spaced at 10×10 m on sandy loam soil at Rehmankhera Farm, Lucknow, Uttar Pradesh during 2015-18. Seven treatments were replicated thrice in a randomized block design. Initial nutrient status of the experimental field was poor. The treatments applied were as T_1: control, T_2: 0.25% $ZnSO_4$, T_3: 0.50% $ZnSO_4$, T_4: 0.75% $ZnSO_4$ T_5: 1.0% $ZnSO_4$ T_6: 1.5% $ZnSO_4$ and T_7: 2.0% $ZnSO_4$. The foliar spray was done in the last week of September, before flowering (3rd week of February), at marble stage of fruit and second spray after 25 days interval. The $ZnSO_4$ was sourced as fertilizer (15%) and the volume of spray per tree was 10-liter volume of solution. Field layout and basin preparation was done as per recommended package of practices. Irrigation water was applied on critical stage wise and based on weather inputs. Tree protection measures were also taken care of. Soil samples were collected randomly from the selected trees. Leaf samples were taken from N-S and E-W directions within the canopy. Fruit samples were collected from different directions in the canopy to represent the overall performance of the tree. Fruits were harvested during 2nd week of June. Yield was reported kg $tree^{-1}$ basis. Quality components were analyzed as par Ranganna (1986). All standard procedures were followed for preparation of soil and leaf and pulp samples for chemical analysis. Leaf digestion and soil digestion was completed following laboratory protocol and micronutrients were analysed using AAS. Statistical analysis *viz*., significance, standard error of mean, standard error of difference and coefficient of variations were computed in OPSTAT (Sheoran *et al*., 1998).

RESULTS AND DISCUSSION

The study reveals the effectiveness of different Zn levels on the Dashehari mango grown on sandy loam soils in Indo-Gangetic plains under subtropical climate. The results showed significant response among mango trees treated with different foliar Zn levels (Table 1). Lowest $ZnSO_4$ application yield of 33.17±2.25 kg $tree^{-1}$ was noted. In general, yield improved up to 1%. Beyond that T_5, the response was not significant. Highest yield of 43.50±2.00 and 50.72±2.40 kg $tree^{-1}$ was noted. TSS of 19.90±0.31 (T_1) to 19.63±0.25 (T_5) and 19.67±0.21 (T_1) to 20.03±0.21°Brix (T_5) was estimated. Similarly, acidity of 0.158±0.03, 0.200±0.02 (T_5) to 0.175±0.03 to 0.158±0.01% (T_1) was recorded. Ascorbic acid content was ranging from 35.17±1.32 (T_5) to 30.58±3.50 mg per 100 g (T_1). Variable content of quality attributes suggested possible nutrient interaction in the mango trees. The enhanced nutrient concentration in fruit pulp was also recorded (Table 2). Lowest Zn concentration of 1.17±0.10 (T_1) to 1.73±0.10 (T_4), 1.60±0.06 mg kg^{-1} (T_5) was recorded. Cu concentration of 3.50±0.015 mg kg^{-1} (T_1) to 3.93±0.015 mg kg^{-1} (T_5), B concentration of 2.01± 0.09 mg kg^{-1} (T_1) to 3.13 ±0.18 mg kg^{-1} (T_5) followed by 2.56±0.12 mg kg^{-1} (T_4) were recorded. Non-significant response was observed in some mineral composition like Fe that varied between 16.20±0.15 to 20.10 ±0.15 mg kg^{-1}. A narrow range of 0.021 to 0.026% P and 0.26 to 0.28% K was observed. The observed results suggested strong response of Zn levels on fruit pulp Zn content.

The mineral contentions of leaf tissue showed Zn variations between 29.7±5.51 (T_1) to 52.0± 5.29 mg kg^{-1} (T_5), Cu content of 13.7±0.58 (T_1) to 19.7±1.53 mg kg^{-1} (T_4), B content of 32.367±3.11 (T_1) to 35.93±1.79 mg kg^{-1} (T_5) (Table 3). However, Fe and Mn contents were non-significant with a narrow range of 170.3±11.59 mg kg^{-1} to 206.7±10.26 mg kg^{-1} and 137.7±5.13 mg kg^{-1} to 158.0±8.72 mg kg^{-1} was observed. Similarly, P and K content were recorded as 0.147 to 0.159% and 0.936 to 1.022% respectively. Soil organic matter in general was low i.e. 0.316 to

Table 1. Effect of foliar application of Zn on fruit yield and quality of mango

Treatment	Fruit yield (kg /tree)		TSS (^{0} B)		Acidity (%)		Ascorbic acid (mg/100g)	
T_1	33.17±2.25	38.32±2.48	19.90±0.31	19.67±0.21	0.175±0.03	0.158±0.01	29.46±2.92	30.58±3.50
T_2	34.83±3.00	44.40±3.60	19.93±0.21	19.87±0.21	0.175±0.01	0.183±0.01	31.14±1.45	31.34±1.32
T_3	37.00±1.53	45.83±1.68	20.03±0.36	19.77±0.15	0.158±0.04	0.167±0.01	29.46±5.26	33.64±2.65
T_4	41.67±1.50	47.85±1.65	19.70±0.38	20.27±0.40	0.167±0.01	0.175±0.03	29.46±2.53	33.64±3.50
T_5	43.50±2.00	50.72±2.40	19.63±0.25	20.03±0.32	0.158±0.03	0.200±0.02	30.30±5.26	35.17±1.32
T_6	42.27±1.26	46.60±1.51	19.83±1.07	19.73±0.15	0.150±0.01	0.183±0.03	31.98±5.26	35.17±1.32
T_7	38.83±2.75	43.12±3.58	20.36±0.20	19.83±0.23	0.167±0.03	0.183±0.01	33.67±1.45	34.40±6.07
$CD_{0.05}$	2.944	3.546	NS	NS	NS	NS	NS	NS
SE(m)	0.945	1.138	0.299	0.151	0.009	0.013	1.982	1.721
SE(d)	1.336	1.610	0.423	0.214	0.013	0.018	2.803	2.434
CV(%)	4.224	4.355	2.603	1.316	9.776	12.16	11.152	8.921

SE(m) stands for standard error of mean and SE(d) stands for standard error of difference.
CV is the coefficient of variations; values in mean ± standard deviations

Table 2. Effect of foliar application of Zn on nutrient concentration of mango pulp

Treatment	P	K	Fe	Mn	Zn	Cu	B
	%		mg kg^{-1}				
T_1	0.023±0.0004	0.28±0.002	17.77±0.50	6.97±0.12	1.17±0.10	3.50±0.015	2.01±0.09
T_2	0.024±0.0003	0.26±0.005	18.17±0.26	7.73±0.10	1.60±0.06	4.17±0.21	2.59±0.21
T_3	0.026±0.0002	0.27±0.002	16.20±0.15	7.80±0.06	1.67±0.12	4.23±0.06	2.55±0.27
T_4	0.024±0.0005	0.26±0.006	18.57±0.30	7.77±0.12	1.73±0.10	4.37±0.06	2.56±0.12
T_5	0.021±0.0006	0.27±0.008	20.10±0.15	7.83±0.12	1.60±0.06	3.93±0.15	3.13±0.18
T_6	0.021±0.0001	0.28±0.001	17.03±0.15	7.87±0.06	1.53±0.06	3.37±0.21	2.02±0.10
T_7	0.023±0.0002	0.28±0.002	16.43±0.06	7.37±0.21	1.43±0.21	3.13±0.17	2.04±0.23
$CD_{0.05}$	NS	NS	NS	NS	0.21	0.3	NS
SE(m)	0.0001	0.002	0.147	0.069	0.068	0.097	0.108
SE(d)	0.0002	0.003	0.207	0.098	0.096	0.137	0.153
CV(%)	1.525	1.519	1.431	1.577	7.686	4.395	7.755

SE(m) stands for standard error of mean and SE(d) stands for standard error of difference.
CV is the coefficient of variations; values in mean ± standard deviations

0.385%, much lower than critical level of 0.50% (Table 4). Lower SOC content thus recommends for higher organic input remedies to sandy loam soil. Available K of 74.78±3.97 mg kg^{-1} (T_1) to 84.48±3.81 mg kg^{-1} (T_4) to 81.79±15.87 mg kg^{-1} (T_5) was estimated. Fe and Mn availability of 4.78 to 5.87 and 8.21 to 9.31 mg kg^{-1} was observed. Significant difference of Zn and Cu content of 0.52±0.08 mg kg^{-1} (T_1) to 0.93±0.25 mg kg^{-1} (T_5) and 0.43±0.15 (T_1) to 1.29±0.30 mg kg^{-1} (T_5) was evidenced (Table 4). Higher CV (%) of 20.78% (Zn) and 30.75% (Cu) was also noticed.

The observed yield differences in the mango orchards are accounted for different rate of Zn application. Tree nutrition was thus found responsible for obtaining satisfactory yields. Zeng *et al.* (2001) reported the possible soil and leaf K

Table 3. Effect of foliar application of Zn on nutrient concentration of mango leaf

Treatment	P	K	Fe	Mn	Zn	Cu	B
	%		mg kg^{-1}				
T_1	0.157±0.007	0.969±0.03	182.3±20.43	149.0±12.49	29.7±5.51	13.7±0.58	32.367±3.11
T_2	0.159±0.005	0.960±0.03	206.7±10.26	155.7±6.11	35.7±3.51	14.7±3.21	35.100±2.05
T_3	0.147±0.002	0.936±0.03	182.7±9.87	143.7±5.13	38.3±1.53	17.0±4.36	38.800±2.79
T_4	0.148±0.005	0.984±0.01	183.0±9.54	152.7±14.05	46.0±6.24	19.7±1.53	39.967±4.60
T_5	0.158±0.012	1.022±0.06	170.3±11.59	137.7±5.13	52.0±5.29	13.0±1.00	35.933±1.79
T_6	0.156±0.002	1.006±0.01	184.3±29.67	158.0±8.72	55.3±5.13	12.3±0.58	35.300±1.80
T_7	0.160±0.013	0.996±0.03	174.7±12.22	154.0±14.0	55.7±7.02	11.7±2.31	36.367±3.67
$CD_{0.05}$	NS	NS	NS	NS	9.9	4.5	NS
SE(m)	0.005	0.020	9.3	6.27	3.22	1.46	1.84
SE(d)	0.007	0.028	13.15	8.86	4.55	2.07	2.61
CV(%)	5.15	3.45	8.78	7.23	12.47	17.4	8.80

SE(m) stands for standard error of mean and SE(d) stands for standard error of difference.
CV is the coefficient of variations; values in mean ± standard deviations

Table 4. Effect of foliar application of Zn on soil nutrients after harvesting of mango

Treatment	SOC	P	K	Fe	Mn	Zn	Cu
	%		mg kg^{-1}				
T_1	0.316±0.02	0.179±0.03	74.78±3.97	4.78±0.31	8.21±0.35	0.52±0.08	0.43±0.15
T_2	0.370±0.05	0.211±0.02	71.41±4.26	5.37±0.78	9.31±0.51	0.68±0.15	0.72±0.14
T_3	0.385±0.07	0.184±0.02	81.96±2.93	5.13±0.46	8.42±1.05	0.55±0.09	0.62±0.14
T_4	0.370±0.07	0.213±0.03	84.48±3.81	5.87±0.51	8.86±0.77	0.84±0.15	0.92±0.25
T_5	0.375±0.06	0.173±0.03	81.79±5.95	5.14±0.66	8.95±1.00	0.93±0.25	1.29±0.30
T_6	0.331±0.04	0.199±0.02	79.75±3.65	5.62±0.68	8.94±1.04	0.61±0.10	1.19±0.23
T_7	0.375±0.02	0.208±0.03	80.64±4.22	5.66±0.23	8.93±1.04	0.78±0.08	0.83±0.52
$CD_{0.05}$	NS	NS	6.78	NS	NS	0.22	0.39
SE(m)	0.026	0.013	2.26	0.28	0.37	0.073	0.13
SE(d)	0.037	0.018	3.20	0.40	0.52	0.103	0.19
CV(%)	14.61	13.13	5.72	10.55	8.39	20.78	30.75

SE(m) stands for standard error of mean and SE(d) stands for standard error of difference.
CV is the coefficient of variations; values in mean ± standard deviations

concentration variations along with nut yield and quality in pistachio tree. Perry *et al.* (2010) exhibited the pear orchard tree characteristics and its variations with yield. The soil condition is always questionable for solute transport ability. Asghari *et al.* (2011) reported the effect of soil conditioners in a sandy loam soil in terms of physical quality and bromide transport while Yadav *et al.* (2011) recorded statistically significant improvement in Amrapali mango with nutrient transformation mechanisms. In fact, the fitness of soil for tree plantations with potential yield is always top most priority on long-term basis to sustain land productivity (Ganeshamurthy and Reddy, 2015). Recently, Vallentin *et al.* (2022) opined that the satellite remote sensing data could

potentially be used for yield estimation and infrared spectroscopy could also be scientifically applied for quality assurances in mango and apple (Li *et al.,* 2021). The role of foliar spray of nutrients is beneficial in fruit trees as observed by Pal *et al.* (2018) in Arka Neelamani grape, Kumar *et al.* (2017) on guava, Hamze *et al.* (2018) on pistachio tree. Talang *et al.* (2017) found the effectiveness of calcium, boron and sorbitol on fruit-set, yield and quality in Himsagar mango. Adak *et al.* (2020) experimentally proved the beneficial effects of foliar nutrient technology on the yield performance, fruit quality and nutrient status of guava. In fact, the technological innovations should efficiently be disseminated to small and marginal growers for harnessing the benefits (Adak *et al.*, 2022). Since, soil properties also influence the yield performances, particularly organic carbon recognized as effective indicator, soil organic carbon stock should be estimated (Hinge *et al.,* 2018) and digital soil mapping of soil properties (Dharumarajan *et al.,* 2020), should also emphasized for future precision orchard management. Thus, the response recorded within the current trial showed 1% $ZnSO_4$ should be applied to mango trees for better statistically higher yield, quality component and nutrient richness. Beyond 1% $ZnSO_4$, economical benefit may not be availed.

CONCLUSION

Harvesting of optimum fruit yield from orchard is the sole objective of mango farmers. Fruit yield and fruit quality increased significantly with application of 1.0% $ZnSO_4$ over control. In the current study yield of 50.72 kg $tree^{-1}$ indicated that there is enormous scope to increase the yield of mango in this region through zinc application through foliar sprays. The study recommends foliar spray of 1.0% $ZnSO_4$ for mango in Indo-Gangetic plain region for higher yields and improvement of fruit quality. Study further shows the scope for improvement in soil management to get a desirable potential yield.

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Original Research Paper

Genotypic variations in biomass production and nutrient removal pattern in gladiolus raised from cormels

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ABSTRACT

The present study was conducted at ICAR-IIHR, Bengaluru, India during 2018-2019 to quantify resource use efficiency in 11 genotypes of gladiolus propagated through cormels based on growth, biomass partitioning and nutrient removal pattern. Growth and yield parameters differed significantly among genotypes. The leaf number was significantly higher in Arka Shobha (9.67) and Arka Manorama (9.00) than other genotypes (6.33-8.67). The spike length was higher in Arka Naveen (102.9 cm) and lesser in Arka Kumkum (66.2 cm). The pattern of biomass partitioning indicated that below ground biomass (corm) accounted for 71.5% of total biomass (3990 kg ha^{-1}), while above ground biomass (leaf and spike) was 28.5% of total biomass (1137 kg ha^{-1}). In gladiolus genotypes, the nutrient profile indicated that the accumulation of N was higher in corms followed by leaves and spikes. The accumulation of P (0.13-0.14%), Mn (29.8-43.5 mg kg^{-1}), Zn (15.3-23.4 mg kg^{-1}) and Cu (5.2-6.0 mg kg^{-1}) was similar. Spikes accumulated higher K and Mg than leaves and corms. The accumulation of Ca was more in leaves (2.39%) followed by flower stalks (1.95 %). The average Fe concentration (mg kg^{-1}) was more in corms (293) followed by leaves (269) and flower stalks (160). The average nutrient removal in genotypes was quantified at 122 kg N, 10.8 kg P and 71.7 kg K per ha per crop. The nutrient demand (g ha^{-1}) of Fe was more (1062.4) than Mn (152.5), Zn (23.8) and Cu (23.0). The data implies that gladiolus is a heavy feeder of N and K. Nutrient removal of K and Fe influenced the biomass production with high degree of variability ($Y = -541.858 + 24.097\ K_{uptake} + 1.405\ Fe_{uptake}$ R^2=0.995). The present study gives scope for precision nutrient use by avoiding blanket recommendations.

Keywords: Biomass partitioning, cormels, genotypes, gladiolus and nutrient removal

INTRODUCTION

Gladiolus (*Gladiolus hybrida*) is a popular cut flower crop and innumerable cultivars with attractive colors are available for cultivation. It belongs to the family *Iridaceae*. However, the area increase under gladiolus cultivation was negligible during the last decade despite huge demand for this flower crop both at national and international levels. The area under gladiolus cultivation was 11, 660 ha during 2011-12 and increased to 11,850 ha during 2017-18 (NHB, 2018). Commercial production of gladiolus depends largely on the availability of propagating material especially corms. Large size corm helps in better plant growth and development by supplying storage nutrients in the corm. The slow multiplication rate of quality planting material either corms or cormels is a recurring problem and is hindering the area expansion of this flower crop. Due to slow multiplication rate, dormancy of corms/cormels and high percentage of spoilage of corms during storage, there is an insufficient supply of planting material (Memon et al., 2009; Priyakumari and Sheela, 2005; Swapnil et al., 2017). As cormel production in terms of number per plant is more than corm number and the resource use efficiency of cormels as propagating material needs to be assessed either for corm/cormel production or for production of flower spikes in gladiolus to address the problems of short supply of planting material and huge domestic demand.

Balanced nutrition is required for getting optimum yields of both spikes and corms/cormels in gladiolus cultivation. Though several reports highlighted the importance of major and micronutrients especially boron and zinc for increased weight and number of corms and cormels per hill in gladiolus, present nutrient recommendations are highly variable (Afify, 1983, Shah et al.,1984; Mukherjee et al., 1998; Singh, 1996 and Das, 1998; Shankar and Dubey, 2005; Singh et al., 2013; Satpathy et al., 2016). No data exist on nutrient requirement of gladiolus varieties based on biomass production and nutrient removal pattern. Soil health is another crucial factor for obtaining higher production of below ground biomass (Baldotto and Baldotto, 2013). Thus, precision farming approach with adequate nutrient supply is essential by assessing the nutrient demand of gladiolus genotypes through biomass and nutrient partitioning, and nutrient removal pattern. With this background, the present study was carried out to precisely assess the demand of various nutrients for different plant components especially corms and cormels in various gladiolus genotypes.

MATERIALS AND METHODS

Description of study site

The present study was carried out in experimental field at ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India (13°7'N latitude and 77° 29'E longitude, 890 m above MSL). The climate of the experimental site is semi-arid. The soil of the experimental site is red sandy loam.

Experimental details

The study was carried out in the ongoing breeding experiment comprising of identified genotypes and advanced breeding lines at ICAR-IIHR during 2018-2019. Uniform cormels of different genotypes were planted in fourth week of January, 2018 at a spacing of 30 cm x 20 cm on raised soil beds. Recommended plant protection measures were followed for control of major pests and diseases. Nutrients were applied @ 200:200:200 kg NPK ha^{-1} in two splits in addition to application 10 t of FYM per hectare before planting. The trait specific genotypes identified at ICAR-IIHR were used to find out overall picture of resource use in gladiolus raised from cormels as propagating material. About eleven IIHR identified genotypes were selected and were evaluated in randomized block design (RBD) with three replications. The desirable traits of genotypes are given in Table S1.

Growth parameters and biomass estimation

Growth observations such as plant height, leaf number, tiller number and spike length were recorded in three plants in each replication of all genotypes. For estimation of both above ground and below ground biomass, destructive sampling method was adopted. Three plants from each genotype were collected in 2018 just before initiation of flowering and at harvestable stage of spikes. Fresh weight was recorded separately for leaves and spikes/flower stems. Similarly, the fresh biomass of corm/cormels was estimated by collecting all corms/cormels from each plant separately. Samples were cleaned with distilled water, air dried, packed in brown paper bags, oven dried at 60^0C to a constant weight and dry weight was recorded after drying. After recording oven dry weight, same samples were ground and kept in labeled butter paper bags for nutrient analysis to find out nutrient accumulation and removal pattern. The average biomass of each part was multiplied with total number of leaves and flower stems/spikes to arrive at total above ground biomass. The below ground biomass was also estimated in a similar manner by multiplying corm/cormel number per plant with biomass per plant. The biomass of leaf, flower stem and corms/cormels was considered to arrive at total biomass production.

Collection and analysis of soil and plant samples

Soil samples were collected at 0-25 cm depth in the root zone at 15 cm distance from the base after harvesting of corms. The air-dried soil samples were ground to pass through a 2.0-mm sieve and kept in labelled plastic bags for further analysis. Soil chemical/fertility parameters like pH, organic carbon, available P and K were analysed for using standard procedures (Jackson 1973). Soil organic carbon was measured by titration method (Walkley and Black, 1934). Soil test P was estimated by ascorbic acid reductant method (Watanabe and Olsen 1965) for colour development after extraction with Olsen's reagent. Available K, Ca and Mg were estimated in Flame Photometer using ammonium acetate extract. The concentration of micronutrients was estimated in AAS using diethylene triamine penta acetic acid (DTPA) extract (Lindsay and Novell 1978).

The leaf, flower stem and corm samples were analysed separately for total N using micro-Kjeldahl digestion method (Jackson 1973). The

plant samples were digested using 1:3 perchloric-nitric acid mixture for estimation of total P, K and micronutrients in different plant parts of flower stalk. Total P (vanadomolybdate) was determined following Piper (1966). Estimation of total K, Ca, Mg was done in flame photometer and micronutrients like copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) was done in AAS. Nutrient removal was computed by multiplying nutrient concentration in each plant part with respective oven-dry biomass and presented per hectare basis.

Statistical Analysis

All data were analyzed using SPSS and Microsoft Excel. The significant differences between the two means are indicated by LSD (5%) values in the tables. Correlations and regressions among different biomass parameters and nutrients were worked out for better understanding of results. Differences among genotypes were tested with ANOVA and LSD.

RESULTS AND DISCUSSION

Growth parameters in different genotypes

The growth parameters such as plant height, leaf number and spike length differed significantly among genotypes. The plant height of genotypes varied from 70.5 cm in Arka Kumkum to 107.7 cm in Arka Naveen. The flower stem length also differed significantly among genotypes. The flower stem length was higher in Arka Naveen (102.9 cm) and lesser in Arka Kumkum (66.2 cm). The leaf number was significantly higher in Arka Shobha (9.67) and Arka Manorama (9.00) than other genotypes with medium leaf number (7.50-8.67) and genotypes with less leaf number (6.33-6.67). The corm number was similar in all genotypes (2), but the corm weight varied significantly due to different size corms. Cormel number and weight were significant among genotypes. The average dry weights of all components per plant varied significantly among genotypes and were used for computing total biomass production per hectare (Table 1).

Biomass partitioning to different components

With respect to biomass production in different genotypes raised from cormels (Fig. 1), the biomass partitioning to spikes (15.9%) was more than partitioning to leaves (12.6%) except in four genotypes (Arka Amar, Arka Aarathi, Arka Shobha and Arka Gold). The partitioning of total biomass (3990 kg ha^{-1}) was maximum to below ground corm biomass (71.5%). In gladiolus genotypes raised from corms, the partitioning to corms is only 46% of the total biomass (Sujatha et al., 2020c). The average partitioning of biomass to both leaves and

Table 1. Growth and biomass partitioning in gladiolus genotypes propagated from cormels

Variety	Plant height (cm)	Flower stem length (cm)	No. of leaves	Above ground biomass (g $plant^{-1}$)			Below ground biomass (g $plant^{-1}$)			
				Leaf	Flower stalk	Total	Corm	Cormel*	Total	Root
Arka Aarti	79.3	74.8	6.33	4.40	2.97	10.30	13.4	10.9(14)	14.3	0.07
Arka Aayush	77.7	73.5	8.33	3.60	5.30	10.20	16.7	10.4(17)	17.4	0.30
Arka Amar	94.3	88.9	8.67	5.70	5.17	17.90	22.2	3.6(10)	24.6	0.33
Arka Darshan	77.0	73.1	6.33	3.73	5.70	12.17	18.3	10.3(19)	19.7	0.40
Arka Gold	94.7	89.6	6.67	4.90	3.50	18.40	29.1	3.4(8)	33.6	0.07
Arka Kumkum	70.5	66.2	7.50	1.83	5.07	9.27	14.3	7.4(11)	15.5	0.37
Arka Manorama	85.3	80.7	9.00	2.53	5.37	11.10	15.6	2.4(7)	17.5	0.07
Arka Naveen	107.7	102.9	8.33	5.40	6.03	13.07	27.2	5.5(8)	27.9	0.40
Arka Poonam	104.3	99.5	8.33	3.23	7.20	15.67	25.9	3.6 (8)	28.9	0.27
Arka Shobha	94.7	89.9	9.67	6.53	3.87	21.00	31.9	3.0(7)	36.4	0.20
Arka Tilak	80.3	75.5	8.33	4.33	7.83	14.87	24.2	4.6(5)	25.8	0.13
Mean	87.8	83.2	7.95	4.20	5.27	13.99	21.73	2.06	23.8	0.236
CD (*p=0.05*)	15.32	14.11	NS	0.880	0.923	2.438	4.03	0.863	4.68	0.085

*Figures in the parenthesis indicate cormel number

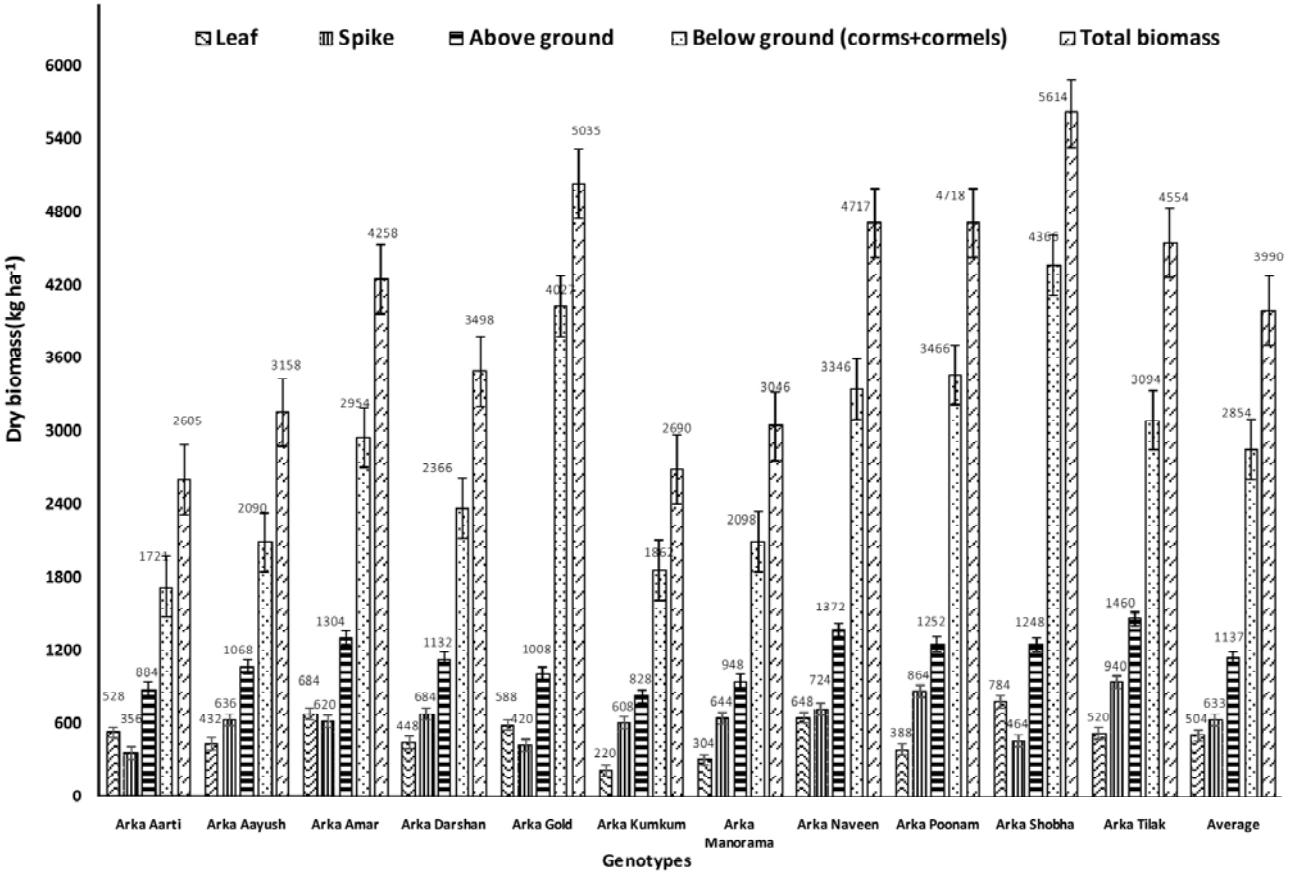

Fig. 1. Biomass partitioning in different genotypes of gladiolus with cormels as planting material (CD (*p=0.05*) for leaf biomass: 112.8; flower stalk biomass: 118.3; aboveground biomass: 139.2; Below ground biomass: 601.1)

spikes was 1137 kg ha^{-1} (28.5% of total). Leaf biomass was significantly higher in Arka Shobha (784 kg ha^{-1}), while spike biomass production was significantly higher in Arka Tilak (940 kg ha^{-1}). The biomass of corms was higher in Arka Shobha (4366 kg ha^{-1}). It is clear that higher biomass partitioning to spikes resulted in reduced corm biomass in genotypes, while higher biomass partitioning to leaves is required for higher corm biomass production. For planting material multiplication, this aspect needs to be considered. The availability of recyclable biomass as leaf and spikes was 28.5% of total biomass in case of recycling after utilization.

Nutrient accumulation in different plant components

There were significant variations in nutrient accumulation of major and secondary nutrients in all plant parts such as leaves, spikes and corms among genotypes (Fig. 2 and 3). In gladiolus genotypes, the nutrient profile among different plant parts also showed distinct pattern. The accumulation of N was higher in corms followed by leaves and spikes in all genotypes. The P accumulation was similar in all plant parts (0.13-0.14%). Spikes accumulated higher K than leaves and corms. The accumulation of Ca was more in leaves (2.39%) followed by spikes (1.95 %) and corms (0.39%). The Mg accumulation was higher in flower stalks (0.38%) followed by leaves (0.34%) and corms (0.16%) more Ca and Mg than corms. Among micronutrients, the average Fe concentration (mg kg^{-1}) was more in corms (293) followed by leaves (269) and flower stalks (160). The range in concentrations (mg kg^{-1}) of Mn, Zn and Cu were 29.8-43.5, 15.3-23.4 and 5.2-6.0, respectively. The previous reports indicated that genotype variability in nutrient content and nutrient uptake is crucial for genetic improvement (Dierig et al., 2003 Feil et al., 2005 Brink et al., 2001).

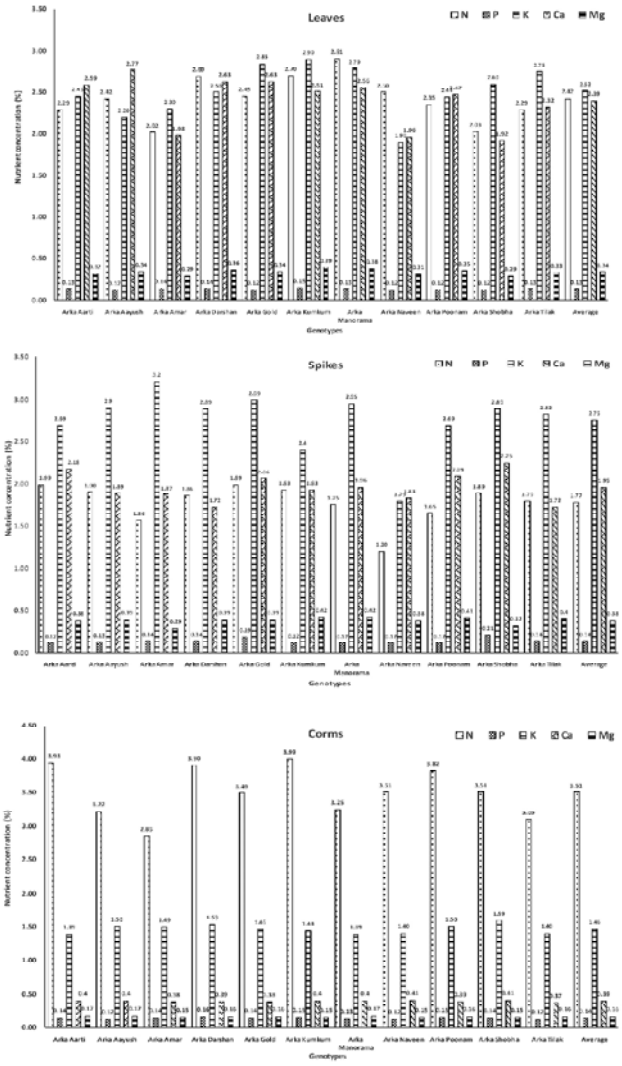

Fig. 2. Nutrient accumulation pattern in gladiolus genotypes raised from cormels

CD (*p=0.05*) for nutrients in leaf (N: 0.172; P: 0.005; K: 0.192;Ca:0.188 Mg: NS), flower stalk (N: 0.147; P: 0.019; K: 0.237;Ca:0.194 Mg:0.06) and corms (N: 0.237; P: 0.008; K: 0.041;Ca: NS Mg: NS)

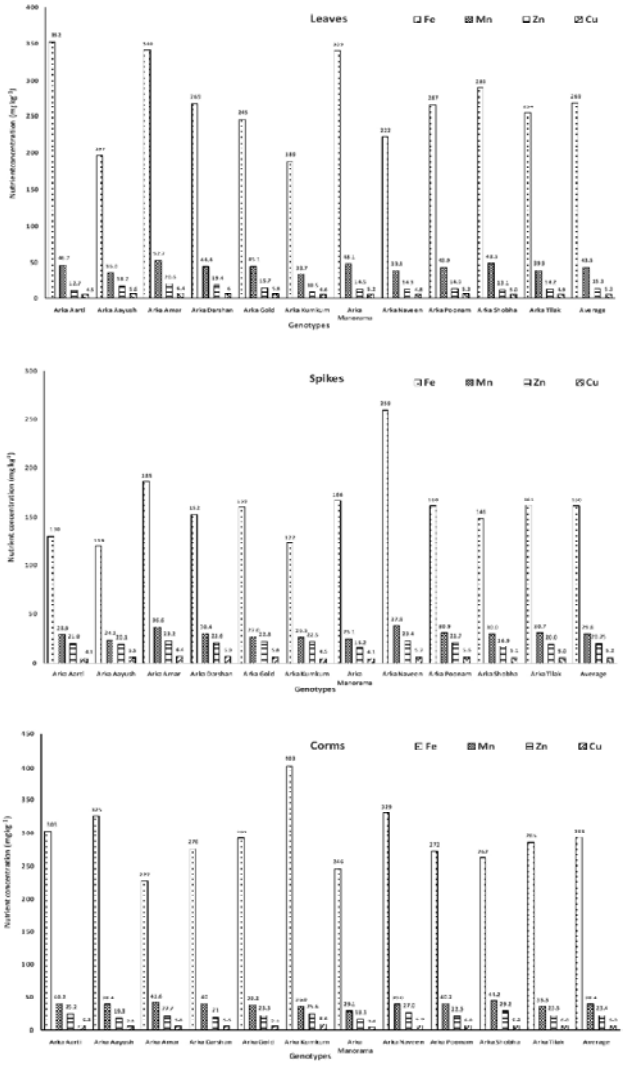

Fig. 3. Micronutrient accumulation pattern in gladiolus genotypes raised from cormels

CD (*p=0.05*) for nutrients in leaf (Fe:38.8, Mn: 6.9, Zn: NS, Cu; NS), flower stalk (Fe:29.9, Mn: NS, Zn:NS, Cu: NS) and corms (Fe:49.5, Mn:5.1, Zn: NS, Cu: NS)

Nutrient uptake pattern

The uptake of major, secondary and micro nutrients differed significantly among different genotypes of gladiolus (Fig. 4). The total N removal ranged from 87 kg ha^{-1} in Arka Aarti to 178 kg ha^{-1} in Arka Shobha. The removal of P and K ranged between 6.1-15.2 kg ha^{-1} and 46.4-103.2 kg ha^{-1}. The average nutrient removal per hectare per year in genotypes raised from cormels was quantified at 122 kg N, 10.8 kg P and 71.7 kg K. The nutrient removal for secondary nutrients ranged between 24.7-43.4 kg for Ca and 6.2-10.8 kg for Mg. Among micronutrients, the demand in terms of grams per hectare for Fe was more (1062.4 g ha^{-1}) than Mn (152.5), Zn (23.8) and Cu (23.0). The order of nutrient uptake in gladiolus in all genotypes was N>K>Ca>P>Mg>Fe>Mn>Zn>Cu. The data implies that gladiolus is a heavy feeder of N and K. In comparison to gladiolus raised from corms (Sujatha et al., 2020c), the biomass production and nutrient uptake by gladiolus genotypes raised from cormels are considerably lower. Higher corm biomass production with less nutrient uptake in gladiolus genotypes raised from cormels gives scope for largescale planting material multiplication utilizing cormels.

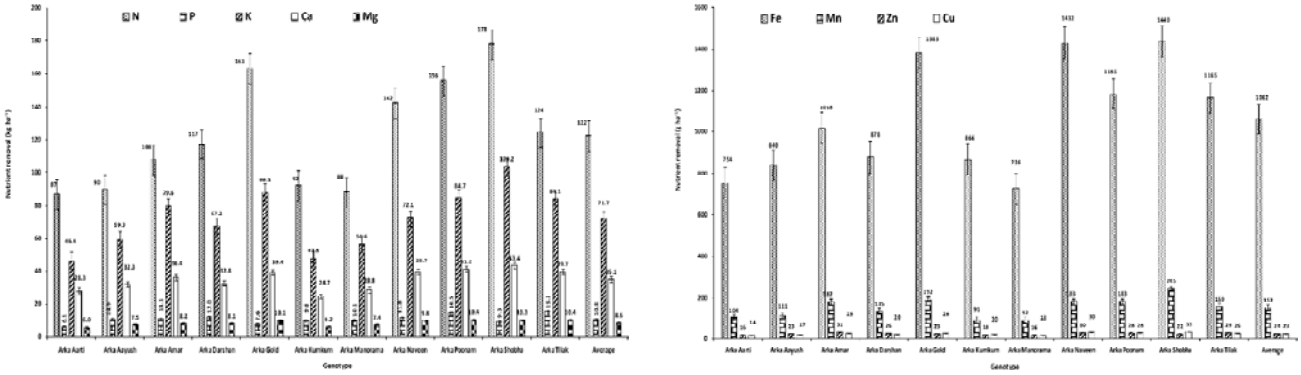

Fig. 4. Nutrient removal pattern in gladiolus genotypes raised from cormels

Table 2. Correlation matrix for biomass and nutrient uptake

Parameter	Total biomass	Leaf biomass	Spike biomass	Above ground biomass	Below ground biomass	N uptake	P uptake	K uptake	Ca uptake	Mg uptake	Fe uptake	Mn uptake	Zn uptake
Leaf biomass	0.71**												
Spike biomass	0.15	-0.27											
Above ground biomass	0.71**	0.58*	0.63**										
Below ground biomass	0.99**	0.69**	0.04	0.59*									
N uptake	0.94**	0.59*	0.04	0.51*	0.96**								
P uptake	0.29	-0.14	0.98**	0.72**	0.10	0.17							
K uptake	0.97**	0.68**	0.16	0.69**	0.95**	0.89**	0.31						
Ca uptake	0.97**	0.71**	0.26	0.80**	0.93**	0.89**	0.39	0.94**					
Mg uptake	0.94**	0.52*	0.41	0.77**	0.90**	0.88**	0.52*	0.91**	0.96**				
Fe uptake	0.94**	0.67**	0.08	0.61**	0.94**	0.92**	0.18	0.83**	0.88**	0.87**			
Mn uptake	0.97**	0.81**	0.01	0.67**	0.96**	0.92**	0.17	0.95**	0.93**	0.84**	0.90**		
Zn uptake	0.62**	0.47*	0.61*	0.89**	0.50*	0.43*	0.70**	0.59*	0.69**	0.66*	0.54*	0.59*	
Cu uptake	0.93**	0.68**	0.19	0.71**	0.91**	0.89**	0.32	0.86**	0.88**	0.84**	0.94**	0.93**	0.66*

In gladiolus genotypes, the correlations (Table 2) were highly significant for total biomass production and removal of N (r=0.938), K (r=0.968), Ca (r=0.967) and Mg (r=0.941) and P removal did not influence significantly total biomass production (r=0.292). Application of stepwise regression technique to identify the nutrient variables with a significant influence on the total biomass (Y) resulted in the following equation, where the variables are written in the increasing order of p-level. Multiple regression analysis of removal/ uptake of nutrients with total biomass production showed high degree of relation and nutrient removal of K and Fe influenced the biomass production with high degree of variability.

Y (total biomass) = -541.858 + 24.097 K_{uptake} + 1.405 Fe_{uptake} (R^2=0.995)

Soil fertility status

The soil fertility status at the end of experimentation was above optimum with soil pH near to neutral (7.07) and the soil organic carbon was 1.32%. The availability of soil nutrients was quantified at 22.3 ppm of P, 335 ppm of K, 4799 ppm of Ca, 1208 ppm of Mg, 11.3 ppm of Fe, 8.5 ppm of Mn, 5.6 ppm of Zn and 4.1 ppm of Cu. The soil fertility status implies that gladiolus system maintains optimum soil fertility despite higher biomass removal in the form of corms/ cormels and the nutrient application can be adjusted based on nutrient uptake pattern to save critical inputs.

CONCLUSIONS

The present study assessed the pattern of biomass and nutrient partitioning, and nutrient demand of different genotypes in gladiolus when cormels were used as planting material. The major influence of N, K, Ca and Fe on biomass production in gladiolus was evident in this study. The results of the present study can be used as basis for assessing the nutrient requirement of gladiolus when raised through

cormels. The results give scope for precision nutrient application that would reduce the cost of production and avoid soil fertility buildup due to excess nutrient application.

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Original Research Paper

Performance of parthenocarpic and non-parthenocarpic grafts of cucumber

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ABSTRACT

Effect of rootstock on yield and quality of cucumber scion was studied at Department of Vegetable Science, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala during February-May, 2021. Parthenocarpic and non-parthenocarpic cucumber scions were grafted onto five different cultivated cucurbit species i.e. pumpkin, bottle gourd, oriental pickling melon, culinary melon and ash gourd. Significant variations were observed for all the traits under this study. The highest vine length (4.37 m) was observed in Heera scion grafted onto *Lagenaria siceraria* rootstock followed by Heera scion grafted onto *Cucurbita moschata* rootstock (4.13 m). The diameter of rootstock hypocotyl was higher in case of KPCH-1 grafted onto bottle gourd (1.48 mm) and Heera grafted onto bottle gourd (1.43 mm). KPCH-1 grafted on bottle gourd (29.33 days) and culinary melon (31 days) rootstocks showed early female flower initiation. The greater number of fruits was observed in graft combination of KPCH-1 and bottle gourd (32) followed by parthenocarpic grafts with pumpkin (30.33) and ash gourd (30.33) rootstocks. A greater fruit weight was observed in graft combination of Heera and bottle gourd (7.51 kg) followed by Heera grafted onto pumpkin (7.38 kg). Results of this experiment suggest that these graft combinations can be employed in sustainable vegetable cultivation.

Keywords: Cucumber, grafts, non-parthenocarpic, parthenocarpic, rootstock and scion

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is a popular and second important cucurbit grown throughout tropical and sub-tropical region. Due to the rising demand for salad cucumber in off-season, protected cultivation can be followed to increase the yield and quality (Singh *et al*., 2012). Parthenocarpy along with gynoecious sex expression is an asset for protected cultivation of cucumber. Cultivation of parthenocarpic hybrids is gaining attention of the growers as it is a reliable and profitable venture. The major factors limiting cucumber cultivation are soil-borne root knot nematodes and soil salinity. To overcome the problems in cucumber cultivation, an eco-friendly technique exploited is vegetable grafting with resistant rootstocks.

Grafting was confined to woody perennials but now vegetable grafting has gained importance to combat biotic and abiotic stress. Though cultivated area of grafted cucubitaceous plants has increased tremendously in foreign countries, but the commercial use of vegetable grafting is a relatively recent innovation in India and scientific information is meagre. Sakata *et al*. (2008) showed that cucumber could be grafted onto different wild and cultivated rootstocks, including *Cucurbita* interspecific hybrids, *Cucumis* spp., bottle gourd, wax gourd, fig-leaf gourd, African horned cucumber, sponge gourd and ridge gourd. However, survival, growth and yield of grafted plants depend on stock-scion compatibility, grafting method and post-grafting management. Due to change in root system, the physiology and metabolic process of plants are affected in grafted plants. The studies on use of parthenocarpic variety as scion in grafting is limited.

Grafting can also increase yield since grafted plants are resistant to soil borne diseases, have strong root systems and increased photosynthesis (Davis *et al*., 2008). Cucumber adapts well to grafting and has few compatibility problems with the usual rootstocks

(Echebarria, 2001). Edelstein *et al*. (2004) observed that number of leaves, stem length, and fresh weight of melon plants increased when grafted onto other cucurbitaceous rootstocks. Chao and Yen (2013) observed that cucumber grafted onto *Cucumis* rootstock showed good rootstock scion combination, better tolerance to soil-borne diseases, better growth, yield and quality. Hang *et al*. (2005) observed that when scion and rootstock have hollow hypocotyls as in cucurbits, the hole insertion and one cotyledon grafting methods are preferred.

MATERIALS AND METHODS

The experiment was conducted in rain shelter during February-May, 2021 at Department of Vegetable Science, College of Agriculture, Vellayani, Kerala. The experimental site was located at 8.5°30' North latitude and 76.9°54' East longitude at an altitude of 29 m above mean sea level. Predominant soil type of the experimental site was red loam of Vellayani series, texturally classified as sandy clay loam.

In this experiment, two different scions were used: a parthenocarpic hybrid KPCH-1 and a non-parthenocarpic variety, Heera. Five rootstocks were used, namely, pumpkin (*Cucurbita moschata*) var. Ambili, bottle gourd (*Lagenaria siceraria*) var. Arka Bahar, ash gourd (*Benincasa hispida*) var. KAU Local, culinary melon (*Cucumis melo* var. acidulus) var. Mudicode local and oriental pickling melon (*Cucumis melo* var. conomon) var. Vishal.

Considering the early germination of cucumber (scion) compared to rootstocks, rootstocks were sown four days earlier than scions. Depending on the result of standardization, ten days old scion was grafted onto fourteen days old rootstocks. Alar and cycocel 20 ppm each were used to control height of rootstocks. Based on the stem grith of rootstocks and scion, grafting methods were employed. For culinary melon, oriental pickling and ash gourd where the stem size were similar to scion, one cotyledon grafting was used. In case of pumpkin and bottle gourd whose stem girth is higher than that of cucumber, hole insertion method was employed. The protrays were shifted to graft healing chamber (e"85 % humidity) immediately after grafting. Graft union formation was noticed within seven days and thereafter the grafts were shifted to 75 % shaded net house. The grafted plants were planted in the main field (rain shelter) after twelve days of grafting. The experiments were laid in a randomized complete block design with three replications of ten plants each at a spacing of 1.5 m × 0.5 m on raised beds. Standard cultural practices were followed to raise a healthy crop under protected condition.

Diameter of rootstock hypocotyls was recorded using vernier calliper. The vine length of each graft was measured using a scale after final harvest. For determining earliness, the node number and days at which the first pistillate flower appeared was recorded for each plant. The number of fruits per plant and yield per plant was recorded as an average of all ten plants in each replication. For quality assessment, five random fruits were selected from each replication. Fruit length and diameter were measured. The total soluble solids (TSS) content was measured using a handheld refractometer (ERMA, Japan). The data obtained in evaluation trial was analyzed using WASP (Web Agriculture Statistical Package) 2.0 software through ANOVA techniques.

RESULTS AND DISCUSSION

The present study revealed that the vegetative and yield parameters of the grafted plants were significantly affected by scion-rootstock combinations (Table 1 and 2). Significant difference was observed in vine length with respect to rootstocks and scions used in this study. Among the ten graft combinations, the highest vine length (4.37 m) was observed in Heera scion grafted onto bottle gourd rootstock followed by Heera scion grafted onto pumpkin rootstock (4.13 m). Generally, vigorous rootstocks increase the vine length of scions. However, the root and shoot vigour imparted by these rootstocks did not reflect in higher yield. This is confirmed by a lack of correlation between yield and root parameters. Similar differences in vine length were also obtained by Mohamed *et al*. (2012) who stated that grafted watermelon plants were more vigorous than self-rooted ones and had a larger central stem diameter and recorded 32 per cent higher main vine length than that of non-grafted counterpart. Selvi and Pugalendhi (2018) also observed the increase in vine length through grafting in bitter gourd. Improved plant growth of grafts is measured by phenomena of stronger and more extensive root growth, increased water and plant nutrient uptake as well as endogenous hormone production (Islam *et al*., 2013).

The diameter of rootstock hypocotyl reflects the vigor of the grafts. The diameter of rootstock hypocotyl was higher in case of KPCH-1 grafted onto bottle gourd (1.48 mm) and Heera grafted onto bottle gourd (1.43 mm). Growth and development of grafted plants was better than that of non-grafted plants throughout the growing period. High vigor was noticed in grafts with high diameter of rootstock hypocotyl. Similar results were observed by Aishwarya (2019).

Earliness coupled with high yield is an important trait for commercial cultivation of vegetable crops. A significant influence of the rootstocks was observed on earliness in terms of the appearance of pistillate flower at the lower nodes and also number of days to female flower initiation. Early flowering was observed in grafts than the control. KPCH-1 grafted on bottle gourd (29.33 days) and culinary melon (31 days) rootstocks showed early female flower initiation whereas, Heera grafted onto pumpkin took greater number of days (48.33 days). These results are similar to Pal *et al*. (2020) and Bigdelo *et al*. (2017). However, a reverse trend of delayed flowering in grafted plants was observed by Hamed *et al*. (2012) and Selvi and Pugalendhi (2018). In cucurbits, node at which first female flower appears is also considered as important trait to measure earliness. The number of nodes of female flower initiation was lower for the graft of KPCH-1 on bottle gourd (3.33) and oriental pickling melon (3.67) than the non-grafted control (4th node). Parthenocarpic gynoecious hybrid bears only female flowers and in case of Heera, the graft combinations of Heera on ash gourd (8) and culinary melon (8.33) showed female flowers at lower nodes (Table 1).

Significant difference was observed for traits like number of fruits per vine, fruit yield, average fruit weight and days to first fruit harvest. Number of fruits were higher in case of parthenocarpic grafts than the non-parthenocarpic graft combinations. The greater number of fruits was observed in graft combination of KPCH-1 and bottle gourd (32.00) followed by parthenocarpic grafts with pumpkin (30.33) and ash gourd (30.33) rootstocks. In non-parthenocarpic graft combination, the grafts with oriental pickling melon (27.67) followed by ash gourd (26.33) and bottle gourd (26.00) produced a greater number of fruits than the control (25.67). These graft combinations produced 5 to 10 per cent higher fruits per plant. In cucumber,

Table 1. Vegetative and flowering parameters of grafts

Graft combinations	Diameter of rootstock hypocotyl (cm)	Vine length (m)	Days to 1st female flower	Node of 1st female flower
KPCH-1 on culinary melon	1.20	1.77	31.00	4.00
Heera on culinary melon	1.21	4.00	47.30	8.33
KPCH-1 on oriental pickling melon	1.24	1.91	32.00	3.67
Heera on oriental pickling melon	1.23	2.30	47.00	11.00
KPCH-1 on pumpkin	1.36	2.53	31.67	4.67
Heera on pumpkin	1.37	4.13	48.33	9.00
KPCH-1 on ash gourd	1.34	2.63	31.67	4.67
Heera on ash gourd	1.26	2.27	45.33	8.00
KPCH-1 on bottle gourd	1.48	2.46	29.33	3.30
Heera on bottle gourd	1.43	4.37	44.33	9.00
KPCH-1	0.90	2.73	33.67	4.00
Heera	0.97	3.60	46.00	10.00
CD (0.05)	0.10	0.34	3.17	1.50
SEm	0.05	0.25	2.27	0.81
SD	0.17	0.89	7.86	2.82
CV	4.94	7.10	4.84	13.34

'Shelper' rootstock provided increase in the number of marketable fruits and 35.5 and 39.5 % increase in yield, compared to non-grafted plants (Kohatsu *et al*., 2013).

A greater fruit weight was observed in graft combination of Heera and bottle gourd (7.51 kg) followed by Heera grafted onto pumpkin (7.38 kg). However, in case of KPCH-1, higher fruit yield was observed in the graft combination of bottle gourd (5.25 kg), followed by ash gourd (Table 2). Fruit yield depends on number of fruits and average fruit weight. In the present study, fruit yield was directly proportional to the average fruit weight. Higher number of fruits was noticed in parthenocarpic grafts whereas higher fruit yield was observed in non-parthenocarpic grafts, which is due to high average fruit weight of non-parthenocarpic grafts (Fig.1).

Early fruit harvesting was noticed in case of parthenocarpic graft combinations with bottle gourd (40 days) followed by ash gourd (41 days) and culinary melon (41 days) than the control (43 days). The graft combination of Heera with bottle gourd (54 days) and ash gourd (54.67 days) rootstock showed early fruit harvesting than that of non-parthenocarpic control (Table 2). Earliness of any vegetable crop is directly measured through days to first harvest which could fetch premium price and catch the early market. Days to first harvest had the positive direct effect with days to female flower initiation and node of pistillate flower appearance. Five to ten per cent increase in yield

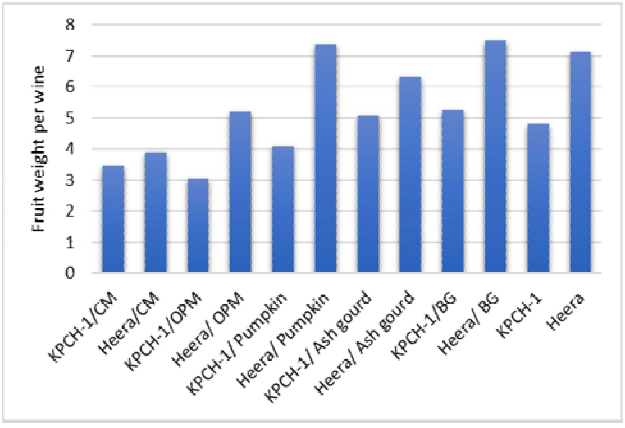

Fig. 1. Graphical representation of average fruit yield per vine
CM - Culinary melon, OPM - Oriental pickling melon, BG - Bottle gourd

Table 2. Fruit yield parameters of cucumber grafts

Graft combinations	Fruits per vine	Fruit yield (kg)	Days to first harvest	Fruit TSS (°B)
KPCH-1 on culinary melon	28.00	3.48	41.00	2.20
Heera on culinary melon	25.00	3.89	57.00	3.60
KPCH-1 on oriental pickling melon	25.67	3.06	40.67	2.07
Heera on oriental pickling melon	27.67	5.20	56.67	2.20
KPCH-1 on pumpkin	30.33	4.09	41.33	2.27
Heera on pumpkin	24.67	7.38	57.67	2.97
KPCH-1 on ash gourd	30.33	5.09	41.00	2.17
Heera on ash gourd	26.33	6.33	54.67	1.97
KPCH-1 on bottle gourd	32.00	5.25	40.00	2.03
Heera on bottle gourd	26.00	7.51	54.00	2.27
KPCH-1	29.00	4.82	43.00	2.07
Heera	25.67	7.14	55.67	2.00
CD (0.05)	3.63	0.89	2.66	0.63
SEm	0.69	0.44	2.25	0.13
SD	2.40	1.52	7.80	0.48
CV	7.78	10.06	3.24	16.12

was observed in grafted plants than the non-grafted control. Fruit TSS was observed to be higher with non-parthenocarpic scion combined with culinary melon rootstock (3.6 °B) and pumpkin (2.96 °B) against the control (2.0 °B). Parthenocarpic non-grafted cucumber has total soluble solids of 2.06 °B which was lower than that of grafts with rootstock pumpkin (2.26 °B) and culinary melon (2.20 °B). Quality parameters are not affected by rootstock-scion combination as previously reported by Selvi and Pugalendhi (2018) in bitter gourd.

CONCLUSION

It can be concluded that grafted plants performed better than non-grafted control in cucumber. Grafting can be commercialized in protected cultivation of cucumber for parthenocarpic and non-parthenocarpic cultivars. According to this study, both scions, KPCH-1 and Heera performed better with the bottle gourd rootstock for almost all vegetative and yield attributing traits. Therefore, this graft combination can be used in sustainable horticulture with higher yield. Further, grafting can be utilized to combat biotic and abiotic stress in cucurbitaceous vegetables.

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Original Research Paper

Heat unit requirement and performances of litchi under Sub-Himalayan terai region of West Bengal

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ABSTRACT

To determine the heat unit requirement and assess its subsequent effects on flowering and fruiting characteristics, a field experiment was conducted during 2018-19 with seven cultivars of litchi viz., Calcuttia, Elaichi, Bedana, Bombai, China, Shahi and Muzaffarpur in randomized block design. Bedana showed better result in terms of maximum fruit weight (17.88g), lowest seed content (10.84%), maximum fruit diameter (3.01 cm), maximum fruit volume (18.70 ml), highest TSS (15.87^0 Brix), total sugar (15.96%), reducing sugar (12.61%), and ascorbic acid (29.47 mg/100g) content.

Keywords: Bud break, flowering, fruiting, growing degree days and panicle.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.), belonging to the family Sapindaceae is an evergreen, subtropical fruit and popularly known as '*Queen of sub-tropical fruits*', '*Pearl of India'* for its excellent aromatic flavour and sweet aril taste (Nakasone and Paull, 1998). Litchi flowers are of three types, staminate or purely male flowers, female or hermaphrodite functioning as female and hermaphrodite flower functioning as male or pseudo-hermaphrodite (Chaudari, 1940, Mustard *et al.,* 1953 and Menzel, 1984). Litchi is very specific to its climatic requirement and requires seasonal temperature variation for best flowering and fruiting (Garcia-Perez and Martins, 2006). It is considered as the essential sub-tropical fruit crops and requires diurnal variation for flowering and fruiting. Litchi cultivation is successful in areas having average minimum temperature of 10^{o}C from December to February and 32^{o}C in April to June is considered more congenial. Climate is the most important limiting factor in the expansion of area under this fruit. The role of growing degree days (GDD) or heat units are often used to predict the growth stages including the date when a flower will bloom or a crop will reach to the maturity. Heat units or growing degree days is the number of temperature degrees above a certain threshold base temperature within consecutive 24 hours period. The heat unit varies among the crops or even within cultivars of the same crop (Kanzaria *et al*., 2015). Heat units have an important role in horticultural crops and it helps in estimating the growth stages of plants, determines harvesting index. It also assesses the suitability of a region for growing a crop to estimate the length of various phenological stages and to predict maturity and quality characteristics of fruits (Khan *et al*., 2007; Shinohara, 2013; Koshita, 2014). The effect of temperature on the phenological development, flowering and fruiting performance was studied by the different workers for predicting the growth stages, yield and physiological maturity (Swan *et al.,*1989, Singh *et al.,* 2007). The prevailing agro-climatic condition in the sub-Himalayan Terai region is suitable for litchi cultivation. However, the information regarding the influence of heat units on flower bud development, panicle emergence, flowering and fruiting characteristics of litchi is very limited in literature. Keeping in view the utmost importance of heat units, the present study was designed to determine the effect of heat units and performance of different litchi cultivars in the foothills of eastern Indian sub-Himalayan Terai region of West Bengal.

MATERIALS AND METHODS

To determine the heat unit requirement and assess its subsequent effects on flowering and fruiting characteristics of litchi, the experiment was conducted at Instructional Farm under the Dept. of Pomology and Post-Harvest Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, situated in the foothills of eastern Indian Himalayas. Seven important commercial litchi cultivars (T_1- Calcuttia, T_2 – Elaichi, T_3 – Bedana, T_4- Bombai, T_5- China, T_6- Shahi and T_7- Muzaffarpur) were selected as treatments and the experiment was carried out in randomized block design with three replication and two plants per replication. Heat unit was calculated by taking the average of the daily maximum and minimum temperatures compared to a base temperature, T_{base} (usually 10°C). The formula applied for calculation of heat unit is $(T_{max}+T_{min})/2- T_{base}$(Monteith, 1984; Rai *et al*., 2002). The heat units for different phonological phases were recorded. The different parameters for flowering and fruiting characteristics were recorded as per the standard methodology. The bio-chemical parameters were determined following Ranganna (1986) and A.O.A. C. (1984). For statistical analysis the mean separation for different parameter were performed using Least Significant Difference (LSD) test (Pd" 0.05).Normality of residuals under the assumption of ANOVA was tested using Kolmogrov-Smirnov, Shapiro-Wilk, Cramer-Von Mises and Anderson Darling procedure using Proc-Univariate procedure of SAS (version 9.3).

RESULTS AND DISCUSSION

Response of heat unit requirement on flowering of litchi

The heat unit requirement of different phenological phases in litchi cultivars were recorded (Table-1). The heat unit requirement for bud break to panicle appearance was maximum in cv. China (144.03°C), which was statistically at par with cvs. Bombai, Calcuttia, Shahi and lowest in Elaichi (94.58°C). Subsequently, it was observed (Table-2a) that cv. Bombai required maximum number of days (17.83 days) for bud break to panicle emergence, statistically at par with cv. China (17.27 days), while the cv. Elaichi recorded the minimum number of days (12.47 days). For panicle appearance to flowering, the heat unit requirement was maximum in cv. Bombai (400.16°C) which was statistically at par with cvs. Elaichi (371.31°C), Bedana (340.20°C), Muzaffarpur (335.26°C) and Calcuttia (329.24°C), whereas, cv. Shahi required lowest (312.38°C) heat unit (Table-1). However, the duration of panicle emergence was not significant among the different cultivars (Table-2a) but maximum number of days (35.10 days) was required in cv. Elaichi and lowest (29.52 days) in cv. Shahi.There was no significant variation observed during flowering to fruit set stage for heat unit requirement, however, it was recorded maximum in cv. Muzaffarpur (347.78°C) and minimum (326.83°C) in cv. Calcuttia. The duration of flowering to fruit set was recorded maximum in cv. Muzaffarpur (28.98 days) which was statistically at par with all other cultivars except for cv. Bombai (23.95 days). The litchi varieties exhibited significant variation for heat units requirement during fruit set to harvesting stages and cv. Bedana registered the maximum heat unit requirement (1133.80°C) which was at par with cv. China (1076.33 °C), whereas, the cv. Shahi required the minimum (950.74°C).

Flowering parameters

It is believed that litchi needs a period of vegetative dormancy to initiate floral buds (Das *et al.,* 2004). The maximum length of panicle (19.00 cm) was observed incv. Muzaffarpur, while the minimum length (16.16 cm) was recorded in cv.Bedana.The flowering duration was statistically similar for different cultivars and cv. Muzaffarpur exhibited the maximum flowering duration (25.83 days).The maximum number of flowers per panicle was produced by the cv. Shahi (593.87) while it was minimum for cv. Bombai (422.80). Number of hermaphrodite flowers as functional male showed significant variation and ranged from 59.19% (Bomabi) to 67.18 %(Muzaffarpur). The highest percentage of hermaphrodite flowers as functional female (21.26%) was recorded in the cv. Bombai while China recorded the lowest percentage (17.94%) of hermaphrodite flowers as functional female. Bedana recorded the highest percentage of male flowers (19.60%) and Muzaffarpur registered the lowest percentage of male flowers (14.46%) (Table 2b).

Table 1. Heat unit requirement by different litchi cultivars

Treatments	Bud break to panicle appearance (°C)	Panicle appearance to flowering (°C)	Flowering to fruit set (°C)	Fruit set to harvesting (°C)
T_1 (Calcuttia)	128.72^{abc}	329.24^{ab}	326.83^{a}	1012.48^{b}
T_2 (Elaichi)	94.58^{c}	371.31^{ab}	329.67^{a}	957.37^{b}
T_3 (Bedana)	104.50^{bc}	340.20^{ab}	334.68^{a}	1133.80^{a}
T_4 (Bombai)	136.85^{ab}	400.16^{a}	336.35^{a}	1010.06^{b}
T_5 (China)	144.03^{a}	322.33^{b}	347.08^{a}	1076.33^{a}
T_6 (Shahi)	121.44^{abc}	312.38^{c}	347.72^{a}	950.74^{b}
T_7 (Muzaffarpur)	100.24^{c}	335.26^{ab}	347.78^{a}	962.54^{b}
S.Em. (±)	11.80	23.94	10.54	20.41
L.S.D (Pd”0.05)	36.35	73.77	NS	62.90

Means followed by same alphabet are not significantly different

Table 2a. Flowering parameters of different litchi cultivars

Treatments	Days taken for bud break to panicle emergence	Duration of panicle emergence (days)	Length of panicle (cm)	Duration of flowering (days)
T_1 (Calcuttia)	12.67^{c}	29.55^{a}	17.42^{bc}	24.48^{a}
T_2 (Elaichi)	12.47^{c}	35.10^{a}	16.69^{cd}	24.12^{a}
T_3 (Bedana)	14.83^{bc}	30.20^{a}	16.16^{d}	23.58^{a}
T_4 (Bombai)	17.83^{a}	31.47^{a}	17.99^{ab}	22.90^{a}
T_5 (China)	17.27^{ab}	29.55^{a}	16.39^{cd}	24.13^{a}
T_6 (Shahi)	13.95^{c}	29.52^{a}	17.32^{bcd}	25.38^{a}
T_7 (Muzaffarpur)	13.65^{c}	30.02^{a}	19.00^{a}	25.83^{a}
S.Em.(±)	0.84	2.41	0.40	1.21
L.S.D (Pd”0.05)	2.59	NS	1.23	NS

Means followed by same alphabet are not significantly different

Table 2b. Flowering parameters of different litchi cultivars

Treatments	Total no of flowers per panicle	Hermaphrodite male (%)	Hermaphrodite female (%)	Male (%)
T_1 (Calcuttia)	476.52^{cd}	64.41^{a}	19.67(4.43)^{ab}	15.84(3.98)^{bcd}
T_2 (Elaichi)	457.35^{de}	65.70^{a}	18.33(4.28)^{ab}	17.20(4.15)^{abcd}
T_3 (Bedana)	518.92^{bc}	59.64^{b}	20.09(4.47)^{ab}	19.60(4.42)^{a}
T_4 (Bombai)	422.80^{e}	59.19^{b}	21.26(4.61)^{a}	18.11(4.24)^{abc}
T_5 (China)	528.83^{b}	63.67^{a}	17.94(4.23)^{b}	18.96(4.35)^{ab}
T_6 (Shahi)	593.87^{a}	64.83^{a}	19.52(4.42)^{ab}	15.56(3.93)^{cd}
T_7 (Muzaffarpur)	593.83^{a}	67.18^{a}	18.48(4.30)^{ab}	14.46(3.80)^{d}
S.Em.(±)	16.97	1.30	0.12	0.12
L.S.D (Pd”0.05)	52.03	4.01	0.36	0.38

Means followed by same alphabet are not significantly different (Value in parenthesis is the square root transformed value)

Fruiting characteristics

Duration of fruit set, fruit set percentage and fruits per panicle was statistically similar for all the cultivars under study. Calcuttia variety showed the maximum duration (11.97 days) for fruit set and was observed minimum in cv. Bombai (10.68 days). The fruit set percentage varied from 3.41% (Muzaffarpur) to 4.79% (Bombai), whereas, number of fruits per panicle was observed from 18.08 (Bedana) to 23.22 (Shahi). Physio-chemical properties of fruits were significantly varied among the different cultivars studied under this experiment. Higher fruit weight (17.88g), fruit diameter (3.01cm) and fruit volume (18.70ml) were observed in cv. Bedana. Small fruit of litchi was observed in cv. China (11.23g). The maximum fruit length (3.17 cm) was observed in cv. Bombai; however, the higher peel content (29.94%) and seed content (27.90%) makes the variety with high waste index (1.51). The fruit pulp content was highest in cv. Muzaffarpur (62.09%) and it was statistically at par with cvs. Calcuttia, Elaichi, Bedana and Shahi. Seed content was lowest in cv. Bedana (10.84%). The seed size (30.65%) and peel content (28.50%) were higher in cv. China resulting maximum waste index (1.66g) among the different varieties studied under this experiment. The minimum fruit length (2.71 cm) was recorded in Bedana. The data on fruit diameter showed that the maximum fruit diameter (3.01 cm) was recorded in Bedana, whereas, Muzaffarpur recorded the minimum fruit diameter (2.33 cm). The maximum specific gravity (1.07) was observed in Elaichi while the minimum specific gravity (0.84) was recorded in Shahi. Highest pulp content (62.09%), and lowest waste index (0.64g) was recorded in Muzaffarpur. China recorded the maximum waste index (1.66g), seed content (30.65%). Percentage of juice was maximum (55.08%) in Shahi while maximum peel (29.94%), minimum juice (30.03%) and was recorded in Bombai (Table 4b).

Bio-chemical characteristics

Litchi cv. Bedana was recorded with maximum total soluble solids (TSS) content (15.87^{O}Brix), total sugar content (15.96%), reducing sugar (12.61%) and ascorbic acid content (29.47 mg/100g), whereas, cvs. Elaichi, China, Muzaffarpur, and Shahi recorded with lowest amount of TSS (13.93^{0} Brix) and total sugar (11.45%), reducing sugar (9.68%), and ascorbic acid (19.72 mg/100g) content, respectively (Table 5).

There was a variation among the litchi cultivars for different parameters studied under this experiment which indicates the genotypic differences. Higher heat unit requirement of cv. Bedana and China for fruit set to harvesting indicates it late maturity in

Table 3. Fruiting characteristics of different litchi cultivars

Treatments	Date of first fruit setting	Duration of flowering to fruit set (days)	Duration of fruit setting (days)	Percent fruit set (%)	Fruits per panicle
T_1 (Calcuttia)	22nd March	27.08^{ab}	11.97^{a}	4.25 (2.06)^{a}	19.83^{a}
T_2 (Elaichi)	22nd March	26.77^{ab}	11.53^{a}	4.29 (2.06)^{a}	19.03^{a}
T_3 (Bedana)	26th March	26.58^{ab}	11.02^{a}	3.56 (1.88)^{a}	18.08^{a}
T_4 (Bombai)	2nd April	23.95^{b}	10.68^{a}	4.79 (2.18)^{a}	19.83^{a}
T_5 (China)	25th March	27.55^{ab}	11.17^{a}	3.86 (1.97)^{a}	20.27^{a}
T_6 (Shahi)	23rd March	28.90^{a}	11.57^{a}	3.85 (1.95)^{a}	23.22^{a}
T_7(Muzaffarpur)	22nd March	28.98^{a}	11.90^{a}	3.41 (1.85)^{a}	19.75^{a}
S.Em.(±)	—-	1.24	0.45	0.11	2.29
L.S.D (Pd"0.05)	—-	3.83	N.S.	N.S.	N.S.

Means followed by same alphabet are not significantly different (Value in parenthesis is the square root transformed value)

Table 4a. Physical characteristics of fruits of different litchi cultivars

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit volume (ml)	Specific gravity
T_1 (Calcuttia)	17.30^{ab}	3.14^{ab}	2.70^{b}	17.63^{ab}	0.99^{ab}
T_2 (Elaichi)	13.85^{bc}	2.86^{bcd}	2.43^{c}	13.07^{c}	1.07^{a}
T_3 (Bedana)	17.88^{a}	2.71^{d}	3.01^{a}	18.70^{a}	0.96^{abc}
T_4 (Bombai)	13.32^{c}	3.17^{a}	2.48^{bc}	13.87^{c}	0.97^{abc}
T_5 (China)	11.23^{c}	3.06^{ab}	2.35^{c}	13.30^{c}	0.87^{bc}
T_6 (Shahi)	12.43^{c}	3.01^{abc}	2.47^{bc}	15.03^{bc}	0.84^{c}
T_7 (Muzaffarpur)	11.78^{c}	2.78^{cd}	2.33^{c}	13.70^{c}	0.88^{bc}
S.Em.(±)	1.26	0.09	0.09	1.11	0.05
L.S.D (Pd”0.05)	3.89	0.27	0.26	3.43	0.14

Means with the same letter are not significantly different

Table 4b. Physical composition and waste index of litchi fruits

Treatments	Juice (%)	Peel (%)	Pulp (%)	Seed (%)	Waste index (g)
T_1 (Calcuttia)	46.12^{ab}	17.80^{c}	59.98^{a}	22.22^{bc}	0.79^{bc}
T_2 (Elaichi)	44.48^{b}	22.16^{c}	58.73^{a}	19.11^{c}	0.92^{bc}
T_3 (Bedana)	44.11^{b}	29.82^{a}	59.34^{a}	10.84^{d}	0.70^{bc}
T_4 (Bombai)	30.03^{c}	29.94^{a}	42.16^{b}	27.90^{ab}	1.51^{a}
T_5 (China)	37.44^{bc}	28.50^{ab}	40.85^{b}	30.65^{a}	1.66^{a}
T_6 (Shahi)	55.08^{a}	23.14^{bc}	52.84^{a}	24.02^{bc}	1.19^{ab}
T_7(Muzaffarpur)	40.65^{b}	18.75^{c}	62.09^{a}	19.16^{c}	0.64^{c}
S.Em.(±)	3.41	1.81	3.43	2.08	0.18
L.S.D (Pd”0.05)	10.50	5.58	10.57	6.42	0.54

Means followed by same alphabet are not significantly different

Table 5. Biochemical properties of litchi fruits

Treatments	TSS (^{o}Brix)	Total sugar (%)	Reducing sugar (%)	Acidity (%)	TSS:acid ratio	Ascorbic acid (mg/100g)
T_1(Calcuttia)	14.63^{bc}	13.15^{abc}	11.55^{b}	0.42^{b}	34.80^{bcd}	23.83^{b}
T_2 (Elaichi)	13.93^{d}	14.71^{ab}	10.87^{c}	0.42^{b}	33.11^{cd}	28.82^{a}
T_3 (Bedana)	15.87^{a}	15.96^{a}	12.61^{a}	0.54^{a}	29.86^{d}	29.47^{a}
T_4 (Bombai)	15.20^{b}	12.60^{bcd}	12.41^{a}	0.40^{b}	38.36^{bc}	20.37^{c}
T_5 (China)	14.37^{cd}	11.45^{b}	10.62^{cd}	0.52^{a}	27.89^{d}	23.62^{b}
T_6 (Shahi)	14.87^{bc}	12.30^{a}	10.95^{cd}	0.32^{c}	47.41^{a}	19.72^{c}
T_7(Muzaffarpur)	14.80^{bc}	11.72^{b}	9.68^{d}	0.36^{bc}	41.34^{ab}	23.40^{b}
S.Em.(±)	0.21	0.17	1.02	0.03	2.50	0.40
L.S.D (Pd”0.05)	0.63	0.53	3.16	0.08	7.71	1.24

Means followed by same alphabet are not significantly different

this agro-climatic situation. The wide variation in heat unit may be due to the varied maturity period of different cultivars indicates that each genotype needs certain amounts of accumulation of heat units for completion of different phenophases which cause the variation in maturity period (Rai *et al*., 2003). Flowering span might be differed in case of climatic condition, but flowering span of particular variety is over only when the required heat units are accumulated (Byrne and Bacon, 1992). The flowering parameters result shows similar trends as observed by Banerjee and Chaudhary (1944), Mustard *et al* (1953), Chadha and Rajpoot (1969), Pivovaro (1974) and Kumar (2000). The data indicated that the maximum titrable acidity (0.54%) was recorded in Bedana while the minimum titrable acidity content (0.32%) was recorded in Shahi.TSS:acid ratio was maximum (47.41) in Shahi and the minimum TSS:acid ratio (27.89) was recorded in China. The differences of fruit physio-chemical properties indicate the relationship between cultivars and heat unit requirement.

CONCLUSION

There was a varietal difference regarding the heat unit requirements for different phonological phases resulting variation on flowering and fruiting characteristics. Bedana required maximum heat unit (1133.80^{o}C) for attaining the harvesting stage from fruiting cultivar and it was lowest in cv. Shahi (950.74^{o}C). Litchi cv. Bedana exhibited promising results in terms of flowering, fruiting and quality parameters with respect to high fruit weight (17.88g), fruit diameter (3.01cm), fruit volume (18.70 ml), TSS (15.87^{O}Brix), total sugar content (15.96%), reducing sugar content (12.61%), ascorbic acid content (29.47 mg/100g) and may be recommended as promising cultivar in terms of better-quality characters under the sub-Himalayan Terai region of West Bengal condition.

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Original Research Paper

Macronutrients and their associated bacterial genera in the soils of Anaimalai block in Tamil Nadu for sustainable vegetable crops cultivation

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ABSTRACT

Meticulous analysis on intertwined interaction among the soil nutrients and microbial communities brings fruitful outcomes on horticulture. This study focuses on identifying well compatible bacterial genera in enhancing soil primary macro-nutrients for sustainable vegetable crops cultivation. The biochemical tests were executed for identification of bacterial genera. Eventually, mathematical models among available NPK nutrients (Nitrogen, Phosphorus and Potassium) and identified bacterial genera were derived to determine most suited bacterial genus for nutrients inhibition. This Study reveals the present nutrient's status of soils at Anaimalai block covering 12,424 ha of Coimbatore district in Tamil Nadu. Seven utilitarian bacterial genera were identified which inhibit the plant nutrients. Among them, *Azotobacter, Arthrobacter* and *Achromobacter* actively inhibit available NPK in the soil. Present study of correlating soil nutrients with bacterial components enriches successful conservation of biosphere through adopting these innovative technologies in horticulture.

Keywords: Correlation, horticultural crops, macronutrients, soil beneficiary microbes, soil fertility, sustainable agriculture and vegetable production.

INTRODUCTION

Soil utilitarian bacteria act as a significant contributing factor for improving diminished soil fertility. Soil bacteria increase the crop production through their inherent characteristics of soil structure improvisation, production of hormones and enzymes, controlling pathogen and heavy metal. Nevertheless, the decline of organic fertilizers usage and soil fertility exploitation through agrochemical affects the horticultural crop yield rate (Malhotra et al., 2017). The incorporation of soil utilitarian bacteria as biofertilizers is needed to overcome the aforementioned issues. Statistical infusion in the soil nutrient and bacterial data analysis would bring out a realistic field study. From the extensive works of literature, it is found that various initiatives have been taken to improvise horticultural crops productivity through soil nutrient and bacterial analysis. Besides, beneficiary microbes such as *A. chroococcum, A. vinelandii, A. beijerinckii, A. paspali, A. armeniacus, A. nigricans, A. salinestri , Azospirillum, Cyanobacteria, Azolla, Gluconacetobacter diazotrophicus, Bacillus aerius, Bacillus amyloliquefaciens, Bacillus mucilaginous, Bacillus subtilis, Enterobacter* contributes for nitrogen fixation in the soil (Dhayalan and Karuppasamy 2021). *Pseudomonas chlororaphiswere, P. putida, P. entomophila, P.koreensis, P.luteola, P. simiae, P.stutzeri, Bacillus* sp.*, Achromobacter, Acinetobacter, Aeromonas hydrophila, Arthroderma cuniculi, Aspergillus niger, Bacillus aerius, B. altitudinis, B. thuringenesis, Enterococcus casseliflavus, E. gallinarum, Lecanicillium psalliotae, Paenibacillus taichungensis, Serratia nematodiphila, Sphingomonas paucimobilis, Azotobacter etc.* are some of the soil phosphorus solubilizing microbes which are used in different horticultural crops for enhanced productivity (Mussa *et al*., 2018). *Pseudomonas azotoformans, Burkholderia, Bacillus mucilaginosus, B. edaphicus, B. circulans, Pseudomonas, Acidithiobacillus ferrooxidans, Paenibacillus* sp., and *Enterobacter hormoecheiin* are some of the beneficiary bacteria that induces the solubilization of potassium (Prajapati and Modi 2016). Correlation among the soil nutrients and their associated bacterial genera is a substantial research gap. Resultant of correlation leads gateway for precise decision making relevant to bio fertilization for horticultural crops.

MATERIALS AND METHODS

Sample site description

Extensive research work was performed recently in Anaimalai at Pollachi, which lies between 10.662°N 77.00650°E, located 40km to the south of Coimbatore, India. The study area consists of vegetable crops such as tomato, bhendi, brinjal, chilies, caspium, paprika, pumpkin, snake gourd, bitter gourd, cluster bean, potato, cabbage, cauliflower, onion etc., Twenty-seven soil samples were collected from the rhizospheric region of horticultural crops for macronutrients analysis.

Macronutrient analysis

The basic physical parameters such as pH, Electrical conductivity, and salinity were measured using pH meter (Elico LI 617), conductivity meter (Elico CM 183), and salinity meter respectively. Available nitrogen in the soil sample was analyzed using the Kjeldahl nitrogen analysis method. Total phosphorus was analyzed using the Bray-1 method for acidic soil samples and Olsen method for alkaline soil samples. Available potassium in the soil was estimated using Flame Photometer (Jenway PFP7).

Bacterial analysis

Enumeration of microorganism

Enumeration of bacteria were carried out through serial dilution method to reduce the microbial load and plated by pour plate method. Bacteria being isolated from the plates are incubated at 37°C in a bacteriological incubator.

Gram staining

Gram staining was done for the isolated bacterial cultures in a slide using Gram's iodine, decolorizing agent and safronin strain. The slides were observed under the trinocular microscope, the purple colors indicated gram positive bacteria reveals the presence of bacterial genera, *Arthrobacter* and most of slides observed pink color indicating the presence of *Azotobacter, Enterobacter, Citrobacter, Achromobacter, E. coli* and *Cornybacterium* which are characterized by gram negative. Further analyses were carried out to confirm the presence of initially traced bacterial genera. The cover slip was taken where its edge was coated with vaseline and the test samples were transferred to the cover slip which was placed over the cavity slide. The slide was viewed under 100X magnification and the organisms' characteristics being motile or non-motile were noted down.

Biochemical test for bacterial genera

For the confirmation of bacterial genera certain biochemical tests were carried. Indole test were carried out for the isolated bacteria culture in which a red colour ring or pink colour ring at the top reviling positive reaction, where yellow colour ring at the top indicate negative reaction. Indole test reveals that most of the identified bacterial genera except *Arthrobacter, Citrobacter* and *Enterobacter* exhibit the positive reaction.

Followed by the indole test, methyl red test were performed, Formations of red colour ring at the top indicate the positive reaction which reveals the existence of *Azotobacter, Citrobacter, Achromobacter, E.coli and Cornybacterium* and formation of yellow colour ring at top shows the negative reaction which indicate the presence of *Arthrobacter* and *Enterobacter*. Further confirmation of bacterial genera was made by Simmons citrate utilization test, so as to determine the ability of the microorganism to use citrate as its sole source of carbon. From the Simmons citrate utilization test it was revealed that the survival of *Azotobacter*, *Citrobacter*, *Enterobacter and Achromobacter* were reverberated by means of green to blue colour changes in the bacterial medium specifying positive reaction. The existence of bacterial genera such as *Arthrobacter, Cornybacterium* and *E.coli* were revealed through green to yellow color changes of bacterial medium indicating the negative reaction (Ishiguro et al., 1979). Voges Proskauer tests were included to analyze the presence of identified bacterial genera at most precise manner. Voges Proskauer tests are used to demonstrate an organism's ability to convert pyruvate to acetoin. Formation of red colour ring exhibits the positive reaction which covey the existence of *Enterobacter* genera and yellow colour ring express the negative reaction exposing the survival of *Azotobacter, Citrobacter, Achromobacter, E.coli, Cornybacterium,* and *Arthrobacter*.

Bacterial genus interpretation

Based on the outcomes of various biochemical tests, identification of bacteria were made with

the help of Bergey's manual of systematic bacteriology. Out of 11,669 different bacterial colonies 31% of *Arthrobacter*, 24% of *Citrobacter*, 17% of *Escherichia coli*, 13% of *Azotobacter*, 7% of *Enterobacter* and 5% of *Achromobacter*, was found as contributing bacterium for promoting soil nutrients. Total colonies of bacterial genus were identified by multiplying the average number of colonies with 10^5 as dilution factor (Table 1).

Table 1. Biochemical characteristic for the identification of bacterial species

S.No.	S.F. No	Latitude	Longitude	Total BC	GS	IT	MRT	SCUT	VPT	IB	CC
1	590	10.588153	76.885931	380	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	157
2	592	10.587975	76.885421	200	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	125
3	596	10.587718	76.885257	511	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
4	600	10.588111	76.883713	561	GNR	(+)	(+)	(+)	(-)	*Achromobacter*	362
5	601	10.587909	76.884139	356	GNR	(+)	(+)	(-)	(-)	*E.coli*	208
6	602	10.587721	76.884218	312	GPR	(-)	(+)	(-)	(-)	*Cornybacterium*	215
7	606	10.587537	76.884144	326	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	225
8	608	10.587163	76.884039	322	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
9	630	10.586454	76.882942	320	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	257
10	637	10.587134	76.883235	320	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
11	638	10.587643	76.883528	320	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
12	641	10.587063	76.88287	312	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
13	642	10.587491	76.882627	245	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
14	648	10.587277	76.882267	478	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
15	649	10.587783	76.88216	520	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425
16	657	10.587926	76.882583	956	GNR	(+)	(+)	(+)	(-)	*Azotobacter*	557
17	656	10.588503	76.882551	288	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	225
18	660	10.588254	76.883272	540	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
19	661	10.588261	76.883422	300	GNR	(+)	(+)	(-)	(-)	*E.coli*	218
20	702	10.587996	76.87914	96	GNR	(+)	(+)	(-)	(-)	*E.coli*	58
21	703	10.588698	76.87921	96	GNR	(+)	(+)	(-)	(-)	*E.coli*	68
22	721	10.589401	76.879324	96	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	55
23	747	10.588187	76.877757	87	GNR	(-)	(-)	(+)	(+)	*Enterobacter*	72
24	750	10.587696	76.877974	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	553
25	751	10.587271	76.878479	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	651
26	756	10.587306	76.878045	956	GPR	(-)	(-)	(-)	(-)	*Arthrobacter*	253
27	758	10.587714	76.877569	859	GNR	(-)	(+)	(+)	(-)	*Citrobacter*	425

BC, Bacterial colonies; GS, Gram Straining; GNR, Gram negative- Rod; GPR, Gram positive- Rod; IT, Indole test; MRT, Methyl red test; SCUT, Simmons Citrate Utilization test; VPT, Voges Proskauer Test; IB, Identified Bacteria; CC, Colonies Count; (+), Positive; (-), Negative.

RESULTS AND DISCUSSION

Statistical Analysis

The resulted characteristics of soil were compared with TNAU standards. Pearson coefficients (Table 2.) were incorporated which represents the relationship among the nutrients measured and the associated microbes. Statistics which includes bacterial symmetry, bacterial counts, mean value, minimum bacterial count were interpreted by descriptive statistical analysis (Table 3).

Physical parameters and primary macronutrients

The study area is observed with clay loam, sandy clay loam and sandy loam types of soil. Loam soil is the mixture of sand, silt and clay having the pH value 4.5 to 6.5. Most of samples in the study area are reddish brown in color indicating fine soil texture that greatly helps in the horticulture. Soil pH value at Anaimalai ranges from 6.41 to 8.72. The overall pH result make a strong report that the pH values which falls above 6.5 in some field area is completely due to the other predominant factors such as water and agrochemicals. The bacterium plays a key role in maintaining the soil pH range (Hoorman, 2016). One of the identified genera *Citrobacter* count holds positive correlation with pH (Table 2). *Citrobacter* can maintain the soil pH ranges from 3 to 11 (Oliveira *et al*., 2016). Electrical conductivity finds its value ranges from 0.08 to 0.9 and the highest EC recorded is 0.9 dS/m. Excess salinity causes a huge hindrance for horticulture (Habib et al., 2016). Beneficial bacteria lower the concentration of ethylene that directs to deduce the salinity stresses in horticulture farmlands. Utilitarian bacterial genuses identified were *Achromobacter* and *Azotobacter* which are positively correlated (Table 2) with Electrical conductivity. Field available nitrogen ranges from 128 to 265 kg/ha. Inspite of two bacterial

Table 2. Pearson correlation among soil available NPK and bacterial species

Pearson Correlations										
	Nitrogen	**Phosphorus**	**Potassium**	**AC**	**AR**	**AZ**	**CI**	**CO**	**E.Coli**	**EN**
Nitrogen	1	0.291	-0.405*	-0.097	-0.366	-0.016	-0.188	0.077	0.150	0.276
Phosphorus	0.291	1	0.322	-0.273	0.175	0.357	-0.239	-0.081	-0.059	0.003
Potassium	-0.405*	0.322	1	0.121	-0.170	-0.031	-0.058	0.186	-0.145	0.136
AC	-0.987	-0.273	0.121	1	-0.093	-0.061	-0.082	-0.038	-0.105	-0.081
AR	-0.366	0.175	-0.175	-0.093	1	-0.147	-0.198	-0.093	-0.255	-0.197
AZ	-0.016	0.357	-0.031	-0.061	-0.147	1	-0.129	-0.061	-0.166	-0.128
CI	-0.118	-0.239	-0.058	-0.082	-0.198	-0.129	1	-0.082	-0.224	-0.172
CO	0.077	-0.081	0.186	-0.038	-0.093	-0.061	-0.082	1	-0.105	-0.081
E.Coli	0.150	-0.059	-0.145	-0.105	-0.255	-0.166	-0.224	-0.105	1	-0.222
EN	0.276	0.003	0.136	-0.081	-0.197	-0.128	-0.172	-0.081	-0.222	1

*. Correlation is significant at the 0.05 level (2-tailed). AC, Achromobacter; AR, Arthrobacter; AZ, Azotobacter; CI, Citrobacter; CO, Cornybacterium; E.Coli, Escherichia Coli; EN, Enterobacter;

Table 3. Descriptive analysis of bacterial species.

	N total	**Mean**	**SD**	**Sum**	**Skewness**	**Kurtosis**	**CV**	**Min**	**Median**	**Max**
Achromobacter	27	13.40	69.66	362	5.19	27	5.19	0	0	362
Arthrobacter	27	82.07	175.8	2216	2.26	4.64	2.14	0	0	651
Azotobacter	27	35.96	118.6	971	3.80	15.28	3.29	0	0	557
Citrobacter	27	62.96	153.8	1700	2.09	2.59	2.44	0	0	425
Cornybacterium	27	7.96	41.37	215	5.19	27	5.19	0	0	215
E.Coli	27	44.66	84.91	1206	1.58	0.72	1.09	0	0	218
Enterobacter	27	26	64.10	702	2.59	5.94	2.46	0	0	225

SD, Standard Deviation; CV, Coefficient of Variation; Min, Minimum; Max, Maximum;

genus presences in the soil, soil available nitrogen indicates low status. This is due to the presence of *E. coli* which is not a nitrogen fixation bacterium and also due to other environmental factors. Nevertheless, the outcomes of the genus action in the soil still contribute 19% of available nitrogen to the plants. Available phosphorus ranges from 15 to 37 kg/ha in the field and fulfills the recommended level which is considered as major growth contributing factor in horticultural crops productivity. Two major bacterial genera *Arthrobacter* and *azotobacter* shows high positive correlation with phosphorus (Table 2).Various literatures show the importance of *azotobacter* and *Arthrobacter* in solubilizing the soil phosphorus (Banerjee *et al*., 2010). Potassium founds to be extracted at higher concentration by horticultural crops (Pimentel *et al*., 2015) which are in the range between 132 to 374 kg/ha. Soil bacteria ensure prolonged crop growth through its production of inorganic acids, acidolysis, polysaccharides and chelation (Archana et al., 2013). Contributing identified bacterial genus *Achromobacter* and *Enterobacter* shows positive correlation with available potassium level (Table 2).

Identified Bacterial genera

Maximum of 362 colonies were found to be *Achromobacter* genus, which gives positive correlation with soil Electrical conductivity and Potassium. *Achromobacter* genus is capable of solubilizing 5.4 µg/ml of potassium in the soil at maximum extend (Gupta *et al*., 2016). Nevertheless, the genus finds negative correlations with nitrogen and phosphorus. *Achromobacter* induce ethylene hormones for contribution of soil available nitrogen (Bangash *et al*., 2021) and helps to expose to transient water stress in horticultural crops more specifically tomato and pepper. *Arthrobacter* genus ranges maximum of 651 in terms of colonies count at the selected study boundary. Distinctive nature of *Arthrobacter* in synthesizing plant hormones alleviates the phosphorus deficiency and stress developed by the salinity in the soil (Etesami and Glick, 2020). The genus finds positive correlation with soil phosphorus whereas shows negative correlation with other soil nutrients. *Arthrobacter* genus induces active mechanism against salt stress in the *Pisum sativum* (pea) crops. Maximum of 557 *Azotobacter* colonies count were identified which implies positive correlations with phosphorus, whereas negative correlation with other soil nutrients. Though *azotobacter* magnifies the nitrogen fixation in the soil through active production of phytopathogenic inhibitors, mathematical model implies negative correlation because of the presence of toxic inorganic fertilizers. *Azotobacter* due to its biological activity helps in increasing the phosphorus solubilization. Compared to *Arthrobacter, azotobacter* implies the indole acidic acids which are responsible for phosphorus solubilization (Aung *et al*., 2020). *Citrobacter* (425 colonies count) maintain the soil pH in recommended range because of its incredible biological activity (Oliveira et al., 2016). Maximum of 218 colonies were found to be *Escherichia coli* genera, which give positive correlation with soil nitrogen and negative correlations with other soil nutrients. *E.coli* is not a nitrogen fixing bacterium but possibly helps in nitrogen cycle by producing urea when *E. coli* utilizes ammonium. Potentiality of *E. coli* in producing iron-chelating compounds helps to promote the growth of potato crops. *Enterobacter* due to ACC deaminase activity induces nitrogen and potassium in the soil (Guo *et al*., 2020). Production of indole-3-acetic acids by the *Enterobacter* helps in phosphorus solubilization thus in turn multiple the yields of tomato, cucumber and pepper crops.

Organic fertilizer selection and dosage recommendations

Identified bacterial genera needs to be formulated for its survival so as to reach the soil for progress of crop productions. BioAtivo, Azotovit, Rhizotorphin, Azotobacterin, Ekophit, Mizorin®, Mamezo, Phylazonit-M, Symbion-N, CALOBIUM, Sardar Biofertilizers and Ferti-Bio are commonly available biofertilizers having identified genera in active state. Biofertilizers are usually bioformulated into two major type viz. liquid and solid with natural carriers.

CONCLUSION

This study implies the necessity of incorporating mathematical models to bring micro investigation on association of Insitu soil nutrient and its correlated bacterial genera so as to perceive the current nutrient status. *Azotobacter, Enterobacter, Citrobacter, Achromobacter, E. coli,* and *Arthrobacter* were some of the identified bacterial species that contribute to the soil primary macronutrients. This can be extemporizing for organic fertilizer formulation consisting aforementioned identified bacterial genera and it is recommended to utilize those organic biofertlizers to attain massive yield in horticulture.

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Original Research Paper

Effect of dehydration methods on quality parameters of drumstick (*Moringa oleifera* Lam.) leaf powder

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ABSTRACT

A study was undertaken to assess the suitable drying methods for retention of quality parameters of drumstick (*Moringa oleifera* Lam.) leaf powder. The experiment was laid out in Factorial CRD involving three methods of drying (T_1: Sun drying, T_2: Shade drying and T_3: Cabinet tray dryer) with three pre-treatments (B_1: Unblanched, B_2: Blanched with plain water and B_3: Blanched followed by KMS dip) replicated three times. All the pre-treatments had significant effect on biochemical characteristics of drumstick leaves. Among the pre-treatments, unblanched leaves (B_1) retained higher nutrient contents compared to other pre-treatments. The results of the investigation revealed that among the three different drying methods, shade dried sample was found to retain better nutritional properties. Significantly maximum values for moisture (11.18 %), ascorbic acid (156.27 mg/100g), vitamin-A (22.71 mg/100g), iron (16.54 mg/100g), oxalate (378.66 mg/100g), antioxidant activity (77.11%) and phenol (140.04 mg/100g) were recorded in shade dried sample. The interaction effect between pre-treatment and drying methods showed variation in results. However, the treatment combination T_1B_1 (Unblanched sun dried) was found to retain high protein (26.43 g/100g), magnesium (318.70 mg/100g) and potassium (1378.79 mg/100g) whereas T_2B_1 (unblanched shade dried) showed higher ascorbic acid (179.47 mg/100g), saponin (3.66 %), oxalate (541.47 mg/100g) and antioxidant (80.33 %) than rest of the treatment combinations.

Keywords: Cabinet tray, nutritional properties, pre-treatments, shade drying and sun drying

INTRODUCTION

Drumstick (*Moringa oleifera* Lam.) is an underexploited perennial tree species that belongs to the family Moringaceae. It is native to the Sub-Himalayan tracts of India, Bangladesh, Afghanistan and Pakistan (Makkar and Becker, 1997). This fast-growing tree is also known as benzolive tree, horseradish tree, marango, mlonge, moonga, kelor, mulangay, saijhan, sajna or ben oil tree. The crop is grown in homesteads for family use or cultivated commercially in the agriculture field for the market. India stands at first position among the drumstick growing countries with an annual production of 2.20 to 2.40 million tonnes of tender fruits from an area of 38,000 ha with productivity of around 63 tonnes per ha. Among the different states, Andhra Pradesh leads in both area and production (15,665 ha) followed by Tamil Nadu (13,250 ha) and Karnataka (10,280 ha) (Sekhar *et al*., 2018).

Drying is one of the traditional methods of preservations, which converts the leafy vegetables into a light weight, easily transportable and storable product. Drumstick is used as a foodstuff in different dishes in India, but their nutritional value is not considered due to lack of information. Most of the research work on the biochemical characteristics of the drumstick tree are mainly focused on the oil from the seeds due to its antioxidant properties but very little is associated to the nutritional value of other edible products of the plant as a traditional important food commodity to improve economic and health condition of the population. Considering the food value, utilization of dried moringa leaves need to be popularized for consumption by rural as well as urban population. In view of the above, the present study was done with the objective to evaluate the effect of dehydration on nutritive value of drumstick leaves dried under different drying methods along with different pretreatments.

MATERIALS AND METHODS

Preparation of sample

Fresh drumstick leaves were collected from the campus of Bishwanath College of Agriculture, Biswanath Chariali. The twigs containing half matured drumstick leaves were taken to the laboratory. The leaves were separated from twigs, washed thoroughly in clean running water, drained and were spread on clean stainless-steel tray to remove surface moisture. After removal of surface moisture, equal quantity of leaves were weighed to impose different pretreatments such as blanched for 2 min, blanching + KMS(0.5%) and control. Pretreated drumstick leaves were dried by different drying methods, by spreading drumstick leaves on stainless steel trays under the sun, shade and cabinet tray drier (60°C) until they were crisp.

Biochemical nalysis

Biochemical analysis of the drumstick leaf powder was carried out immediately after drying following the standard estimation methods.

Moisture content

Moisture content was determined according to AOAC (1980) method. The moisture content of the fresh and dried samples was measured by using the hot air oven method. At first, the weight of the crucible was measured using a digital balance. Then the sample along with crucible was measured and kept in a hot air oven at 105°C for 24 hours. The crucible was then taken out from the oven and cooled in a desiccator. After attaining the room temperature, the weight of the crucible along with the sample was measured. The moisture content was computed using the following formula:

$$\text{Moisture content (\%)} = 100 \times \frac{A - B}{A}$$

Where, A = Sample weight before oven drying,
B = Final weight of the sample.

Protein

Estimation of protein was done by Lowry's method (Lowry *et al.,*1951). For the estimation of protein, 500 mg of the sample was weighed and ground well with a pestle and mortar in 5-10 ml of the buffer. The above sample was centrifuged and the supernatant was used for estimation of protein. The working standard solutions of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken in a series of test tubes. Again, the sample extracts of 0.1 and 0.2 ml were taken in another 2 test tubes and the volume was made up to 1 ml in all the test tubes. A tube with 1 ml of water served as blank. Five ml of alkaline copper solution as reagent was added to each test tube including blank and allowed to stand for 10 minutes. Then, 0.5 ml of folin-ciocalteu reagent was added to test tubes and allowed to stand for 30 min at room temperature. The reading was taken in a spectrophotometer at 750 nm wavelength.

Ascorbic acid

Ascorbic acid content was determined by the visual titration method using 2,6 dichlorophenol indophenol dye (Freed, 1966). Ten grams of sample was taken in 100 ml volumetric flask and volume was made up with 4 per cent oxalic acid and filtered. Ten ml of filtrate was taken and titrated against the standard dye. Ascorbic acid content was calculated by the following formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre value x Dye factor x Volume made up}}{\text{Weight of the sample taken for estimation x Aliquot of sample taken for estimation}} \times 100$$

*Dye factor = 0.5/Titre value

Vitamin A

Vitamin A was determined in terms of beta carotene. Five ml of leaf extract was taken in a separating funnel and 10 ml of acetone and petroleum ether were added and mixed thoroughly. Lower layer was discarded and upper layer was collected and made up the volume to 100 ml with petroleum ether. The reading was taken at 452 nm. using petroleum ether as blank. The amount of Vitamin A was calculated by the following formula and expressed in µg/100g (Srivastava and Kumar, 2007).

$$\beta \text{ carotene (µg/100g)} = \frac{\text{O.D x 13.9 x } 10^4 \text{ x 100}}{\text{wt. of the sample taken x 560 x 1000}}$$

$$\text{Vitamin A (µg/100g)} = \frac{\beta \text{ carotene (µg/100g)}}{6}$$

Mineral compositions of leaves

Calcium (Ca) and Magnesium (Mg)

For the estimation of Ca and Mg, about 1 g of sample was digested by wet ashing method (Saini *et al.,* 2012). Then 5 ml aliquot was taken in a china clay dish and pH of the aliquot was adjusted to 10 by adding 15 ml NH_4Cl + NH_3OH buffer solution. Ten drops of Erichrome black-T indicator was added and

titrated with 0.01 N EDTA solution till the colour changes from red to bright blue. A blank was carried out in the same manner.

Five ml of NaOH solution and 50 mg of murexide indicator were added to 5 ml of aliquot and titrated with 0.01N EDTA solution till the colour changed from pink to purple. Similarly, a blank was also prepared. Both the minerals were calculated by the following formula and expressed as follows,

For Ca + Mg,

Meq. of (Ca+Mg)/100 g of plant material = (0.01 x V_3) x (V/V_1) x (100/1)

Meq. of Ca/100g of plant material = (0.01 x V_2) x (V/V_1) x (100/1)

Where,

V = Volume of the plant digest made

V_1 = Volume of the aliquot taken for analysis

V_2 = Volume of EDTA solution in titration (titre value)

V_3 =Volume of EDTA solution in titration (titre value)

Potassium

Ten ml of aliquot was taken from the pre-digested sample and 25 ml of neutral NH_4OAc solution was added. The content was then shaken on an electric shaker for 5 minutes and filtered. The filtrate was then fed to the atomizer of the flame photometer. The flame photometer reading was set zero for the blank (NH_4OAc solution) and at 100 for 40 ppm K solution. A standard curve was prepared by making different concentrations of K from 5 – 40 ppm. The concentration of K in the sample was calculated using the standard curve (Ward and Johnson,1962).

$$\text{Potassium (mg/100g)} = \frac{\text{ppm found from Standard curve x Volume made up x Dilution x 100}}{\text{Wt. of sample x 100}}$$

Iron

Three test tubes were taken *viz*. one for blank to which 5 ml water, 0.5ml concentrated sulphuric acid, 2 ml potassium persulphate and 4 ml of potassium thiocyanate were added. In the second test tube for standard, 1 ml standard solution, 4 ml water, 0.5 ml concentrated sulphuric acid, 2 ml potassium persulphate and 4 ml potassium thiocyanate were added and to the third test tube, 5 ml of sample, 0.5 ml concentrated sulphuric acid, 2 ml potassium persulphate and 4 ml potassium thiocyanate were added and the absorbance of each was measured at 480 nm.The amount of iron present was calculated by the following formula given by Saini *et al* (2012).

$$\text{Iron (mg/100g)} = \frac{\text{Absorbance of sample x 0.1 x Total volume of ash solution x100}}{\text{Absorbance of standard x Volume of sample x Wt. of sample taken for ashing}}$$

Anti-nutrient analysis of leaves

Saponin

The saponin content of the samples was determined by the double extraction gravimetric method described by Harborne (1973). A measured amount (5g) of powdered sample was mixed with 50 ml of 20 per cent aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55ºC; it was then filtered through Whatman filter paper (No. 42). The residue was extracted with 50 ml of 20 per cent ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90ºC and transferred to a separating funnel. About 40 ml of diethyl ether was added to a separating funnel and shaken vigorously. Re-extraction by partitioning was done repeatedly until the aqueous layer becomes clear in colour. The saponins were extracted with 60 ml of butanol. The combined extracts were washed with 5 per cent aqueous sodium chloride solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60ºC in the oven and reweighed after cooling in a desiccator. Saponin content was determined by difference and calculated as below:

$$\text{Saponin (\%)} = \frac{W_2 - W_1}{\text{Weight of the sample}} \times 100$$

Where,

W_1 = Weight of evaporating dish

W_2 = Weight of evaporating dish + sample

Oxalate

Oxalate was determined by AOAC (2005) method. One gram of the sample was weighed into a 100 ml conical ûask. Then, 75 ml of 3M H_2SO_4 was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 h and then ûltered using Whatman No.1 ûlter paper. The sample ûltrate (extract, 25 mL) was collected and titrated against hot (80-90°C) 0.1 N $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30s. The concentration of oxalate in each sample was

obtained from the calculation: 1 ml of 0.1N $KMnO_4$ = 0.006303 g oxalate.

Antioxidant and Phenolic compounds

Antioxidant Activity

DPPH radical scavenging activity of extracts of *M. oleifera* was measured by the modified method of Brand-Williams *et al.* (1995). DPPH in ethanol is a stable radical, dark violet in colour. Its colour is bleached by its reaction with a hydrogen donor. For analyses, 0.1 ml of each extract was added to 2 ml of 100 µM DPPH solution in ethanol. The control was made of 0.1 ml ethanol in 2 ml DPPH. The reaction mixture was incubated for 30 min in the dark at 25°C and the absorbance was read at 517 nm, against a reagent blank. The percentage of free radical scavenging activity was calculated according to equation.

$$\text{Scavenging activity (\%)} = \frac{\text{Abs. control - Abs. test sample}}{\text{Abs control}} \times 100$$

Where Abs. is the Absorbance at 517 nm.

Phenolic compounds

Phenolic compounds were determined by using the method given by Malik and Singh (1980). Sample of 1.0 g was taken and ground it with a pestle and mortar in 10 times volume of 80 per cent ethanol. The homogenate was then centrifuged at 10,000 rpm for 20 minutes and the supernatant was collected. The residue was re-extracted and the collected supernatants were pooled. Supernatants were evaporated to dryness and the residue was dissolved in distilled water (5 ml). Different aliquots of 0.2 to 2 ml were taken in a series of test tubes and volume was made up to 3 ml with water in each tube. Folin-ciocalteu reagent of 0.5 ml was added and after 3 minutes, 2 ml of 20 per cent Sodium carbonate solution was added in each tube. The tubes were placed in boiling water for exactly 1 minute, cooled and the absorbance was measured at 650 nm against a reagent blank. The standard curve was prepared using different concentrations of catechol.

RESULTS AND DISCUSSION

Moisture content

The initial moisture content of fresh drumstick leaves was 74.50 per cent which decreased significantly from 74.50 to 7.84 per cent irrespective of the drying method. Blanching significantly reduced the level of moisture as compared to unblanched sample (9.04 to 10.67%). This might be due to loss of cell wall integrity in blanched sample where bound water loss was at faster rate compared to unblanched sample as reported by Waldron *et al.* (2003). The lowest moisture level was found in the treatment combination T_3B_3 (7.60%) (Table1).

Protein content

The protein content of drumstick leaves increased on drying from 6.09 g per 100g to maximum level in sun dried sample (25.12 g/100g) and minimum amount in shade dried sample (24.27 g/100g). Removal of moisture leads to an increase in the concentration of nutrients. In the present study, the increase in protein content of dried drumstick leaves compared to fresh leaves as a result of moisture loss might have influenced dry matter content (Oulai *et al.*, 2016). The result was in agreement with the work of Osum *et al.* (2013) who reported that the drying process increased the protein content due to moisture loss. The unblanched leaf sample retained higher protein (25.67g/100g) than that of the blanched sample. The reduced protein content in blanched sample might be due to leaching loss. The treatment combination of T_1B_1 (unblanched sun-dried leaves) showed highest protein (26.43g/100g) from rest of the combinations (Table 1).

Vitamins

Ascorbic Acid content

Ascorbic acid being highly water soluble and heat labile vitamin, was found to decrease significantly on dehydration. However, shade dried sample retained maximum ascorbic acid (156.27 mg/100g). The unblanched sample showed higher ascorbic acid as compared to blanched sample. The reduced level of ascorbic acid might be due to oxidation of ascorbic acid. These results were well supported by Gupta *et al*. (2008) in green leafy vegetables. Among the treatment combinations, unblanched shade dried leaves (T_2B_1) recorded highest amount (179.47 mg/100g) of ascorbic acid (Table 2).

Vitamin-A content

Vitamin-A is a fat soluble and heat stable vitamin but sensitive to light. The β-carotene content increased on drying from 6.76 mg /100g (fresh leaves) to 19.81 mg /100g in cabinet tray drying, 19.85 mg/100g in sun drying and 22.71 mg /100g in shade drying. The variation in β-carotene content due to different drying

Table 1. Effect of pre-treatment and drying methods on moisture and protein content of drumstick leaves

	Moisture (%)				Protein (g/100g)			
Fresh leaves:	**74.50 %**				**6.09 g/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	10.00	9.73	9.73	9.82	26.43	25.07	23.86	25.12
Shade dried (T_2)	13.80	9.93	9.80	11.18	24.97	24.25	23.61	24.27
Cabinet tray (T_3)	8.20	7.73	7.60	7.84	25.61	24.56	24.45	24.87
Mean	10.67	9.13	9.04		25.67	24.62	23.97	
CD (P= 0.05)	T: 0.31, B: 0.31, T x B: 0.53				T: 0.19, B: 0.19, Tx B: 0.32			

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS

Table 2. Effect of pre-treatment and drying methods on ascorbic acid and vitamin-Acontent of drumstick leaves

	Ascorbic acid (mg/100g)				Vitamin A (mg/100g)			
Fresh leaves:	**206.67 mg/100g**				**6.79 mg/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	154.53	132.93	131.47	139.64	18.73	21.19	19.62	19.85
Shade dried (T_2)	179.47	145.60	143.73	156.27	22.02	23.63	22.50	22.71
Cabinet tray (T_3)	134.67	122.67	123.20	126.84	17.78	20.83	20.81	19.81
Mean	156.22	133.73	132.80		19.51	21.88	20.98	
CD (P= 0.05)	T: 2.24, B: 2.24, TxB: 3.87				T: 0.49, B: 0.49, TxB: 0.85			

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS

processes could be attributed to the length of exposure to light, oxygen and heat and also, it has been described as being labile to different drying techniques (convection, sun, vacuum or freeze drying) as reported by Soria *et al.* (2009). Thus, more loss in vitamin A was observed in sun drying than other drying methods. The drumstick leaves retained highest vitamin A content in shade dried leaves and lowest in cabinet drying. Similar results were also reported by Joshi and Mehta (2010) that shade drying retained highest vitamin A content followed by oven drying at 60°C. Blanching resulted in significant loss of β carotene content of drumstick leaves. The β carotene content of blanched leaves (21.88 mg/100g) was higher than those of their corresponding unblanched leaves (19.51 mg/100g). The carotene content on the plant is bounded with protein, the heat treatment such as steaming, cooking and blanching can release the carotene that bounded (Howard *et al*., 1999). The treatment combination T_2B_2 (Blanched and shade dried leaves) showed highest (23.63 mg/100g) vitamin-A content from rest of the combinations (Table 2).

Minerals content

Calcium

The calcium content of fresh drumstick leaves was 438 mg /100g and it was much lower than the dried leaves. Among the drying methods, highest (2078.73 mg/100g) calcium content was noticed in cabinet tray dried leaves than the other two methods. Liman *et al.* (2014) also observed enhanced mineral nutrients in *Moringa oleifera* leaves dried under moisture analyzer drying method as compared to sun and oven drying. The general increase in mineral content with increase in drying temperature is attributable to concentration factor due to moisture removal, which resulted in higher

level of total soluble solid (Alakali *et al*., 2014). The result revealed that drumstick leaves blanched along with KMS dip had the highest calcium content (2062.91 mg/100g) followed by only blanched (2051.56 mg/100g) while unblanched leaves showed the lowest calcium concentration (2031.79 mg/100g) (Table 3).

Magnesium

Magnesium occurs abundantly in chloroplast as a constituent of chlorophyll molecule. The fresh drumstick leaves contain 48.36 mg /100g, which increased significantly upon drying to 289.32 mg 100g in cabinet drying, 294.78 mg /100g in shade drying and 315.41 mg per 100g in sun drying. Buchaillot *et al*.(2009) reported that magnesium could transform into pyropheophytin and pheophytin because of high temperature. Hence, magnesium might have bound in which it inhibited the mineral from leaching when structure of the leaf breaks. The amount of magnesium in drumstick leaves without blanching was found to be much higher (304 mg/100g) than the blanched leaves (Table 3).

Potassium

The result revealed that out of three drying methods, sun dried drumstick leaves had the highest potassium content (1210.06 mg/100g) followed by shade drying and the lowest potassium was observed in cabinet tray dried leaves (1099.87 mg/ 100g). Probably the reason might be due to potassium being cationic in nature that do not polarize on heating but forms oxides when exposed to light and air. Blanching significantly reduced the level of potassium content in leaves. The blanched leaves showed lowest value for potassium while highest potassium content (1242.43 mg/100g) was noticed in unblanched leaves. The reduced potassium content of blanched green leafy vegetable indicated the solubility and the leaching of the minerals into the water because of their highly reactive nature of the metal that readily reacts with water (Michael, 2006) (Table 4).

Iron

The iron concentration of dried drumstick leaves was higher as compared to fresh leaves. Present study revealed that there was an increment of iron content during drying of leaves irrespective of drying procedure. The shade dried leaves showed 16.54 mg / 100g of iron content while cabinet tray dried and sun dried sample exhibited 13.62 and 13.52 mg/100g respectively. However, shade dried leaves significantly retained higher iron content in comparison to sun drying and cabinet tray drying. This might be due to the fact that concentration of solid increases with the removal of moisture from the leaves. A similar trend of iron content in shade dried moringa leaves was reported by Emelike and Ebere (2016). Blanching increased the availability of iron content in leaves. The result revealed that drumstick leaves blanched and dipped in KMS solution retained highest (16.09 mg/100g) iron content while unblanched leaves recorded the lowest amount (13.04 mg/100g) (Table 4).

Table 3. Effect of pre-treatment and drying methods on calcium and magnesium content of drumstick leaves

	Calcium (mg/100g)				Magnesium (mg/100g)			
Fresh leaves:	**438 mg/100g**				**48.36 mg/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	2044.00	2064.67	2082.07	2063.07	318.70	314.43	313.11	315.41
Shade dried (T_2)	1984.03	2004.57	2023.23	2003.94	298.72	294.16	291.57	294.78
Cabinet tray (T_3)	2067.33	2085.43	2083.43	2078.73	294.70	277.60	295.67	289.32
Mean	2031.79	2051.56	2062.91		304.00	295.40	300.11	
CD (P= 0.05)	T: 1.87, B: 1.87, Tx B: 3.25				T: 1.68, B: 1.68, Tx B: 2.92			

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS

Table 4. Effect of pre-treatment and drying methods on magnesium and iron content of drumstick leaves

	Potassium (mg/100g)				Iron (mg/100g)			
Fresh leaves:	**254.71 mg/100g**				**1.07 mg/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	1378.79	1124.69	1126.71	1210.06	12.80	13.38	14.37	12.80
Shade dried (T_2)	1219.79	1121.18	1194.64	1178.54	13.38	16.67	19.61	13.38
Cabinet tray (T_3)	1128.72	1099.03	1071.86	1099.87	12.96	13.61	14.29	12.96
Mean	1242.43	1114.97	1131.07		13.04	14.56	16.09	
CD (P= 0.05)	T: 0.39, B: 0.39, Tx B: 0.68				T: 0.96, B: 0.96, Tx B: 1.67			

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS

Antinutritional Factors

Saponin

Saponin, a naturally occurring glycoside that is widely distributed in the plants. It acts as antinutrient and also as antioxidant in human. Fresh drumstick leaves contain 5.71 % of saponin, which gets reduced to 0.76 -1.76 % upon dehydration by different methods. All three drying method could reduce saponin content drastically. Reduction in saponin content may be attributed to heat induced degeneration involved in drying processs (Ademiluyi *et al*., 2018). Saponin content was also influenced by pretreatments. The decrease in the saponin content upon blanching of drumstick leaves was 89.49% in only blanched and 87.74 % in blanched and dip in KMS solution. Whereas unblanched leaves showed 62.87 % reduction. The treatment combination T_2B_1 (unblanched shade dried leaves) recorded highest (3.66 %) amount of saponin and lowest (0.50 %) in blanched sun-dried leaves (T_1B_2) (Table 5).

Oxalate

The antinutritional constituent oxalate can reduce the bioavailability of some minerals, especially calcium. Oxalate occurs naturally in plants. It occurs as soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron. The total oxalate content of drumstick leaves was found to increase on drying. The oxalate present in fresh leaves was 121.56 mg/100g and in dried sample it ranged from 299.84 to 378.66 mg /100g. The result of the present study was in accordance with Aditi *et al*. (2017) who reported increase in oxalate content of moringa leaves. It has been observed that after drying, the oxalate content increased, which may be due to considerable

Table 5. Effect of pre-treatment and drying methods on saponin and oxalate content of drumstick leaves

	Saponin (%)				Oxalate (mg/100g)			
Fresh leaves:	**5.71%**				**121.56 mg/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	1.54	0.50	0.62	0.89	380.14	265.21	254.16	299.84
Shade dried (T_2)	3.66	0.74	0.88	1.76	541.47	298.36	296.15	378.66
Cabinet tray (T_3)	1.15	0.55	0.59	0.76	413.29	265.21	269.63	316.04
Mean	2.12	0.60	0.70		444.97	276.26	273.32	
CD (P= 0.05)	T: 0.10, B: 0.10, Tx B: 0.18				T: 3.12, B: 3.12, Tx B: 5.40			

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS

loss of moisture content, hence other compounds such as oxalate content of dehydrated sample became concentrated and their value was much greater than those of fresh sample (Paul *et al.*, 2012).

Blanching reduced the oxalate content because the concentration of antinutrients was highest on the superficial layer of vegetable and blanching ruptures this layer (Albinhna and Savage, 2001). In the present findings, oxalate content was at its minimum level in blanched sample (273.32- 276.26 mg/100g) compared to unblanched sample (444.97 mg/100g). Among the treatment combinations, lowest oxalate was found in T_1B_3 (254.16 mg/100g) (Table 5).

Antioxidant and Phenolic compounds

Antioxidant activity

The drumstick leaves were endowed with the added benefits of antioxidants such as vitamin A, C and phenolic compounds. The result of the present findings showed that antioxidant activities of the drumstick leaves were reduced due to dehydration and blanching. Antioxidant activities (DPPH) of the leaves ranged from 63.76 to 77.47 % among the drying methods. The leaves dried under shade had the highest radical scavenging power (77.47%) than the other drying methods. Cabinet tray dried sample showed lowest activity. The drying process lead to deterioration of antioxidants in leaves. It appears that mainly vitamin C involved in free radical scavenging activity while carotenoid and total phenol are mostly implicated but to a lesser extent in the ion reducing power. During drying, exposure to heat, light and oxygen accelerate the rate of oxidation of vitamin A and C present in vegetables (Oulai *et al.*, 2015)

Blanching had significant influence on antioxidant properties. The scavenging power of drumstick leaves reduced on blanching while unblanched leaves recorded highest activity. This may be due to leaching out and thermal degradation of heat sensitive compounds, vitamin C and A. Bamidele *et al.* (2017) also observed similar trend in bitter leaves (*Vernonia amygdalina*) and found a significant decrease in reducing power and the DPPH scavenging activity of blanched sample. The highest activity was observed in T_2B_1 (unblanched shade dried) (80.33 %) (Table 6).

Phenolic compounds

Phenolics are one of the most effective antioxidant constituents of drumstick leaves. The total phenol content of drumstick leaves varies with the drying procedure and pre-treatments. Phenol content was found to decrease from 163.42 mg /100g (fresh leaves) to 140.04 mg /100g in shade dried, 136.05 mg / 100g in sun dried and 130.81 mg /100g in cabinet tray dried leaves. The decrease in the phenolic contents of the moringa leaves exposed to drying processes such as sun and cabinet tray drying could be due to heat-induced degradation of phenolic compounds (Oboh *et al.,* 2010). The maximum (137.32 mg/100g) total phenol content was observed in unblanched sample and the minimum (134.20 mg/100g) was in only blanched sample.

Table 6. Effect of pre-treatment and drying methods on antioxidant activity and phenol content of drumstick leaves

	Antioxidant activity (%)				Total Phenol (mg/100g)			
Fresh leaves:	**85.25 %**				**163.42 mg/100g**			
Drying methods	**Pre-treatments**			**Mean**	**Pre-treatments**			**Mean**
	B_1	**B_2**	**B_3**		**B_1**	**B_2**	**B_3**	
Sun dried (T_1)	73.80	69.48	71.54	71.61	137.67	134.62	135.86	136.05
Shade dried (T_2)	80.33	73.52	77.47	77.11	141.89	138.18	140.05	140.04
Cabinet tray (T_3)	66.07	61.86	63.35	63.76	132.40	129.79	130.23	130.81
Mean	73.40	68.29	70.79		137.32	134.20	135.38	
CD (P= 0.05) T: 0.24, B: 0.24, Tx B: 0.41T: 0.46, B: 0.46, Tx B: NS								

B_1: Unblanched B_2: Blanched B_3: Blanched + KMS NS: Non-Significant

CONCLUSION

The result obtained from the present study indicated that out of three drying methods, shade drying was found to have better retention of nutrients. The pre-treatments had significant effect on physiochemical characteristics of drumstick leaves. The nutrients such as vitamin A, C, iron, anti-nutrients, antioxidant and phenol retention ability of drumstick leaves was better in shade dried samples while protein, calcium, magnesium and potassium retention was more in sun dried samples. The combination of unblanched leaves dried under shade was found superior in terms of retention of nutrients from rest of the treatment combination. Considering the above fact, both shade and sun drying may be considered best for preserving nutrient and also from the point of view of cost involvement.

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Original Research Paper

Effect of various pre-harvest treatments on shelf life and morphological characteristics of fruits of mango (*Mangifera indica* L.) var. 'Amrapali'

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ABSTRACT

The mango is considered as 'king of fruits' in India due to its delicious taste and nutritional status. Extension of fruit shelf life is a prime importance for availability of fresh fruit in market for longer duration and distance transportation. India is the largest producer and a prominent exporter of mango in the world.In this context, the study was conducted to evaluate the effect of preharvest spray of different chemicals and plant growth regulators (PGRs) on mango var. 'Amrapali' for shelf life and its quality. As 'Amrapali' has regular bearer with very good flavor and taste with a late maturing character, selected for shelf life studies. The fruits of mango weresprayed with chemicals *viz.* $CaCl_2$ 1%, $CaCl_2$ 2%, $Ca(NO_3)_2$ 1%, $Ca(NO_3)_2$ 2%, KNO_3 1%, KNO_3 2%, GA_3 25 mg/l, GA_3 50 mg/l, Ethrel 0.1 ml/l and Ethrel 0.2 ml/l prior to harvest. After harvesting, fruits were stored under ambient storage condition. Among all the treatments, GA_3 25 mg/l treatment recorded significantly highest fruit length, fruit diameter, fruit volume and fruit weight at harvest and at fully ripe stage. Application of $CaCl_2$ 2% resulted in significantly minimum physiological loss in weight consistently from 2nd day to 16th day of storage besides significantly highest shelf life and quality. Hence, this intervention can contribute in preserving physical and chemical quality attributes for maximum acceptance by consumers.

Keywords: $CaCl_2$, GA_3, pre-harvest, PGRs and storage

INTRODUCTION

The mango is the national fruit of India and is a highly popular among the masses owing to its excellent flavour, delicious taste, delicate fragrance and attractive colour. Inadequate postharvest handling and management cause major losses in nutritional and quality attributes, pathogenic outbreaks, and economical losses all along the supply chain, from farm to fork. Fresh mangoes are perishable in nature that require coordinated activity by growers, storage operators, processors, and retailers to maintain quality and reduce wastage. In mango, major postharvest losses are due to the loss of quality in terms of firmness, high physiological weight loss and spoilage. In spite of the highest production, India contributes a small share of less than 5% in export market due to its postharvest losses. About 20–30% of the fruits grown in India are lost due to improper handling practices (NHB, 2018). However, It is a climacteric fruit, the upsurge in respiration rate after harvesting becomes faster which shortens the shelf life. The shelf life reduction due to rapid fruit ripening, senescence attack of biotic and abiotic stresses (Zhu *et al*. 2013). The researchers made attempts to extend the shelf life and to reduce spoilage of fruit *viz.* edible coating (Ali *et al.* 2011), modifed or controlled atmosphere storage (Martins and Resende 2015), low temperature storage (Aghdam and Bodbodak, 2013), application of fungicides (Sripong *et al*. 2015), and hot water treatment. Sometimes, due to reduced oxygen level in controlled atmospheric storage develops off-flavor in fruits. There is lacking in availability of storage facilities *viz.* controlled atmospheric storage and modified atmospheric storage at farmers in India and setting up infrastructures for advanced storage facilities is very costly. Also, a cold chain to

manage the time–temperature conditions is adequate for the preservation and transportation of perishables in the proper temperature range to slow down the biological decay processes and deliver safe and high-quality produce to consumers is a lacuna. Hence, preharvest spray of chemicals are very economical to extend the shelf life of fruits.

Potassium plays an important role in photosynthesis, synthesis of carbohydrates, oils, fats and proteins. It is also involved in the transportation of photosynthates towards the sink and enhances the production of protein (Lu *et al*., 2016). Potassium is an important nutrient for fruit weight and quality. Potassium is required for the production and transport of plant sugars that increase the weight of fruit (Jaiswal *et al*. 2021). Ethrel releases ethylene gas, influences the growth and development of fruits. Ethrel is responsible for early development of many fruits characterized by a high rate of ethylene evolution and hastens the ripening process with uniform colour development (Dhillon, 2013). Calcium is known to be essential plant nutrient involved in a number of physiological processes concerning membrane structure, function and enzyme activity (Jones and Lunt, 1967). It has received considerable attention in recent years due to its desirable effects in delaying ripening and senescence, increasing firmness, reduce respiration, extending storage life and reducing the incidence of physiological disorders and storage rots. Preharvest application of these compounds hinders the fruit ripening without affecting the edible quality. Preharvest application of $CaCl_2$ extends the shelf life and restrict the microbial infection without any detrimental effect and protects against post-harvest deterioration and extend shelf life (Saure, 2005). Gibberellic acid has been found to enhance the fruit size, increase the yield, and improve the physico-chemical characteristics of fruits through modification of various physiological and bio-chemical processes (Pandey and Sinha, 2013). Gibberellic acid in proper concentration and at appropriate time have been found to better results in fruits quality, yield, size, decrease fruit drop, increasing sugar content, improve the physico-chemical characteristics and extend the post-harvest life of fruits through modification of various physiological and bio-chemical processes of plant (Pandey and Sinha, 2013). Gibberellins have been useful in enhancing fruit retention and improving the size and quality of fruits. Further, gibberellic acid has anti-senescent property and help in maintaining cell wall integration and prevents growth of pathogen in the fruits and extend shelf life (Prasad, 2006).

Being a climacteric fruit, weight loss increases rapidly during storage period due to surge in respiration rate and transpiration process. However, it can be minimized by supplementary application of chemicals and plant growth regulators on fruits for maintaining fruit quality and extending their shelf life (Vishwakarma and Masu, 2018; Bisen and Thakur, 2012). Now a day, the mango variety 'Amrapali' grown commercially throughout the country because of its dwarf stature. It has very good flavor, taste and high in vitamins and carotenoids content as compared to other verities of mango with a late maturing character, selected for shelf life studies.Considering these points, the present study was designed to study the effect of preharvest spray of different chemicals and plant growth regulators on shelf life extension of mango fruits under ambient storage condition.

MATERIALS AND METHODS

The experiment was conducted at Horticultural Research Farm and Postgraduate Laboratory, Department of Horticulture, Bansilal Amrutlal College of Agriculture, Anand Agricultural University, Anand during summer season of the year 2016. The climate of Anand region is semi-arid and sub-tropical type. The temperature was in the range of 25 to 40 ^{o}C with 52 to 73 % relative humidity during experiment time in the month of June, 2016. There were eleven treatments embedded in Completely Randomized Design replicated thrice. Thirty-three uniform sizedfourteen-year old trees of mango var. 'Amrapali' were selected and preharvest sprayed with different chemicals ($CaCl_2$ 1 %, $CaCl_2$ 2 %, $Ca(NO_3)_2$ 1 %, $Ca(NO_3)_2$ 2 %, KNO_3 1 %, KNO_3 2 %), Ethrel 0.1 ml/l and Ethrel 0.2 ml/l) along with control at twenty days before anticipated date of harvest while, GA_3 25 mg/l and GA_3 50 mg/l were sprayed at marble stage. Mature and uniform sized ten fruits per replication were harvested from the representative trees and kept in ambient storage condition (32±1 ^{o}C). When the outer layer of fruits starts to spoil like discoloration, shriveling and visible sign of biotic spoilage

(anthracnose) considered as end of shelf life and noted as spoiled (Rahman *et al*., 2007).

RESULTS AND DISCUSSION

Effect of preharvest treatments on physical parameters of mango fruit

The fruit size is an important consideration for consumer preference. The effect of treatments on fruit size *viz*. length and diameter were found to be significant at harvest as well as at fully ripe stage (Table 1). The fruit length (10.20 cm) at harvest stage was found significantly maximum with GA_3 25 mg/l treatment followed by the treatments of $KNO_3$1 %, Ethrel 0.2 ml/l, GA_3 50 mg/l, $Ca(NO_3)_2$ 1% and 2% while at fully ripe stage significantly maximum fruit length l (10.16 cm) was recorded with GA_3 25 mg/ followed by treatments of Ethrel 0.2 ml/l, GA_3 50 mg/ l, $Ca(NO_3)_2$ 1% and 2%. The maximum fruit diameter (6.16 cm) at harvest stage was found significant in treatment of GA_3 25 mg/l and $Ca(NO_3)_2$ 1% followed by $Ca(NO_3)_2$ 2%, $CaCl_2$ 1%, GA_3 50 mg/l, Ethrel 0.1 ml/l, KNO_3 2% while, at fully ripe stage after storage under ambient condition the diameter of fruits (6.14 cm) was found significantly maximum in treatment of GA_3 25 mg/l followed by $Ca(NO_3)_2$ 1% and 2%, GA_3 50 mg/l, Ethrel 0.1 ml/l and KNO_3 2%. The significant effect of treatments was found on fruit volume at harvest as well as at fully ripe stage. Preharvest sprayed with GA_3 25 mg/l reported significantly highest fruit volume (150.54 cc) at harvest followed by KNO_3 1% and at fully ripe stage (fruit volume - 130.62 cc) also found maximum in treatments of GA_3 25 mg/l followed by KNO_3 1% and Ethrel 0.2 ml/l under ambient storage condition (Table 1). The fruit weight was significantly influenced by various chemicals and plant growth regulators at everyday up to last ripening stage. Application of GA_3 25 mg/l depicted significantly maximum fruit weight (170.50 g) at harvest and consistently up to 16th day of storage period under ambient condition as compared to rest of the treatments (Table 2). The lowest fruit weight, length, diameter and volume were recorded in the control at both the stages *i.e.* at harvest and fully ripe stage.

The fruit size of mango was greatly influenced by different treatments of chemicals. In comparison to all treatments gibberellic acid influenced significantly in terms of fruit weight, volume, length and diameter. It

Table 1. Effect of preharvest treatments on fruit length (cm.), fruit volume (cc.) and fruit diameter (cm.) in mango fruit var. 'Amrapali'.

Treatments	Fruit length (cm)		Fruit diameter (cm)		Fruit volume (cc)	
	At harvest	At fully ripening stage	At harvest	At fully ripening stage	At harvest	At fully ripening stage
T_1: $CaCl_2$ 1 %	9.45^{cde}	9.41^{cd}	6.05^{ab}	6.02^{ab}	126.33^{de}	101.58^{de}
T_2: $CaCl_2$ 2 %	9.27^{de}	9.25^{d}	5.53^{c}	5.50^{cd}	124.61^{e}	104.16^{cd}
T_3: $Ca(NO_3)_2$ 1%	9.83^{abc}	9.80^{abc}	6.13^{a}	6.08^{ab}	127.87^{cde}	104.12^{cd}
T_4: $Ca(NO_3)_2$ 2%	9.80^{abc}	9.77^{abc}	6.06^{ab}	6.04^{ab}	133.73^{c}	111.29^{b}
T_5: KNO_3 1 %	10.05^{ab}	10.01^{ab}	5.44^{cd}	5.41^{de}	148.88^{ab}	129.95^{a}
T_6: KNO_3 2 %	9.65^{bcd}	9.62^{bcd}	5.90^{ab}	5.87^{ab}	123.10^{e}	98.22^{e}
T_7: Ethrel 0.1 ml/l	9.25^{e}	9.21^{d}	5.95^{ab}	5.93^{ab}	115.43^{f}	101.43^{de}
T_8: Ethrel 0.2 ml/l	9.99^{ab}	9.96^{ab}	5.82^{b}	5.78^{bc}	142.23^{b}	126.33^{a}
T_9: GA_3 25 mg/l	10.20^{a}	10.16^{a}	6.16^{a}	6.14^{a}	150.54^{a}	130.62^{a}
T_{10}: GA_3 50 mg/l	9.85^{abc}	9.82^{abc}	5.96^{ab}	5.93^{ab}	131.88^{cd}	107.42^{bc}
T_{11}: Control	8.32^{f}	8.29^{e}	5.23^{d}	5.21^{e}	101.02^{g}	77.73^{f}
SEm±	0.123	0.126	0.089	0.089	2.112	1.612
C.D.	0.364	0.373	0.264	0.262	6.234	4.758
C. V. %	2.223	2.284	2.653	2.648	2.822	2.574

Note: Treatment means with the letter/letters in common are not significantly different by Duncan's New Multiple Range Test at 5 % level of significance.

Table 2. Effect of preharvest treatments on fruit weight (g) of mango var. 'Amrapali'.

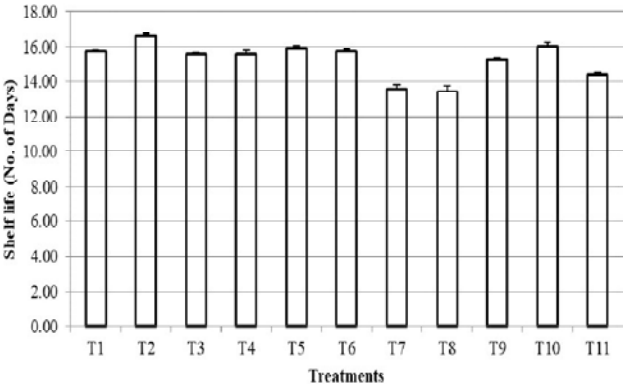
Treatments	Fruit weight (g) in storage at ambient condition (Days)															
	At harvest	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
T_1: $CaCl_2$ 1 %	137.7^{e}	134.64^{de}	132.45^{fg}	129.93 ^{fg}	128.54^{ef}	126.57^{ef}	125.08^{ef}	122.32^{ef}	121.44^{ef}	120.03^{ef}	117.27 ^{fg}	115.05 ^{fg}	113.63^{cd}	112.01^{ef}	110.94^{cd}	108.30^{de}
T_2: $CaCl_2$ 2 %	138.1^{e}	136.28^{d}	134.32^{ef}	132.25^{efg}	130.23^{ef}	128.85^{def}	127.43^{def}	125.36^{def}	123.95^{def}	122.29^{de}	120.92 ^{def}	117.91^{ef}	115.11^{cd}	113.70^{def}	112.17^{cd}	109.83^{cd}
T_3: $Ca(NO_3)_2$ 1%	146.2^{d}	143.68^{c}	141.53^{de}	139.13^{de}	135.99^{de}	133.98^{cde}	131.92^{cde}	129.63^{cde}	127.69^{cde}	125.70^{cde}	124.03^{cdef}	122.52^{cdef}	121.60^{bc}	119.92^{cde}	117.66^{c}	113.82^{cd}
T_4: $Ca(NO_3)_2$ 2%	149.3^{d}	146.78^{c}	144.72^{cd}	142.15^{cd}	140.26^{cd}	137.51 ^{cd}	134.98^{cd}	133.18^{cd}	131.38^{cd}	129.61^{cd}	126.97 ^{cde}	125.35^{cde}	123.47^{bc}	121.87^{bcdee}	119.17^{bc}	117.53^{cd}
T_5: KNO_3 1 %	164.4^{b}	162.63^{a}	158.75^{ab}	156.05^{ab}	153.93^{ab}	151.87^{ab}	147.80^{ab}	145.14^{ab}	143.30^{ab}	141.33^{ab}	138.53 ^{ab}	137.22 ^{ab}	134.76 ^{a}	132.54 ^{ab}	130.53 ^{ab}	128.91^{ab}
T_6: KNO_3 2 %	138.6^{e}	135.89^{d}	134.52^{ef}	132.51^{ef}	131.11^{ef}	129.58^{def}	128.04^{def}	125.98^{de}	123.84^{de}	122.63^{de}	119.95^{ef}	118.77^{def}	116.95^{bc}	115.58^{cde}	113.06^{c}	111.06^{cd}
T_7: Ethrel 0.1ml/l	132.4^{f}	130.26^{e}	126.00^{gh}	124.25^{gh}	122.97^{fg}	120.95^{fg}	118.93^{fg}	116.65^{fg}	114.73^{fg}	112.40^{fg}	109.76^{gh}	107.87^{gh}	106.01^{de}	104.27^{fg}	101.73^{de}	99.36^{ef}
T_8: Ethrel 0.2ml/l	158.4^{c}	155.63^{b}	151.67^{bc}	147.57^{bcd}	145.62^{bc}	143.27^{bc}	140.60^{bc}	136.86^{bc}	134.51^{bc}	133.08^{bc}	130.74^{bc}	128.89^{bc}	125.73^{ab}	123.97^{bcd}	120.67^{bc}	118.65^{bcd}
T_9: GA_3 25 mg/l	170.5^{a}	167.18^{a}	164.14^{a}	160.34^{a}	158.99^{a}	156.38^{a}	153.79^{a}	150.46^{a}	147.31^{a}	145.02^{a}	143.41 ^{a}	142.22 ^{a}	135.37 ^{a}	135.29 ^{a}	132.86 ^{a}	130.92^{a}
T_{10}: GA_3 50 mg/l	155.8^{c}	153.23^{b}	150.06^{c}	148.13^{bc}	145.59^{bc}	142.74^{bc}	140.21^{bc}	137.18^{bc}	135.03 ^{bc}	133.08^{bc}	130.54 ^{bcd}	128.65^{bcd}	126.81^{ab}	124.70^{abc}	120.86^{bc}	119.42^{bc}
T_{11}: Control	124.9^{g}	123.29^{f}	121.83^{h}	119.5^{h}	116.9^{g}	115.8^{g}	113.36^{g}	111.93^{g}	109.60^{g}	107.42^{g}	105.0^{h}	103.9^{h}	102.1^{e}	100.4^{g}	96.65^{e}	96.00^{f}
SEm±	1.550	1.677	2.283	2.533	2.683	2.756	2.828	2.858	2.910	2.784	2.906	2.967	3.005	3.040	3.444	3.144
C.D.	4.575	4.950	6.739	7.478	7.918	8.137	8.347	8.435	8.591	8.217	8.579	8.757	8.869	8.973	10.168	9.280
C. V. %	1.826	2.010	2.788	3.151	3.384	3.530	3.685	3.795	3.925	3.808	4.050	4.192	4.331	4.449	5.142	4.777

Note: Treatment means with the letter/letters in common are not significantly different by Duncan's New Multiple Range Test at 5 % level of significance.

might be due to the involvement of gibberellic acid in promoting cell elongation and cell enlargement of fruit (Jagtap *et al*. 2013; Lal *et al*. 2013). As, GA_3 level in developing cell is low, the exogenous application of GA_3 helps to increase its level in different parts of the fruits, which ultimately helps its growth. The cell elongation stimulated by exogenous gibberellins through altering the rheological properties of the cell wall; as a consequence, the water potential of the cell is lowered allowing for water uptake and greater accumulation of food materials and therefore an increase in cell volume (Derbyshire *et al*., 2007; Brahmachari and Rani, 2000). In the present study, results of GA_3 sprays are in line with those reported by El-Sese (2005) where Balady mandarin trees sprayed with GA_3 resulted in increased yield as of increased fruit weight, length and diameter. The results are also supported by Mostafa *et al.* (2001) on pear and ElSharkawy and Mehaisen (2005) on guava. Marschner (1986) indicated that application of GA_3 and/or IAA on higher plants caused elongation in the primary cells in the young tissues and growth centers. The bigger size and good quality fruits was also observed in plum by González-Rossia *et al.* (2006), Bhomick and Banik (2011) in mango and Singh *et al.* (2009) & Katiyar *et al.* (2008) in guava.

Effect of preharvest treatments on storage studies

There were significant differences observed in physiological loss in weight due to various preharvest treatments of fruits from harvest to everyday up to 16th day (Table 3). Among the treatments, $CaCl_2$ 2 % consistently recorded significantly minimum physiological loss in weight of fruits (1.12 % to 19.91%) from 2nd day to 16th day of storage period respectively, it was found on par with KNO_3 (2%). The significant effect of various treatments was observed on shelf life of mango fruit during storage periods and $CaCl_2$ 2 % was found most effective treatment for extending the shelf life. After storage at ambient temperature $CaCl_2$ 2 % was recorded significantly maximum shelf life (16.60 days) compared to rest of the treatments (Fig. 1) while Ethrel treated fruits were recorded lowest shelf life. There was a significant difference observed in the marketable fruit percentage and spoilage of the fruits during storage under ambient condition. The treatments were significantly influenced at harvest and everyday up to last ripening stage (Table 4). There were 100 % marketable fruits and no spoilage in fruits was recorded in all the treatments up to 12th day of storage



(T_1: $CaCl_2$ 1%, T_2: $CaCl_2$ 2 %, T_3: $Ca(NO_3)_2$ 1%, T_4: $Ca(NO_3)_2$ 2%, T_5: KNO_3 1 %, T_6: KNO_3 2 %, T_7: Ethrel 0.1 ml/l, T_8: Ethrel 0.2 ml/l, T_9: GA_3 25 mg/l, T_{10}: GA_3 50 mg/l and T_{11}: Control)

Fig. 1. Effect of preharvest treatments on shelf life (days) of mango var. 'Amrapali'.

periods. Significantly maximum marketable fruit percentage (90.93%) and minimum spoilage (9.07%) were found in treatment of $CaCl_2$ 2 % followed by $CaCl_2$1% at 13th and 14th day of storage. Significantly maximum marketable fruit percentage and minimum spoilage were found in treatment of $CaCl_2$ 2 %, $CaCl_2$1%, $GA_3$25 mg/l & 50 mg/l after 15th day of storage under ambient condition. Treatment with $CaCl_2$ 2 %, also recorded significantly highest marketable fruit percentage and minimum spoilage at 16th day of storage followed by treatment of $GA_3$25 mg/l and 50 mg/l.

Moisture content of the fruits is an important consideration for its freshness and stability to the storage for a longer duration. The physiological loss in weight in mango fruits was tended to increase during the storage irrespective of the treatments. This could be due to increased moisture loss and enhanced shriveling (Lata *et al.* 2017). Fruits sprayed with $CaCl_2$ 2 % retained the minimum physiological loss in weight and spoilage per cent and maximum shelf life & marketable fruit per cent as compared to rest of the treatments.

As calcium is known to increase fruit cell wall turgidity, serves as a semipermeable membrane, it is also supposed to reduce water diffusion over the fruit cuticle to reduce the differences in osmotic potential, which slows down the evapotranspiration and respiration rate in fruits due to reduced endogenous substrate catabolism and altered membrane permeability (Vercesi *et al*., 2018). Higher concentrations of $CaCl_2$ might be require for the driving force for water diffusion, and to

Table 3. Effect of preharvest treatments on physiological loss in weight (%)of fruit of mango var. 'Amrapali'

Treatments	Storage period (Days)														
	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
T_1: $CaCl_2$ 1 %	2.24^{a}	3.85^{c}	5.68^{c}	6.68^{de}	8.11^{bcd}	9.19^{b}	11.22^{bc}	11.85^{c}	12.85^{d}	14.87^{d}	16.48^{bc}	17.51^{cde}	18.68^{def}	19.47^{cd}	21.38^{cd}
T_2: $CaCl_2$ 2 %	1.12^{f}	2.13^{h}	4.33^{g}	5.43^{h}	6.53^{g}	7.66^{c}	9.15^{d}	10.26^{d}	11.47^{e}	12.44^{e}	14.35^{d}	15.66^{f}	16.66^{g}	18.48^{d}	19.91^{e}
T_3: $Ca(NO_3)_2$1%	1.76^{d}	3.23^{e}	4.87^{de}	7.02^{bc}	8.40^{bc}	9.80^{b}	11.37^{bc}	12.69^{bc}	14.05^{bc}	15.19^{cd}	16.22^{c}	16.85^{def}	18.00^{efg}	19.55^{cd}	22.18^{bc}
T_4: $Ca(NO_3)_2$2%	1.68^{d}	3.10^{e}	4.81^{ef}	6.07^{f}	7.90^{cd}	9.62^{b}	10.82^{bc}	12.01^{c}	13.19^{cd}	14.95^{cd}	16.04^{c}	17.30^{cde}	18.36^{ef}	20.18^{c}	21.29^{cd}
T_5: $KNO_3$1 %	1.97^{b}	3.48^{d}	5.12^{d}	6.41^{e}	7.66^{de}	10.13^{b}	11.74^{b}	12.87^{bc}	14.64^{b}	15.77^{bcd}	16.56^{bc}	18.06^{cde}	19.41^{cde}	20.63^{c}	21.62^{cd}
T_6: KNO_3 2 %	2.06^{c}	2.72^{f}	4.42^{g}	5.71^{g}	6.71^{fg}	7.74^{c}	9.24^{d}	10.68^{d}	11.56^{e}	13.50^{e}	14.62^{d}	16.65^{ef}	17.67^{fg}	18.78^{d}	20.47^{de}
T_7: Ethrel 0.1 ml/l	1.63^{ab}	4.83^{a}	6.16^{b}	7.13^{b}	8.66^{b}	10.18^{b}	11.90^{b}	13.35^{b}	15.12^{ab}	17.11^{ab}	18.53^{a}	19.95^{ab}	21.26^{ab}	23.18^{ab}	24.97^{a}
T_8: Ethrel 0.2 ml/l	1.81^{cd}	4.31^{b}	6.90^{a}	8.13^{a}	9.60^{a}	11.29^{a}	13.65^{a}	15.13^{a}	16.04^{a}	17.52^{a}	18.69^{a}	20.68^{a}	21.79^{a}	23.88^{a}	25.16^{a}
T_9: $GA_3$25 mg/l	2.00^{bc}	3.79^{c}	6.02^{b}	6.82^{cd}	8.35^{bc}	9.86^{b}	11.81^{b}	13.66^{b}	15.00^{ab}	15.94^{bcd}	16.64^{bc}	18.70^{bc}	20.70^{abc}	22.12^{b}	23.25^{b}
T_{10}: $GA_3$50 mg/l	1.70^{d}	3.74^{c}	4.98^{de}	6.60^{de}	8.43^{b}c	10.06^{b}	11.99^{b}	13.37^{b}	14.63^{b}	16.25^{abc}	17.47^{b}	18.65^{bc}	20.04^{bcd}	22.48^{b}	23.41^{b}
T_{11}: Control	1.30^{e}	2.35^{g}	4.57^{fg}	6.42^{e}	7.25^{ef}	9.25^{b}	10.40^{c}	12.26^{c}	13.99^{bc}	15.93^{bcd}	16.77^{bc}	18.20^{cd}	19.59^{bcde}	22.63^{ab}	23.14^{b}
SEm±	0.059	0.052	0.079	0.092	0.190	0.303	0.375	0.312	0.352	0.404	0.299	0.449	0.500	0.384	0.427
C.D.	0.175	0.155	0.232	0.273	0.562	0.893	1.106	0.920	1.038	1.191	0.882	1.325	1.475	1.134	1.260
C. V. %	5.86	2.659	2.585	2.433	4.137	5.502	5.791	4.301	4.391	4.538	3.122	4.315	4.486	3.163	3.295

Note: Treatment means with the letter/letters in common are not significantly different by Duncan's New Multiple Range Test at 5 % level of significance.

Table 4. Effect of preharvest treatments on marketable fruit (%) and spoilage fruit (%) of mango var. 'Amrapali'

Treatments	Marketable fruit (%)					Spoilage fruit (%)				
	Storage period (Days)					Storage period (Days)				
	1 to 12th	13th	14th	15th	16th	1 to 12th	13th	14th	15th	16th
T_1: $CaCl_2$ 1 %	100	90.10^{ab}	76.67^{b}	54.41^{a}	42.40^{b}	00	9.90^{fg}	23.33^{d}	45.59^{c}	57.60^{e}
T_2: $CaCl_2$ 2 %	100	90.93^{a}	79.55^{a}	56.63^{a}	45.16^{a}	00	9.07^{g}	20.45^{e}	43.37^{c}	54.84^{f}
T_3: $Ca(NO_3)_2$ 1%	100	85.87^{f}	71.95^{e}	42.86^{bc}	36.43^{c}	00	14.13^{b}	28.05^{b}	57.14^{ab}	63.57^{d}
T_4: $Ca(NO_3)_2$ 2%	100	86.56^{ef}	73.99^{cd}	44.11^{bc}	34.40^{cd}	00	13.44^{bc}	26.0^{c}	55.89^{ab}	65.60^{cd}
T_5: KNO_3 1 %	100	87.04^{def}	73.44^{de}	42.48^{c}	32.34^{de}	00	12.96^{bcd}	26.56^{bc}	57.52^{a}	67.66^{bc}
T_6: KNO_3 2 %	100	86.84^{def}	73.81^{cd}	45.45^{b}	33.68^{d}	00	13.16^{bcd}	26.19^{c}	54.55^{b}	66.32^{c}
T_7: Ethrel 0.1 ml/l	100	86.18^{ef}	74.35^{cd}	43.15^{bc}	30.63^{ef}	00	13.82^{bc}	25.65^{c}	56.85^{ab}	69.37^{ab}
T_8: Ethrel 0.2 ml/l	100	88.22^{cd}	75.12^{bc}	43.05^{bc}	30.00^{f}	00	11.78^{de}	24.88^{cd}	56.95^{ab}	70.00^{a}
T_9: GA_3 25 mg/l	100	87.50^{de}	74.51^{cd}	56.26^{a}	43.10^{ab}	00	12.50^{cd}	25.49^{c}	43.74^{c}	56.91^{ef}
T_{10}: GA_3 50 mg/l	100	89.15^{bc}	76.66^{b}	56.55^{a}	43.59^{ab}	00	10.85^{ef}	23.34^{d}	43.45^{c}	56.41^{ef}
T_{11}: Control	100	80.14^{g}	68.03^{f}	43.42^{bc}	30.91^{ef}	00	19.86^{a}	31.97^{a}	56.58^{ab}	69.09^{ab}
SEm±		0.437	0.503	0.822	0.684		0.437	0.503	0.822	0.684
C.D.		1.290	1.484	2.426	2.020		1.290	1.484	2.426	2.020
C. V. %		**0.869**	**1.171**	**2.964**	**3.238**		**5.887**	**3.398**	**2.740**	**1.870**

Note: Treatment means with the letter/letters in common are not significantly different by Duncan's New Multiple Range Test at 5 % level of significance.

strengthen the walls of epidermal cells that might had resulted in improved resistance to the fruit cell degradation, when the cells meet free flow of water (Sekse, 1997). Preharvest spray of $CaCl_2$ restricts the microbial infection without any detrimental effect, maintains cell turgor and delays lipid peroxidation, thereby extending shelf life of fruits (Saure, 2005). The calcium compounds significantly thickened the middle lamella of fruit cells owing to increased deposition of calcium pectate and thereby maintained the cell wall rigidity which inhibits the penetration and spread of pathogens in fruits (Gupta *et al.* 1987). This could be one of the reasons for reduction in physiological loss in weight and biotic and abiotic spoilage during storage under ambient condition for 2% calcium chloride treated mango fruits. The similar view of results was also reported in persimmon cv. Karaj (Bagheri *et al.* (2015), in pear cv. Leconte (Sajid *et al.*, 2014), in papaya (Lata *et al.*, 2018; Yadav and Varu, 2013; Ramkrishna *et al.*, 2001), in plum (Kirmani *et al.*, 2013), in mango(Bhusan *et al.*, 2015; Karemera *et al.,* 2014; Singh *et al.,* 2012) and ber (Jawandha*et al.,* 2009; Yadav *et al.,* 2009) for physiological loss in weight, shelf life and reduce spoilage during ambient storage after calcium treatments.

CONCLUSION

Quality evaluation and maintenance is must to be realized in all segments as consumers will not accept a product when it does not have the requirements or desired quality attributes that may cause major impact on the commercialization chain, especially exportation.The results obtained from present investigation concluded that, GA_3 25 mg/l treatment found better in response to improve the physical charactertics of fruit like fruit length, fruit diameter, fruit volume and fruit weight during storage period. Whereas, application of $CaCl_2$ 2% effectively improved the shelf life of fruits and marketable fruit percentage while, minimizing the physiological loss in weight and spoilage percentage of fruits under ambient storage condition. The study

shows that preharvest spray of calcium chloride is eco-safe and could be done for improving shelf life of mango fruits for better marketability.

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Original Research Paper

Maturity determination of red and white pulp dragon fruit

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ABSTRACT

There is a huge potential for dragon fruits grown in India but insufficient information may hamper its production and postharvest handling. The aim of this study was to investigate the right harvest time and maturity indices for red and white pulp dragon fruit. Growth and developmental studies were undertaken using destructive (total soluble solids (TSS), titratable acidity and TSS: acid ratio) and non-destructive methods (fruit weight, specific gravity, peel colour and heat units). Fruits were collected at seven intervals (7, 14, 21, 26, 31, 36 and 41 days after flowering) to assess the right maturity. All these methods were used to standardize the optimum maturity and right time for the harvest of red and white pulp dragon fruit. Harvesting dragon fruits between 31-36 days after flowering (DAF) was found ideal for optimum maturity and quality. Both red and white pulp fruits harvested at 31 DAF showed better quality in terms of physic-chemical and sensory attributes.

Keywords: Dragon fruit, heat units, maturity and physico-chemical properties

INTRODUCTION

Dragon fruit (*Hylocereus* sp.) also known as pitaya belongs to the family Cactaceae. It has originated from Mexico and spread to Central and South America (Britton and Rose, 1963). Pitaya is considered a super fruit due to its rich nutraceuticals and high economic value. A variety of colours exist in dragon fruit such as white pulp with red and yellow peel, red and violet-red pulp with red peel colour (Grimaldo-Juarez *et al*., 2007). The red pulp fruit is rich in betalains which is a natural food colour and an excellent source of antioxidants (Le Bellec *et al*., 2006; Baker *et al*., 2013; Mello *et al*., 2015).

Presently, the area under dragon fruit production is expanding rapidly. Vietnam is the leading producer (shares 51% of the world production) of dragon fruit followed by China India produces about 12113 metric tons of dragon fruit annually from an area of approximately 3084 ha (Merten, 2003; Wakchaure *et al*., 2020).

Harvesting fruits at their optimum maturity provides the utmost quality to consumers and better profit to growers. Fruits should be harvested at an appropriate time and developmental stage for the highest fruit quality. Harvesting prior to full maturity is a common practice to get an extended storage life in the international trade of several fruit crops. This often leads to compromise on the potential quality of the concerned fruit in the interest of trade. Although maturity standards exist for dragon fruit, they vary with location and growing conditions. The stage of optimum maturity can be determined using destructive and non-destructive methods. Destructive methods include physiochemical and mechanical parameters such as total soluble solids (TSS), titratable acidity, and TSS: acid ratio. Physical parameters such as fruit weight, external peel colour, days after flowering to harvest and specific gravity are extensively used as non-destructive methods for indices of maturity in many fruits (Wanitchang and Jarimopas, 2008; Fawole and Opara 2013; Kapilan and Anpalagan 2015).

Dragon fruit is a recently introduced crop in India and its demand has been increasing due to its high nutritional and economic values. Harvesting fruits at optimum maturity helps in better post-harvest management of fruits. The maturity of fruits depends on edaphic factors such as soil, climate, temperature, rainfall *etc*. The aim of this study was to understand the growth and development pattern of red and white

pulp dragon fruit for optimum maturity and harvest time in the region of Bengaluru, Karnataka, India.

MATERIALS AND METHODS

White (*Hylocereus undatus*) and red pulp (*Hylocereus polyrhizus*) dragon fruit cultivars were selected from the experimental block of research farm, Hirehalli, ICAR-IIHR, Hessarghatta, Bengaluru, Karnataka, India. It is situated at the longitude 77°11' East and latitude 28°38' North at an altitude of 845 meters above mean sea level and about 40 km from ICAR-IIHR campus, Bengaluru in south India. It falls under a tropical humid climate and is characterized by pleasant summer, moderate rainfall and mild winter.

For the experiment, four-year-old and well-maintained plants were selected. Both cultivars were tagged at the time of bud initiation and at flowering. The fruits were harvested between the 7 and 41 days after flowering (DAF) from May to June 2019.The fruit samples were collected at 7, 14, 21, 26, 31, 36 and 41 DAF to study the maturity pattern. At each interval, fruits were harvested and immediately brought to the laboratory for further analysis.

Collection of weather data

Weather data for growing conditions were collected from the Weather station of Karnataka State Natural Disaster Monitoring Centre, Yelahanka, Bengaluru, Karnataka. Temperature, rainfall and relative humidity (RH) were taken from1st May to 30th June 2019 for calculating heat units (Fig. 1).

Non-destructive parameters

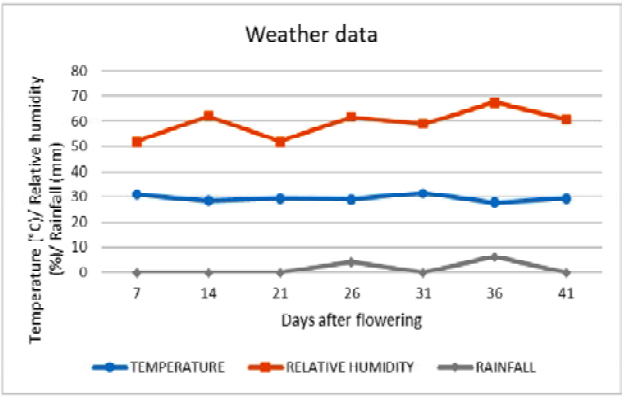

Fig. 1. Weather data (temperature, relative humidity and rainfall) during the study on growth, development and maturity of dragon fruit.

Physical parameters such as average days after flowering (DAF), fruit weight, specific gravity, and heat units were used for the determination of maturity. Weight of each fruit was weighed by electronic balance (Sartorius GPA 5202, Germany). Specific gravity was calculated by measuring the volume of the individual fruit by water displacement method (Mohsenin, 1986). Degree days accumulated were calculated using following formula given by McMaster and Wilhelm (1997) :

Degree days = Sum of (Maximum temperature + Minimum temperature) / 2- Base temperature

Peel colour of fruits was recorded by colorimeter (Minolta RS- 232C, Japan) and represented by 'L', 'a' and 'b' values. The L value represents brightness and its value ranges between 0 (black) to 100 (white). Green colour of peel is indicated by negative or smaller value of 'a' whereas positive or higher 'a' value denotes red colour. The b value represents variations from blue (-b) to yellow (+b). For each colour parameter, two values from opposite sides of individual fruit were recorded and averaged. Redness of peel colour was calculated using *L**, *a** and *b** values as per Minolta (2019).

Redness index was calculated by this formula:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2}$$

Destructive parameters

Physiochemical parameters such as total soluble solids (TSS), Titratable acidity (TA) and TSS: acid ratio was measured by destructive methods. Fruits were cut into pieces and then squeezed to extract the juice. Juice was used to measure TSS through a digital refractometer (ATAGO PAL-3, Japan). Titratable acidity was estimated by juice of white pulp and titrated against 0.1 N NaOH till light pink colour as end point (AOAC, 2000). Red pulp extract was titrated against 0.1 N NaOH till pH 8.1 using microprocessor-based pH system (ESICO RS232PC, India)(Zahid *et al*., 2012).

Sensory properties

A panel of semi-trained and trained judges was selected for the sensory evaluation. Fruits harvested at 31, 36 and 41days intervals were cut into uniform pieces and served for sensory evaluation. Different sensory attributes of red and white pulp dragon fruit such as fruit colour, texture or crispiness, taste and

overall acceptance were taken using a nine-point-Headonic scale (1 = extremely dislike and 9 = extremely like) (Stone *et al*., 2012).

Statistical analysis

Data were analysed using software WINDOSTAT 9.3 version as per factorial randomized block design (FRBD) with two factors (colour and interval of harvesting days) having four replication sand eight fruits in each.

RESULTS AND DISCUSSION

Non-destructive parameters

Growth pattern and days after flowering

The growth rate increased rapidly during early stages of fruit development and slowed down after full maturity. Both red and white pulp types followed a sigmoid growth pattern (Fig. 2). Previous studies suggested that both red and white pulp followed a sigmoid growth pattern (Nerd *et al*., 1999; Jamaludin *et al*., 2011; Magalhaes *et al*., 2019). It was observed that 80% of fruit development such as fresh weight and pulp percentage was completed before colour break stage. Colour break stage to full red colour stage was found crucial for development of optimum biochemical attributes (TSS and acidity). After full red colour development in the peel, variation in fruit shape and size was almost stopped (Nerd *et al*., 1999).

In dragon fruit, bud initiation was started from the last week of March and continued till last week of August. Both *Hylocereus* spp. required 17-20 days for bud initiation to flowering (Table 1). For optimum fruit maturity, red pulp fruits needed 29-31 DAF and white pulp needed 31-33 DAF. A previous study done by Merten (2003) represented immature (23-27 DAF), mature (28-30 DAF) and over-mature (31-40 DAF) stages of dragon fruit in USA condition. Similar type of results were found in a study done in Thailand (Wanitchang *et al*., 2010). Kishore (2016) studied the growth and development of dragon fruit in Orissa (Bhubaneshwar), India and found that it is a fast-growing crop and takes only a month for attaining optimum maturity. Previous studies have also confirmed the similar maturity period of pithaya (To *et al*., 2002; Centurion-Yah *et al*., 2008; Martinez-Chavez, 2011). Determination of optimum maturity based on DAF was observed as a crucial parameter in many fruit crops (Fawole and Opara, 2013: Patel *et al*., 2014; Kapilan and Anpalagan, 2015).

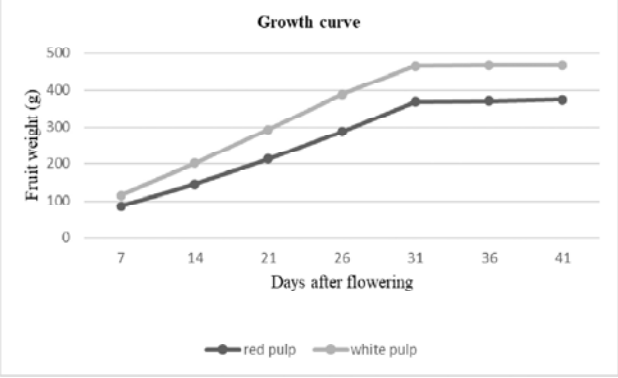

Fig. 2. Growth curve drawn using fruit weight of dragon fruit harvested at seven intervals (7,14,21,26,31,36 and 41 DAF). Presented values are mean± SEm of four replications.

Heat units

Heat units denote the heat requirement of fruits for reaching a particular developmental stage during their growth and development (Lysiak, 2012; Matzneller *et al*. 2014). The heat units accumulated during the period from flowering to optimum maturity was 731.6 (at 29 DAF, data not presented) and 782.2 heat units (31 DAF) in red and white pulp types, respectively (Table 2). Red colour type required lesser heat units for optimum maturity than white pulp fruits. Further studies are required as no reports are available on heat units required during optimum maturity of dragon fruit in India or any other country.

Fruit weight

Table 1. Days required from flower bud emergence to flowering in red and white pulp dragon fruit.

Days required from bud emergence to flowering		
	Red pulp	**White pulp**
Days after bud emergence to flowering	18.02	19.31
SE (m)±	0.045	
CD (0.05)	0.213	

Fruit weight of white pulp was more than red pulp fruit at all harvesting intervals. In white pulp, fruit weight increased from116.44 to 467.37 g and in red pulp it was 56.31 to 367.23 g. A considerable increase in fruit weight was noticed up to 31 DAF in both red and white pulp. Whereas rate of increase in fruit weight was least and almost constant during 36 to 41 days of harvest (Table 1).Therefore, both colour types

gained optimum fruit weight up to 31 DAF and recorded 348.44 g in red pulp and 465.5 g in white pulp type at optimum maturity. Many studies have reported a significant increase in fruit weight of dragon fruits and then growth was almost ceased after full maturity (Centurion Yah *et al*., 2008; Martinez Chavez, 2011; Ortiz and Takahashi, 2015). Red pulp fruits had significantly lower fruit weight than white pulp. This result was in accordance with Nerd *et al*. (1999).

Specific gravity

The photosynthates (soluble solids) accumulates from source to sink during growth and development of fruits (Zhang *et al*., 2005). The specific gravity showed a significant difference between colour types and different harvesting intervals (Table 2). A sharp increase in specific gravity was recorded till 31 DAF and it was almost stable during last intervals of harvest. It was maximum on 31st day for both red and white pulp types1.08 and 1.12 g/cc, respectively. This finding was in accordance with Wanitchang *et al*., (2010) and Fawole and Opara, (2013).

Peel colour

The brightness of Peel colour was shown by *L**value which gradually declined up to full maturity and then somewhat constant at last harvest (Table 3). The value of a* ranged from 10 (green colour) at immature stage to 46 (red colour) in over-mature red pulp fruit and 12 (immature) to 38 (over-mature) in white pulp (Table 2). The *b** value was relatively constant up to 26th DAF and suddenly decreased on 31 DAF then remained constant till over-mature stage. Redness index increased significantly as maturity progressed and found higher at 31 DAF and it was at par with 36 and 41 DAF. These values indicated that optimum red colour development in peel occurred on 31 DAF in both red and white pulp fruits. Red pulp fruits had a significantly higher redness index of peel than white pulp fruits (Table 3).

Manifestation of red colour in the peel initiated after 26 days of flowering in both pithaya species. This result was in accordance with Centurion Yah *et al*. (2008). Both cultivars took 4–5 days to develop full red colour from colour break stage. Fruit peel colour was found as a crucial parameter for determining the

Table 2. Changes in fruit weight, specific gravity and heat units during growth and development of dragon fruit harvested at seven days intervals.

Harvest days days intervals	Fruit weight		Specific gravity		Heat units
	Red pulp	White pulp	Red pulp	White pulp	Red pulp/White pulp
7	56.31	116.44	0.58	0.68	166.85
14	135.04	238.43	0.79	0.79	346.85
21	176.12	252.73	0.87	0.92	534.80
26	231.15	288.74	0.98	1.02	659.40
31	348.44	465.50	1.08	1.12	782.20
36	361.42	468.04	1.10	1.15	897.65
41	367.23	467.37	1.11	1.14	1015.05
C.D. (0.05)					
Colour	28.22	0.025	-		
Days	52.78	0.046	-		
Colour*Days	NA	NA	-		
S.Em±					
Colour	9.83	0.009	-		
Days	18.38	0.016	-		
Colour*Days	26.00	0.023	-		

Table 3. Changes in peel colour (*L, *a**, *b** value and redness of peel index) during growth and development of dragon fruit harvested at seven days intervals.**

Harvest days intervals	L* value		a* value		b* value		Redness index	
	Red pulp	White pulp	Red pulp	White pulp	Red pulp	White pulp	Red pulp	White pulp
7	50.71± 2.51	51.18± 1.49	10.46± 1.23	9.58± 0.30	31.18± 1.81	32.88± 1.67	0.00	0.00
14	47.19± 1.21	50.61± 1.05	10.96± 0.38	10.21± 0.76	29.01± 0.92	33.89± 0.28	4.16± 0.12	1.32± 0.07
21	46.41± 0.99	49.28± 1.45	12.23± 0.52	11.66± 0.34	27.73± 1.22	31.83± 1.33	5.57± 0.19	3.01± 0.11
26	44.73± 1.72	48.75± 1.67	13.02± 0.83	6.37± 2.07	29.37± 1.34	30.32± 1.93	5.89± 0.14	4.78± 0.15
31	36.82± 1.73	41.34± 0.85	43.70± 3.58	36.92± 3.32	9.94± 2.31	9.94± 0.87	40.02± 2.06	37.02± 1.41
36	35.03± 1.80	39.51± 0.89	44.23± 2.47	37.71± 1.20	9.79± 0.71	10.28± 1.03	42.24± 1.42	37.93± 1.33
41	36.88± 0.24	37.93± 0.90	46.08± 1.78	38.08± 2.19	9.10± 0.43	10.38± 1.45	44.13± 1.93	38.66± 2.41

maturity in plum (Usenik *et al*., 2009), citrus (Singh *et al*., 2017), sweet cherry (Chelpinski *et al*., 2019), tomato (Goisser *et al*., 2020) and apple (Pourdarbani *et al*., 2020).

Destructive parameters

Total soluble solids and titratable acidity

A significant rise in TSS was recorded during fruit maturity (Table 4). TSS was higher in red pulp than white pulp fruits. TSS values ranged from 4.5 to 14.3°B and 4.1 to 13.4°B in red pulp and white pulp fruits during different maturity stages (7 to 41 DAF). It remained increasing till optimum maturity and started decreasing during over-mature stages (Table-4). TSS recorded highest on 31 DAF in red (14.2°B) and white pulp fruits (13.2°B). Rise in TSS during maturity indicated that it was a suitable indicator of optimum maturity of dragon fruit (Nerd *et al*., 1999). Many studies have reported that TSS ranged from 10 to 17°B in different genotypes of dragon fruits (Marquez-Guzman *et al*., 2005; Livera-Munoz *et al*., 2010). TA of both *Hylocereus* spp. had likely to increase and reached highest on 26th DAF (0.6% in red and 0.7% in white) and then suddenly decreased on 31st day. Acidity was constantly decreasing in over-mature fruit and reached to a minimum at 41 DAF (Table 4). Optimum acidity (0.23% in red pulp and 0.31% in white pulp) was recorded on 31 DAF. The increasing trend of TA before the colour development of immature fruits and then decline in acidity is associated with the commencement of maturity (Arevalo-Galarza and Ortiz-Hernandez, 2004;Ortiz and Takahashi, 2015). The optimum concentration of TA imparts a good flavor and blend in dragon fruit. Similar pattern of changes in TA during fruit maturity has been reported in various studies (Sornyatha and Anprung, 2009; Osuna-Enciso *et al*., 2011; Kienzle *et al*., 2011; Babu *et al*., 2017; Bakshi *et al*., 2018). Nerd *et al*. (1999) had found that highest acidity was less than 1% in mature fruits of red and white pulp pithaya.

TSS: acid ratio

For a better palatability, TSS: acid ratio of fruits is important. The TSS: acid ratio of both red and white pulp dragon fruit exhibited an increasing trend during fruit maturity. TSS/acid ratio was lowest at immature stage (18.2 and 12.5) and significantly higher at mature (61.3 and 41.9) and over mature stages (76 and 55) in red and white pulp fruits respectively (Table 4). The percentage increase in TSS/acid ratio was highest at 31 DAF (64 and 69%) in red and white pulp fruits, respectively. At immature stages TSS was less and acidity was more while at mature stage it was vice-versa. The higher TSS: acid ratio at 31 DAF was

Table 4. Changes in TSS, titratable acidity and TSS: acid ratio during growth and development of dragon fruit harvested at seven days intervals.

Harvest days intervals	TSS (°B)		Titratable acidity (%)		TSS: acid ratio	
	Red pulp	White pulp	Red pulp	White pulp	Red pulp	White pulp
7	4.55	4.15	0.25	0.33	18.20	12.57
14	5.57	5.15	0.31	0.34	17.96	15.14
21	8.85	7.90	0.43	0.55	20.58	14.36
26	11.56	10.15	0.50	0.62	23.12	16.37
31	14.20	13.20	0.23	0.31	61.30	41.93
36	14.30	13.40	0.20	0.28	68.09	47.85
41	13.70	13.20	0.18	0.24	76.11	55.00
C.D. (0.05)						
Colour	0.12		0.019		3.32	
Days	0.21		0.036		6.21	
Colour*Days	0.31		0.051		8.78	
SEm±						
Colour	0.04		0.007		1.16	
Days	0.08		0.013		2.16	
Colour*Days	0.11		0.018		3.06	

a result of decline in acidity and rise in TSS (Martinez Chavez, 2011; Osuna-Enciso *et al*., 2011).This result concurred with the findings of Centurion-Yah *et al*. (2008), Martinez Chavez (2011) and Ortiz and Takahashi(2015).

Sensory properties

The sensory scores given for different attributes such as fruit appearance, pulp colour, texture and taste are summarized in Fig. 3. Evaluation of sensory quality of red and white pulp dragon fruit indicated that fruits harvested on 31 DAF had maximum consumer acceptance followed by 36 DAF in both colour types. Sensory attributes scored highest at optimum mature stage (31DAF) and least at over mature stage (41 DAF). Sensory properties are important for deciding the optimum maturity of fruits as each attribute is related to fruit quality (Shahbaz *et al*., 2014; Taiti *et al*., 2017).

Days after flowering (DAF) to optimum maturity

The optimum maturity was considered to have reached when the incremental fruit growth rate was

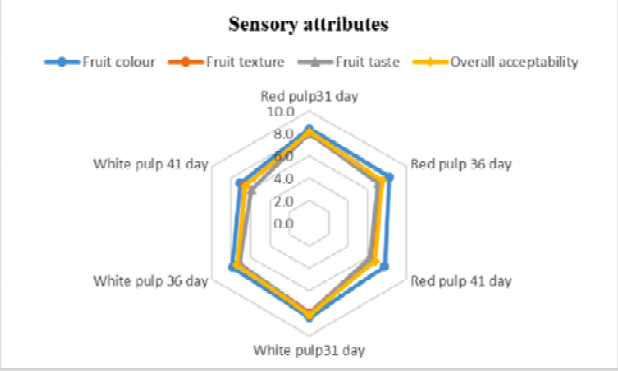

Fig. 3. Changes in fruit colour, texture, taste and overall acceptability during growth and development of dragon fruit harvested at seven days intervals. Presented values are mean of fifteen replications.

significantly lower. All the physico-chemical and sensory parameters were considered to calculate the days required from flowering to optimum maturity. Red pulp fruits required comparatively less duration for attainment of optimum maturity compared to white pulp. Above parameters showed that both colour types needed 31 DAF for attaining the optimum maturity.

Over-mature fruits were prone to cracking, postharvest losses and had lesser shelf life. Fruit cracking was more prevalent in red pulp than white pulp fruits.

CONCLUSION

Dragon fruit is an exotic fruit crop having rich nutraceutical properties. This crop has a great potential in both domestic and export market. Harvest at optimum maturity is an important factor for improving quality and shelf life of fruits. Results of the study reported that physico- chemical parameters were helpful to predict the optimum maturity of red and white pulp dragon fruits. Growth and development of both *Hylocereus* spp. followed a sigmoid growth pattern. The results showed that all the parameters were highest or optimum on 31DAF in both colour types. Red pulp fruits needed comparatively lesser time (29-31 DAF) than white pulp fruits (31-33 DAF) for optimum maturity. At optimum maturity, TSS was higher in red pulp and acidity, fruit weight and specific gravity was higher in white pulp fruits. Sensory attributes scored highest in optimum mature fruits (31DAF) and lowest in over mature fruits (41 DAF). Fruit weight, specific gravity, TSS, acidity and days after flowering can be used as important maturity indices for determining the optimum maturity of dragon fruit.

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Conflict of interest

Author reports no conflicts of interest.

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Original Research Paper

Development and evaluation of ready to serve (RTS) beverage from bael (*Aegle marmelose* Correa.)

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ABSTRACT

A research study was carried out to develop a RTS beverage by exploiting the nutritional and organoleptic properties of bael fruit pulp. Six treatment combinationsof bael RTS with 10, 15 and 20% of pulp concentration and 10 and 15°B of TSS were prepared based on the review of literature. The biochemical and organoleptic properties of the prepared RTS were evaluated during storage. The pH, ascorbic acid and antioxidant activity of the RTS decreased with the storage, while acidity and total sugars increased. Results of the sensory evaluation showed that there was a significant difference between treatments in terms of color, flavor, taste, body and overall acceptability. From the results of quality assessments, the formulated bael RTS beverage with 15% pulp and 15°B TSS was found to be superior and suitable for consumption up to 12 weeks without any significant changes in the quality characteristics.

Keywords: Bael, beverage, biochemical, nutritional, organoleptic properties and RTS

INTRODUCTION

The demand for processed tropical fruit products is increasing in domestic and international markets, however less than 15 fruits are commercially processed. As these fruits are seasonal and perishable in nature, their seasonal surpluses in different regions are wasted in bulk due to improper handling, distribution, marketing, and inadequate storage facilities. For this reason, fruits in excess need immediate processing for value-added products to minimize postharvest losses, which are about 30–35% according to National Horticultural Board. (NHB 2016). The bael is increasingly becoming an important crop in functional food production and is of economic importance. Although the pulp is mainly consumed fresh, the juice prepared from bael fruit was rich in bioactive compounds such as carotenoids, phenols, alkaloids, coumarins, flavonoids, terpenoids and other antioxidants (Thakur, 2014). Fruit beverages are processed food products that are conveniently used and liked by all age group consumers. They also provide a better chance of meeting the daily requirement of nutrients in a healthy diet. There are many different product variants marketed in India, such as sweetened carbonated soft drinks, clarified juice beverages, pulpy beverages, and soda water. Among these non-alcoholic beverages, the share of fruit-based beverages is presently very small as compared to synthetic carbonated beverages. Consumers are now gradually shifting towards the consumption of natural fruit-based beverages because of their nutritional quality, medicinal importance, and good calorific value over synthetic beverages). The advantage of RTS beverage is that there is no need to dilute it further with a required quantity of water, unlike other concentrated beverages such as squash, or syrup, which are diluted judiciously with water before consumption. At present, bael is an underutilized fruit in India and has a limited shelf life in fresh form. Therefore, there is a need for processing it into a value-added product like RTS beverage with extended storage life so that the product can be consumed throughout the year and consumers may relish its unique taste and flavour and quench their thirst. The demand for natural fruit-based beverages with high nutritional value and other health-imparting attributes are immense in the global market.

Bael *(Aegle marmelose* Correa.) is one of the ancient, nutritious minor fruit crops that belongs to the family Rutaceae. Indo-Malayan region is believed to be the centre of origin of this tree and it is found growing in many South East Asian countries including Sri Lanka, Pakistan, Nepal, Bangladesh, Myanmar, Vietnam, Thailand, Cambodia, Malaysia, Philippines, Java and Fiji. The tree grows up to 6-8m height, leaves are trifoliate and deciduous in nature, fruits are aromatic, bark is thick, branches are spiny in some varieties and lower branches are drooping. Young leaf of the tree is glossy shiny and pinkish maroon in color, the flowers are bisexual, 4 curved fleshy petals with green outside and yellowish inside, fragrant having sweet aroma, cluster blooming (4-7), and stamens are 50 or more in number. Fruits are hard-shelled berries, greenish yellow inearly immature stage and turn yellow when mature. It consists of thin or hard woody or soft rind dotted with oil glands, a hard central core with triangular segments and dark orange walls. Segments of fruit are filled with aromatic pale orange, pasty, sweet resinous, more or less astringent pulp, seeds are embedded in the pulp, have round-oblong structure bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying. The shape and size of the fruit varies with varieties and round, pyriform, oval, or oblong fruit shape having 5-20 cm diameter have been reported.

The bael is well-known for its organoleptic properties with special reference to its unique flavour and color. The pulp is highly nutritious and very good source of vitamins, minerals, fiber and pectin (Table 1). Further, the bael fruit was found to be antispasmodic, diuretic, antiseptic, sedative, and analgesi. Epidemiological studies revealed that increased consumption of bael could lead to lower the risk of developing chronic degenerative diseases (Reddy *et al.,* 2010). Studies indicate that consumption of bael have a significant effect on blood glucose and lipid parameters and bael can alleviate the symptoms of diabetes in a natural manner (Sharma *et al.,* 2016).

Therefore, the present study was focused with an objective to optimize the process conditions for the preparation of RTS beverage from bael fruit and to evaluate the physicochemical and sensory characteristics during storage period.

MATERIALS AND METHODS

Preparation of RTS beverage of bael fruit

The ripe bael fruits were collected from the IIHR field gene bank and washed with tap water in the laboratory. The fruits were opened by hitting with hammer due to its hard outer shell. The fruit pulp along with seeds and fiber was scooped with the help of stainless-steel spoon manually. Amount of water equal to the weight of pulp was added. The mixture was heated up to 70°C for 1min and cooled. The pulp was then passed through stainless-steel sieve (800µm) to separate seeds and fibres. The beverage was prepared by varying the pulp concentration and TSS. Acidity was maintained at 0.3% and KMS was added at 120ppm in all the treatmentsas per the formulations given bellow.

Experimental formulations for RTS preparation

T_1- 10% bael fruit pulp + 10°B TSS with sugar syrup.

T_2- 10% bael fruit pulp + 15°B TSS with sugar syrup.

T_3- 15% bael fruit pulp + 10°B TSS with sugar syrup.

T_4- 15% bael fruit pulp + 15°B TSS with sugar syrup.

T_5- 20% bael fruit pulp + 10°B TSS with sugar syrup.

T_6- 20% bael fruit pulp + 10°B TSS with sugar syrup.

The requisite amount of sugar and citric acid were dissolved in requisite amount of water to prepare sugar syrup in heating condition and then mixed with bael fruit pulp in RTS beverage. It was removed from the gas burner and was allowed to cool for 10 min at room temperature of 28 - 30°C. Subsequently, 70 ppm of KMS was added and mixed well with the solution. Just after addition of KMS, hot filling was done into already oven sterilized (160°C for 45 min) glass bottles and caped with stopper immediately. The sealed bottles were put on the hot water bath at 80°C for 30min for pasteurization. Then bottles were removed from the hot water bath and allowed to cool.

Determination of sensory properties

Sensory evaluation was conducted to evaluate the organoleptic properties of the RTS by semi-trained panelists. The color, taste, flavor, body and overall acceptability was evaluated using 9 points Hedonic scale. Samples were evaluated between 10.00 to 11.00am for morning session and 2.00 to 3.00 pm for evening session for effective assessment by the panelists. Each panelist was asked to evaluate the

samples which were arranged randomly to judge the organoleptic properties. The samples were served to the panelist at 10°C as this temperature is commonly used for serving RTS.

Quality analysis of Ready to Serve (RTS)

pH

The pH of the sample was taken using a pH meter (Model: EUTECH Instruments-pH Tutor, Singapore). Twenty mL of the RTS beverage sample was taken to dip the calibrated electrode of the pH meter and the observations were recorded in triplicate for each sample.

Titratable Acidity

Acidity was determined by titration method (AOAC, 942.15, 2000). Homogenized sample of 5 g was mixed with distilled water, squeezed through a muslin cloth and volume was made up to 50 ml. A known volume of the filtrate (25 ml) was titrated against 0.01N NaOH using 0.5% phenolphthalein (3 to 4 drops) as indicator. Acidity was calculated as percentage of citric acid equivalent using citric acid standard curve.

$$\text{Titratable Acidity (\%)} = \frac{\text{TV. (mL)} \times \text{N NaOH} \times \text{Volume(mL)} \times \text{Eq. Wt. (Citric Acid)}}{\text{Sample Weight (g)} \times \text{Aliquot Taken (mL)} \times 1000} \times 100$$

Ascorbic acid

Ascorbic acid content was determined by 2,6-Dichlorophenol indophenol method (AOAC, 967.21, 2006). About 5ml of sample was mixed with 4% oxalic acid solution and volume was made up to 50 ml and was then estimated by titrating a 25ml of the extract against DCPIP. Vitamin C content was calculated as mg of ascorbic acid per 100ml RTS using a standard curve of L-Ascorbic acid.

Total Sugar

Total sugar was estimated by the standard method of AOAC (1980). The sugar extract was hydrolysed with concentrated hydrochloric acid and titrated against 10ml of mixed Fehling's solution (5ml Fehling A + 5ml Fehling solution B) using methylene blue as indicator. Results were expressed as per cent total sugar.

Total Antioxidant activity

2, 2 – diphenyl-1-picrylhydrazyl (DPPH)assay was done according to the method of Williams *et al.* (1995) with some modifications. The DPPH stock solution was prepared by dissolving 19.7 mg of DPPH in 100 mL of 80% methanol. RTS (200 µL) was allowed to react with 50 µL of DPPH solution for 30 min in dark conditions. Readings were taken at 517 nm. The calibration curve was linear from 50 to 500 µL of Trolox. The results were expressed in µM Trolox equivalents (µM TE/g dry weight). Additional dilutions were made when the values obtained from the samples were outside the linear range of the calibration curve.

Sensory evaluation (9-point Hedonic scale)

Samples of appetizers were presented to a panel of 8 judges. For evaluating the RTS, nine-point hedonic scale was used. The samples were served at room temperature.

Statistical analysis

Biochemical and quality analysis data were subjected to statistical analysis, level of significance (LOS). Critical difference (CD) at 5 per cent level of probability was used for comparison among treatments. The results were presented by way of tables. Analysis of quantitative data (biochemical and quality analysis) was done in statistical tool OPSTAT, Statistical Software.

RESULTS AND DISCUSSION

Qualitative analysis of Ready to Serve (RTS) beverage from fruit pulp of bael

pH

There was a significant decrease in pH during storage (Table 1). This might be due to increase in acidity, as acidity and pH are inversely proportional to each other. It was observed that the maximum pH (3.38) was recorded in T_2 (10% pulp + 15°Brix). The decrease in pH was due to increase in titrable acidity which affects the organoleptic quality of juice. Similar effect of ingredients on pH of the value-added product of fruit was observed by Jain and Nema (2007), Elbelazi *et al.* (2015).

Titratable acidity

There was a significant increase in acidity content during storage (Table 1). It was observed that maximum acidity (0.53%) was recorded in T_5 (20% Pulp + 10° Brix). The minimum increase (0.36%) in acidity was observed in T_1 treatment which might be due to addition of citric acid. Similar effect of ingredients on titratable acidity of value-added product of fruit was observed by Jain and Nema (2007), Elbelazi *et al.* (2015), Asghar *et al.* (2016).

Table 1. Influence of pulp level and TSS on physio-chemical attributes of bael RTS.

Treatments	pH	Titrable Acidity (%)	Ascorbic acid (mg/100 ml)	Total Sugar (%)	Total Antioxidant activity (mg AEAC/100ml)
T_1: 10% Pulp + 10°Brix	3.35	0.28	30.54	22.18	81.58
T_2: 10% Pulp + 15°Brix	3.38	0.31	32.42	22.59	82.60
T_3: 15% Pulp + 10°Brix	3.22	0.34	34.50	23.20	83.52
T_4: 15% Pulp + 15°Brix	3.23	0.38	37.60	23.54	84.52
T_5: 20% Pulp + 10°Brix	3.05	0.45	44.50	24.05	86.90
T_6: 20% Pulp + 15°Brix	3.00	0.42	41.50	24.34	85.50
Mean	3.21	0.36	36.84	23.32	84.10
SEm±	0.070	0.008	0.817	0.074	0.768
CD at 5%	0.211	0.024	2.447	0.221	2.301
4 weeks after storage					
T_1: 10% Pulp + 10°Brix	3.32	0.30	30.00	22.90	81.08
T_2: 10% Pulp + 15°Brix	3.34	0.34	31.95	23.34	82.13
T_3: 15% Pulp + 10°Brix	3.21	0.37	33.94	23.90	83.03
T_4: 15% Pulp + 15°Brix	3.20	0.41	37.15	24.28	84.04
T_5: 20% Pulp + 10°Brix	3.02	0.48	43.98	24.75	86.42
T_6: 20% Pulp + 15°Brix	2.98	0.45	40.97	25.04	84.97
Mean	3.178	0.392	36.332	24.036	83.612
SEm±	0.067	0.008	0.764	0.501	1.741
CD at 5%	0.199	0.024	2.287	N/A	N/A
8 weeks after storage					
T_1: 10% Pulp + 10°Brix	3.29	0.33	29.54	23.65	80.54
T_2: 10% Pulp + 15°Brix	3.30	0.36	31.46	24.15	81.64
T_3: 15% Pulp + 10°Brix	3.17	0.40	33.39	24.65	82.51
T_4: 15% Pulp + 15°Brix	3.18	0.45	36.65	25.00	83.56
T_5: 20% Pulp + 10°Brix	3.00	0.50	43.48	25.40	85.94
T_6: 20% Pulp + 15°Brix	2.95	0.47	40.48	25.81	84.45
Mean	3.149	0.419	35.833	24.777	83.107
SEm±	0.065	0.009	0.752	0.516	1.731
CD at 5%	0.196	0.026	2.252	N/A	N/A
12 weeks after storage					
T_1: 10% Pulp + 10°Brix	3.25	0.36	28.03	24.19	80.02
T_2: 10% Pulp + 15°Brix	3.27	0.39	30.89	24.90	81.06
T_3: 15% Pulp + 10°Brix	3.15	0.43	32.85	25.35	82.00
T_4: 15% Pulp + 15°Brix	3.16	0.48	35.98	25.78	83.08
T_5: 20% Pulp + 10°Brix	2.98	0.53	42.94	26.18	85.48
T_6: 20% Pulp + 15°Brix	2.92	0.49	39.95	26.52	83.97
Mean	3.122	0.447	35.107	25.487	82.602
SEm±	0.065	0.009	0.739	0.531	1.719
CD at 5%	0.195	0.027	2.212	N/A	N/A

Ascorbic acid content

The ascorbic acid (vitamin C) content of the juice decreased during storage with the advancement of storage period, which was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat gets easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst. Maximum ascorbic acid content (44.50 mg/100 ml juice) was recorded in T_5 initially, and decreased to 42.94 mg/100 ml juice at the end of the storage. Each ingredient used in preparation of RTS has its own organic acid composition which affect the ascorbic acid of RTS. Jain and Nema (2007) and Abhangrao *et al.* (2017) also reported the similar effect of ingredients on ascorbic acid content of the fruit-based value-added product.

Total sugars

The results revealed that the total sugars content was significantly affected as a result the total sugars content in the juice increased apparently during storage (Table 1), which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides. The minimum increase (24.19%) in total sugar content was recorded in T_1 treatment. The change in total sugar content of beverage was almost negligible during storage, the different ingredients used for RTS preparation vary in their total sugar content which affects the total sugar content of RTS. The effect of ingredients on total sugar of other value-added products was also reported by Asghar *et al.* (2016) in functional bael jam and Chauhan *et al.* (2016) in bael vermouth.

Total antioxidant activity

Decreased antioxidant activity in the juice was observed during storage (Table 1), which might be due to increase in pulp content. The Maximum total antioxidant activity recorded in T_5 (86.90 mg AEAC/100ml); the minimum total antioxidant activity recorded in T_1 (81.58 mg AEAC/100ml). The different pulp concentration used for RTS preparation vary in their antioxidant activity which affects the total antioxidant content of RTS. Similar effect of ingredients on antioxidant activity of the value-added product of fruit was observed by Asghar *et al.* (2016), Bhatt and Verma (2016), Chauhan *et al.* (2016), and Bisen *et al.* (2017).

Sensory evaluation

Sensory assessment is a scientific discipline that uses the concepts of experimental design and statistical analysis to evaluate consumer products through the use of human senses (sight, smell, taste, touch and hearing). It necessitates the use of human assessors, who test the product and keep track of the results. It is therefore feasible to generate insights and judgments about the products under test by using statistical approaches to the results acquired from human assessors. It is the final judge of a product's quality from the consumer's perspective, and it is a significant factor in determining quality. It's all about the product's colour, flavour, taste, texture, and overall acceptability. RTS sensory evaluation is described in depth in the following sections (Table 2).

Table 2. Influence of pulp level and TSS on sensory evaluation (9-point Hedonic scale) scores of bael RTS.

Treatments	Colour	Flavour	Taste	Body	Overall acceptability
T_1: 10% Pulp + 10°Brix	6.45	6.40	6.45	6.10	6.60
T_2: 10% Pulp + 15°Brix	6.65	6.65	6.70	6.90	7.05
T_3: 15% Pulp + 10°Brix	6.80	6.25	6.10	6.65	6.85
T_4: 15% Pulp + 15°Brix	7.60	7.30	7.6	7.10	7.70
T_5: 20% Pulp + 10°Brix	7.60	7.00	7.20	7.05	7.30
T_6: 20% Pulp + 15°Brix	7.65	6.90	6.85	6.95	7.15
Mean	7.13	6.75	6.81	6.79	7.10
SEm±	0.065	0.062	0.142	0.141	0.148
CD at 5%	0.196	0.185	0.425	0.423	0.443

Appearance/Colour

Appearance/colour differed significantly among the treatments with mean value of 7.13 (Table 2). Maximum appearance/ colour recorded in T_6 (7.65) which was on par with T_4 (7.6) and T_5 (7.6). The minimum appearance/ colour recorded in T_1 (6.45).The colour attracts the consumers towards the product and can help in impulse purchases. At the point of purchase, consumers use mostly appearance factor as an indication of quality. Colour is derived from the natural pigments present in fruits. The primary pigments which impart colour are the fat-soluble chlorophylls (green), carotenoids (orange, yellow and red) and the water-soluble anthocyanins (red, blue), flavonoids (yellow), and betalains (red). An effect of ingredients on colour of product was reported by Kaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017). The best product with respect to colour was obtained when 50% level of aonla pulp and 50% of bael pulp were used for preparation of mixed fruit leather by Uttarwar *et al.* (2018).

Flavour

Flavour differed significantly among the treatments with mean value of 6.75 (Table 2). Maximum flavour was recorded in T_4 (7.3) and the minimum flavour was recorded in T_3 (6.25). Flavour is a mingled but a unitary experience which includes sensations of taste, smell, and pressure. Flavour is typically described by aroma and taste. Similar findings on effect of ingredients on flavour of bael based value added product was reported by Kaur and Kochhar (2017), Thukral (2017) and Ullikashi *et al.* (2017). Similar effect was also reported by Uttarwar *et al.* (2018) in preparation of mixed fruit leather and the highest score obtained from 50% level of aonla pulp and 50% of bael pulp with respect to flavour.

Taste

Taste differed significantly among the treatments with mean value of 6.81 (Table 2). Maximum taste recorded in T_4 (7.6) was on par with T_5 (7.2). The minimum taste was recorded in T_3 (6.1). The sensation that is perceived in the mouth and throat on contact with a substance is called as taste. It includes the sweet, sour, salty and bitter quality of a thing that can sense when it is in the mouth. Taste is important for acceptability of any product. Similar effect of ingredients on the bael based value added product was reported by Liyanaduragc *et al.* (2007), Kaur and Kochhar (2017),

Body

Body differed significantly among the treatments with mean value of 6.79 (Table 2). Maximum body recorded in T4 (7.1) which was on par with T5 (7.05), T-6(6.95) and T-2(6.9). The minimum value for an attribute body was recorded in T1 (6.1). Body is another important sensory parameter to judge the quality of product. The body parameters are perceived with the sense of touch or either when the product is picked up by hand or placed in the mouth and swirled. Similar effect on body of different bael based value added product was found by Liyanaduragc *et al.* (2007), Pingale and Dighe (2015),

Overall acceptability

Overall acceptability differed significantly among the treatments with mean value of 7.1 (Table 2). Maximum overall acceptability recorded in T4 (7.7) which was on par with T5 (7.3). The minimum overall acceptability recorded in T1 (6.6).The colour and appearance decides the first purchase of the product but ultimately the overall acceptability of the product is the most important factor for its further future purchase. A similar effect on overall acceptability of different bael based value added product was found by Liyanaduragc *et al.* (2007), Pingale and Dighe (2015),

CONCLUSION

This research was designed to utilize the bael fruit pulp to formulate RTS beverage. The range of pulp and sugar concentration used for the development of RTS beverage was in combination of 10, 15 and 20% pulp and 10 and 15ºB TSS. RTS beverage formulation with 15% pulp and 15ºB having pH 3.23, acidity 0.38%, ascorbic acid 37.60 mg/100g, total sugar 23.54%, and total antioxidant activity of 84.52 mg AEAC/100ml was found best. The RTS beverage T_4 with 15% pulp and 15ºB showed highest overall acceptability (7.7) along with colour 7.60, flavour 7.30, taste 7.6 and body 7.10.

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Original Research Paper

Comparative effect of different sugars instigating non-enzymatic browning and Maillard reaction products in guava fruit leather

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ABSTRACT

Browning is a major quality deterioration process affecting both visual colour and nutritional value of guava leather. The aim of the study was to determine the role of different sugars *viz*., sucrose, fructose, glucose and sorbitol in non-enzymatic browning and antioxidant activity of guava fruit leather. The total free amino acids, ascorbic acid and antioxidant activities were at significantly lower levels in glucose and fructose treated guava leather, while the sorbitol added samples had all of above parameters at the highest level; while a reverse trend was observed in browning index and non-enzymatic browning. Among the browning intermediate products, Hydroxymethylfurfural was present at higher concentration (12.80-32.32 ng/g) than furfural (0.29-0.95 ng/g) in guava leather samples. Among the treatments, hydroxymethylfurfural was found lowest in sorbitol (12.8 ng/g) and highest in fructose (32.3 ng/g). In brief, this paper describes a novel effort in bringing the *in-vitro* studies related to sugars and total free amino acids, influencing the biochemical and nutritional attributes which are responsible for browning in guava fruit leather.

Keywords: Total free amino acids, ascorbic acid, browning, furfural, hydroxymethylfurfural, non-enzymatic and sugars

INTRODUCTION

Guava (*Psidium gujava L.*) a species of *Myrtaceae* family is cultivated widely around tropical and subtropical regions. It is known for pleasant flavour, refreshing taste and nutritional value. Guava is abundant in vitamins, especially vitamin C (ascorbic acid) other vitamins include vitamin A, thiamine, riboflavin, niacin, and pyridoxine (Kumari *et al*., 2017). Dietary fibres and bioactive compound contribute to prevention of chronic degenerative diseases (Blancas-Benitez *et al*., 2015). The fruit is also rich in considerable amounts of minerals *i.e*., phosphorus, calcium, iron (Kumari *et al*., 2017).

Guava fruits are often consumed fresh and are also suitable for processing into jelly, jam, juice, nectar, wine and fruit leather among other products (Kumari *et al*., 2017). Guava fruit leather is one among the popular processed products. Fruit leather is a dehydrated fruit-based confectionery dietary product which is often eaten as a snack or dessert. Fruit leathers are made by combining fruit puree with other ingredients such as sugar, pectin, acid, glucose syrup, colour, and potassium metabisulphite, then dehydrating them under controlled conditions.

Browning is an important biochemical reactions taking place during processing and storage of fruit leather. Browning not only affects the sensory attributes (colour; off flavour) but also deplete the nutritional quality. Decline in quality and color due to browning was the major hindrance in production of guava fruit leather (Singh *et al*., 2019). Similar claims were done for apple leather (Demarchi *et al*., 2013). Non-enzymatic browning is primarily caused by the Maillard reaction, caramelization, and ascorbic acid degradation at the product development stage by production of hydroxymethylfurfural (HMF) and

furfural (FUR) (Akyildiz *et al*., 2021). HMF and FUR could be used as the non-enzymatic browning indicators in dehydrated products (Kus *et al*., 2005). Specific sugars and amino acids, as well as their concentrations, play an important role in the Maillard reaction, determining the severity of browning, which is a reflection of the product's nutritional quality (Murata, 2021). In this regard, the role of different sugars (sucrose, fructose, glucose, and sorbitol) and their interactions with biomolecules in determining non-enzymatic browning in guava fruit leather was investigated.

MATERIALS AND METHODS

Raw material

This study employed firm ripe guava (cv. Arka Poorna) fruits produced from a guava plantation at the ICAR-Indian Institute of Horticultural Research in Bengaluru.

Preparation of leather

The selected guava fruits were washed thoroughly using potable water. Fruits were subjected to manual peeling, cut into halves, pulp was extracted in a laboratory grade pulper and seeds were removed by passing the pulp through a sieve. The extracted pulp without pasteurizing was incorporated directly with 15% sugars *viz*., sucrose, fructose, glucose and sorbitol in separate lots (treatments) followed by addition of 0.3 % citric acid and 700 ppm potassium metabisulphite to maintain the desirable acidity and as a preservative respectively. Further, the mixture was stirred gently for five minutes. The mixtures were spread on a tray and dried at 60 ± 5 °C in a cabinet dryer. The drying process continued till the moisture content reached ~15%. The guava leather sheets were cut into 8 x 4 cm bars and later subjected to various analyses.

Physico-chemical analysis

Moisture content was analyzed in a thermo-ventilated oven gravimetrically to obtain a consistent weight consecutively in three measurements at 12 h interval. Water activity was measured using an electric water activity meter (Rotronic Hydrolab, UK) at 25±2 °C. Titratable acidity was estimated by titrating against 0.1N NaOH with phenolphthalein as an indicator (AOAC,1990). Reducing and total sugars were estimated as suggested by Lane and Eynon (1923) as reported by Ranganna (1986). Non- reducing sugars was calculated from the difference between of total sugars and reducing sugars. Total free amino acid was estimated using ninhydrin reagent (Moore and Stein, 1948) and expressed as mg leucine/100g. The 2, 6-dichlorophenol indophenol dye technique was used to determine the vitamin C content suggested by Johnson (1948) and described by Ranganna (1986)**.** The total phenolic content was estimated as per Folin - Ciocalteu spectrophotometric method and expressed in gallic acid equivalent (mg GAE/100g) (Yilmaz *et al*., 2017). Ferric Reducing Antioxidant Potential (FRAP) was used to determine antioxidant activity (Ndou *et al*., 2019) and expressed in ascorbic acid equivalents (mg AAE/100g). Non-enzymatic browning was recorded by submerging the samples in 60 per cent ethanol overnight and reading the OD values at 440 nm (Ranganna, 1986).

Color

The color (*L* a* b* C* h°)* was measured using colorimeter (Model: Colour Reader, CR-10, Konica Minolta, Japan). Browning Index was calculated based on *L*a*b** co-ordinates. The browning index is generated using the following equation to capture this variance in a single index that is associated to a brown color. (Pathare *et al*., 2013)

$$BI = 100\,\frac{(X-0.31)}{0.17}$$

$$X = \frac{(a* + (1.75 \times L)) \times a*}{((5.645 \times L) + a* - (3.012 \times b))}$$

Furfural and hydroxymethylfurfural

To extract furfural (FUR) and hydroxymethylfurfural **(**HMF), 2g of material was homogenized in 15 ml of HPLC grade water. The extract was filtered using 0.45 µm nylon filters. The HPLC studies were carried out on a Shimadzu Series LC-20AT system (Shimadzu, Kyoto, Japan), which included a liquid chromatograph coupled to a UV-VIS detector (SPD-10A), binary pump (LC-10AT), auto sampler (SIL-20A HT), and LC solution Workstation software, Kinetex, column of dimension 250 x 4.6 mm, 5m C18 (Phenomenex, USA) was used, along with a security guard column made of the same material. Samples were injected using the auto sampler. At 32°C, the column and guard column were thermostatically controlled. The flow rate was 1 ml/min, and the mobile phase was 0.3

percent tetrahydrofuran. The instrument was operated in isocratic mode and elutants were detected at 280 nm. The retention time for HMF was 10.80 minutes, whereas the retention time for FUR was 11.64 minutes (Zhong-Fu *et al*., 2016). The values were expressed in ng/g.

Statistical analysis

The analysis was done in triplicates and the results were presented in Mean ± SE (standard error). One-way ANOVA was used to determine the CD of means and variance among different sugars. Duncan multiple range test (DMRT) was performed at $\alpha = 0.05$ level of significance of using R software.

RESULTS AND DISCUSSION

Physico-chemical composition of guava pulp

Table 1. Physico-chemical composition of fresh guava pulp

Colour	*L**	57.07
	*a**	3.20
	*b**	12.48
	*C**	12.89
	h°	75.61
Moisture (%)		84.15
Water activity		0.824
TSS (°Brix)		12.5
Titratable acidity (%)		0.4
Reducing sugar (%)		5.53
Total sugar (%)		9.77
Non-reducing sugar (%)		4.24
Total free amino acids (mg Leu/100g)		1.06
Ascorbic acid (mg/100g)		206.62
Total Phenols (mg GAE/100g)		591.67
Antioxidant Activity (mg AAE/100g)		1574.19

The Physico-chemical composition of the fresh guava (cv. Arka Poorna) pulp is given in Table 1.

Effect of different sugars on the properties of guava leather

Moisture content and water activity

The moisture content and water activity did not show any significant ($p>0.05$) difference among guava leather developed using different sugars (Table 2). The moisture content and water activity was ~15 and ~0.6 respectively. Moisture content in guava leather was in agreement with food safety and standards regulations, 2011 *i.e.*, not more than 20%. That moisture contents at15% and water activity of 0.6 is found to be safe with respect to microbiological activity and adverse biochemical and deteriorative reactions (Suna *et al*., 2014). In this regard the guava leather developed had acceptable moisture content and water activity levels.

Titratable acidity

The titratable acidity in guava leathers did not vary significantly among different sugars ($p > 0.05$). The values ranged from 1.62 ± 0.02 % to 1.70 ± 0.03% (Table 2).

Sugar

The sugar composition of guava leather is presented in Table 2. Total sugars values in guava leather ranged from 29.15 ± 0.31 to71.30± 1.19%. The highest total sugar was on par in sucrose (71.12 ± 0.84%), fructose (70.26 ± 0.57%) and glucose (71.30 ± 1.19%), and the lowest was found in sorbitol (29.15± 0.31%). As sorbitol is a sugar alcohol its addition even at 15% did not contribute to the total sugar content (Choi *et al*., 2013). Reducing sugars content varied significantly ($p > 0.05$) in guava leather as the base material used was different sugars. Guava leather with fructose (41.99± 0.86%) reported to have a highest reducing sugar which was statistically on par with glucose (41.21± 0.21%) and the lowest was recorded in sorbitol (13.07 ± 0.60%). Reducing sugars are capable of producing reactive carbonyl species (RCS) which aid in development of Maillard reactions products (Picouet *et al*., 2009) which further cause non-enzymatic browning. The highest non-reducing sugar was found in guava leather with sucrose (53.79 ± 0.49%) and the lowest in sorbitol (16.08± 0.51%). Sucrose has an acetal structure with anomeric carbons combined together by a glycosidic bond. This is a stable structure that cannot be oxidised.

Total free amino acids

Incorporation of different sugar in guava leather had a significant ($p>0.05$) impact on total free amino acids (TFAA) (Table 2). Guava leather with sorbitol (2.91± 0.02 mg/100g), which was on par with sucrose

(2.86±0.05mg Leu/100g), had the highest TFAA, while fructose (2.26± 0.02 mg Leu/100g), which was on par with glucose (2.32± 0.09 mg Leu/100g), had the lowest. The decline in TFAA was found to be higher in guava leather incorporated with fructose and glucose; this is due to differential reaction between amino acids and RCS, resulting in the production of a variety of Maillard reaction products depending on the affinity and reactivity of individual amino acids. Among the amino acids, leucine, glutamic acid, tryptophan and lysine contributed more for Maillard reaction. Leucine, alanine, aspartic acid, glutamic acid and glycine was comparatively found high in guava fruit (Chen *et al*., 2007).

Ascorbic acid

Ascorbic acid (Vitamin-C) plays an important role in human nutrition due to its antioxidant nature (Cruz *et al*., 2009). It is thermo-labile and considered as a quality indicator in dehydration process (Ali *et al*., 2016). Guava leather developed using different sugars showed significant ($p>0.05$) difference in of ascorbic acid levels (Table 3.) The highest ascorbic acid level was found in sorbitol (136.13 ± 3.27mg/100g) which was statistically on par with sucrose (132.47± 2.38 mg/100g), while the lowest was found in fructose (116.7± 1.50mg/100g) and glucose (119.64± 0.60 mg/100g). Ascorbic acid would have been degraded to dehydroascorbic acid, then hydrolyzed to 2,3-diketogulonic acid, and lastly polymerized as a result of the Maillard reaction product, which is catalysed by multiple oxidation and reduction processes involving reducing sugars (Chuah *et al*., 2008) Mango juices with the highest glucose: fructose ratio showed decreased ascorbic acid concentration (Pithava and Pandey, 2018). Furthermore, amino acids have the ability to act as catalytic agents in the decomposition of ascorbic acid (Shinoda *et al*., 2005). According to Yu *et al*. (2017), the interaction of ascorbic acid with lysine, arginine, and histidine was more important in the synthesis of browning pigments.

Total phenols

The total phenols content of guava fruit leathers showed significant ($p>0.05$) difference among different sugar source (Table 3). The highest total phenols were found in Sorbitol (436.23 ± 12.2 % mg GAE/100 g) and sucrose (427.95 ± 6.61 mg GAE/100g). whereas, fructose, and glucose significantly reported low values for total phenol content of 392.09 ± 2.85and 410.87 ± 2.11mg GAE/100g respectively. The degradation of total phenols was high in samples with fructose and glucose. Phenols are also common substrates for Maillard reaction (Amaya-Farfan and Rodriguez-Amaya, 2021). This browning reaction also involves various oxidation and reduction process which will degraded the total phenol content severely. In addition to this, the RCS formed by reducing sugars bind to phenols and make them biologically unavailable.

Antioxidant activity

Varying the sugar forms had significantly different antioxidant activity in guava fruit leathers ($p>0.05$) (Table 3). Sorbitol (1,146.20± 41.02 mg AAE/100g) had the highest antioxidant activity, which was on par with sucrose (1,086.35 ± 35.13 mg AAE/100g). The samples with fructose (935.97 ± 9.81 mg AAE/100g) and glucose (949.36 ± 6.30mg AAE/100g) significantly deprived the antioxidant activity. Ascorbic acid and phenolics contribute the lion share of antioxidant activity (Eyiz *et al*., 2020). It can be inferred that guava leather processed using fructose and glucose resulted in highest degradation of ascorbic acid and loss of phenols and thus adversely affected the antioxidant activity of the guava leather.

Color:

L* a* b*

The colour values of guava fruit leather are presented in Table 4. The lightness (L^*) values varied significantly among the different guava leather developed using different sugar sources. The highest lightness was reported in samples containing sorbitol (61.40± 0.78) and the lowest values were reported in sucrose (58.90±0.72), which was on par with glucose (58.70 ± 0.66), and fructose (58.27± 0.35). The decrease in the L^* values indicates the product is comparatively darker, this occurred in the samples with reducing sugars (fructose and glucose) and the highest luminance was reported in guava leather containing sorbitol. The redness (a^*) values varied significantly among the different guava leather developed using different sugar sources. Redness indicates the occurrence of browning in the product. The highest redness was reported in samples containing fructose (4.63± 0.46) and the lowest values were reported in sorbitol (3.13 ± 0.12). The highest yellowness (b^*) was reported samples containing fructose (34.37± 0.25) which was found on par with

Table 2. Physico-chemical composition of guava leather

Treatment	Moisture (%)	Water activity	Reducing Sugar (%)	Non - Reducing Sugar (%)	Total Sugar (%)	Titratable acidity (%)	Total free amino acids (mg Leu/100g)
Sucrose	15.23[a] ± 0.22	0.672[a] ± 0.01	17.32[b] ± 0.13	53.79[a] ± 0.86	71.12[a] ± 0.84	1.62[a] ± 0.04	2.86[a] ± 0.05
Fructose	15.38[a] ± 0.12	0.677[a] ± 0.01	41.99[a] ± 0.86	28.27[b] ± 1.43	70.26[a] ± 0.57	1.65 [a]± 0.02	2.26[b] ± 0.02
Glucose	15.08[a] ± 0.17	0.665[a] ± 0.01	41.21[a] ± 0.36	30.09[b] ± 1.19	71.30[a] ± 1.19	1.70[a] ± 0.05	2.32[b] ± 0.09
Sorbitol	15.28[a] ± 0.03	0.675[a] ± 0.01	13.07[c] ± 0.60	16.08[c] ± 0.51	29.15[b] ± 0.31	1.65[a] ± 0.35	2.91[a] ± 0.02
C.D.	NS	NS	1.07	2.01	1.52	NS	0.18
SE(m)	0.09	0.004	0.32	0.61	0.46	0.10	0.05

Note: Mean values followed by different letters in the same column differs significantly (α = 0.05 level).

Table 3. Functional attributes of guava leather

Treatment	Ascorbic Acid (mg/100g)	Total Phenols (mg GAE/100g)	Antioxidant Activity (mg AAE/100g)
Sucrose	132.47[a] ± 2.38	427.95[a] ± 6.61	1,086.35[b] ± 35.13
Fructose	116.70[b] ± 1.50	392.09[c] ± 2.85	935.97[c] ± 9.81
Glucose	119.64[b] ± 0.60	410.87[b] ± 2.11	949.36[c] ± 6.30
Sorbitol	136.13[a] ± 3.27	436.23[a] ± 12.2	1,146.20[a] ± 41.02
C.D.	4.16	13.72	52.71
SE(m)	1.26	4.14	15.92

Note: Mean values followed by different letters in the same column differs significantly (α = 0.05 level).

Table 4. Color (*L* a* b* C* h°*) and non-enzymatic browning (NEB) in guava leather

Treatment	Color						NEB
	L	a	b	C	H	Browning Index	
Sucrose	58.90[a]±0.72	3.43[b]± 0.15	34.20[a]± 0.26	34.00[a]± 1.75	84.53[a]± 0.47	84.61[b] ± 1.97	0.193[c] ± 0.01
Fructose	58.27[a] ± 0.35	4.63[a] ± 0.46	34.37[a] ± 0.25	34.43[a]± 0.58	83.00[b]± 0.36	90.58[a] ± 0.82	0.232[a] ± 0.01
Glucose	58.70[a]± 0.66	3.83[b]± 0.06	34.13[a]± 0.41	34.27[a] ± 0.46	84.07[a]± 0.25	89.40[a] ± 0.98	0.211[b] ± 0.01
Sorbitol	61.40[b]± 0.78	3.13[bc]± 0.12	33.07[b]± 0.15	32.93[b]± 0.21	84.47[a]± 0.23	77.72[c] ± 1.74	0.181[d] ± 0.01
C.D.	1.24	0.48	0.54	1.01	0.66	1.99	0.011
SE(m)	**0.37**	0.15	0.16	0.30	0.20	0.60	0.003

Note: Mean values followed by different letters in the same column differs significantly (α = 0.05 level); Browning index was estimated using average values of L*, a*, b*

sucrose (34.20 ± 0.26) and glucose (34.13 ± 0.41) and lowest values were reported in sorbitol (33.07 ± 0.15). Lower *L** values and high *a** and *b** values will indicate the intensity of browning. Decreasing *L** values in combination with decreasing *b** values, indicating the occurrence of mild browning due to non-enzymatic browning (Korley *et al*., 2015). In guava leather yellowness indicate the undesirable colour change towards browning. In addition to it *a** values also significantly contribute to non-enzymatic browning.

Cand **h**°*

Chroma values indicate the purity of color, in guava leather fructose had the highest value indicating more browning attributes (low-lightness; high-redness; high-yellowness). The Chroma (*C**) values varied significantly among the different guava leather developed using different sugar sources (Table 4). The highest Chroma was reported samples containing fructose (34.43±0.58) which was found on par with sucrose (34.00 ±1.75) and glucose (34.27± 0.46) and lowest values were reported in sorbitol (32.93±0.21). The Hue (*h*°) values varied significantly among the different guava leather developed using different sugar sources. The highest hue value was reported in samples containing sucrose (84.53±0.47) which was found on par with sorbitol (84.47±0.23) and glucose (84.07±0.25) and lowest values were reported in fructose (83.00± 0.36). Lower hue value indicates redder colour of the product (Korley *et al*., 2015). The shift in hue values from 90 to 0° indicate change in color from yellow to red, which was predominant in fructose followed by glucose containing samples. Hue angle ~90°suggests that the product has more yellowness than redness (Pedisic *et al*., 2009)

Browning index (BI)

To determine the change in visual quality, colour co-ordinates (*L* a* b**) were utilized to derive browning index. BI aid in determining the degree of brown colour occurred during dehydration. BI changed between 77.72 ± 1.74and 90.58 ± 0.82 with different sugars (Table 4). The highest BI in leather was recorded in sample containing fructose (90.58 ±0.82) and glucose (89.40 ± 0.98). As discussed earlier and supported by literature, reducing sugars play an important role in determining the colour of the final product as they are the potential source of reactive carbonyl species which contribute significantly to Maillard reaction (Fu *et al*., 2020; Calin-Sanchez *et al*., 2020). The total free amino acids also decreased in guava leather containing fructose (2.26 ± 0.02) and glucose (2.32 ± 0.09) (Table 3). As a result, these reactive carbonyl species and amino acids are likely to have interacted to form various Maillard reaction products, resulting in greater BI in fructose and glucose samples. Furthermore, ascorbic acid degradation in guava leather has contributed to the creation of HMF, which in turn produces Maillard reaction product which caused the browning. Yu *et al.* (2017) reported that the degree of browning was only related to the total amount of L-ascorbic acid in the reaction system. Similar results were observed in citrus and apple juices (Burdurlu *et al*., 2006).

Non-enzymatic browning (NEB)

The absorbance at 440 nm is commonly used to determine the degree of browning in a non-enzymatic browning reaction, often caused by Maillard reaction (Paravisini and Peterson, 2016). NEB indicates the intensity of browning in processed product through spectrometric OD values. NEB values were reported between 0.232 ± 0.01 and 0.181± 0.01in guava fruit leather (Table 4). Among different sugars investigated, highest NEB values were recorded in fructose (0.232±0.01) and glucose (0.211±0.01) treated samples and the lowest was reported in sorbitol (0.181±0.01) and sucrose (0.193±0.01). Degradation of ascorbic acid (Table 3) and production of reactive carbonyl groups from the reducing sugars (Table 2) contributed to higher NEB values in guava leather. Browning is complex biochemical reaction which involves numerous biological compounds to take part in the reaction to yield varied degree of browning in processed products. Our results were in confirmation with, Paravisini and Peterson, (2016) who reported decomposition of sugars under acidic conditions to form reactive intermediates. Major mechanisms, being ascorbic acid degradation, acid-catalyzed sugar degradation, and Maillard reactions, have been identified as the main reaction pathways responsible for NEB (Bharate and Bharate, 2014). Maillard reaction rate is highest in intermediate moisture foods with water activity range of 0.5 - 0.7 (Malec *et al.*, 2002). The physico chemical composition of guava leather mentioned in Table 2, 3 and 4 shows that in this product all above mentioned favorable environment for browning reactions were present.

Furfural and hydroxymethylfurfural

Maillard reaction products such as Furfural (FUR) and Hydroxymethylfurfural (HMF) are considered as the biochemical markers for non-enzymatic browning (ErtekinFiliz and Seydim, 2018). Among the two Maillard products, HMF (32.3 -12.8 ng/g) content was found to be higher than FUR (0.95-0.29 ng/g) in all guava leather samples (Fig. 1; Table 5). HMF production occur in product high in reducing sugar *i.e.*, fructose and glucose, whereas FUR production occur in xylose and arabinose rich product (Machado *et al*., 2016). Among the treatments, guava leather with fructose and glucose reported remarked higher HMF of 32.3 and 29.3 ng/g respectively than sucrose **(**14.32 ng/g**)** and sorbitol (12.8 ng/g) treated samples. The ascorbic acid degradation in guava fruit leather containing fructose and glucose (Table 3) and production of RCS for maillard reaction has also contributed to HMF formation (Chen *et al*., 2022). Similar results were found in apple leather (Ruiz *et al*., 2012) reporting degradation of ascorbic acid caused higher levels of HMF which in turn produced brown pigments (Helyes *et al*., 2006).

Table 5. Biochemical markers of non-enzymatic browning in guava leather

Treatment	Furfural (ng/g)	Hydroxymethylfurfural (ng/g)
Sucrose	0.33	14.32
Fructose	0.95	32.3
Glucose	0.73	29.3
Sorbitol	0.29	12.8

Note: The values presented are mean values of two replicates

Besides being identified as thermal processing indicator, HMF is instrumental in imparting certain typical flavors to the food products. However, the toxicity of compound has been much discussed as a carcinogen (Severin *et al*., 2010). The estimates of HMF for human daily intake range from 2 to 150 mg/person (Capuano and Fogliano, 2011). It is understood from this study that HMF generation couples with loss of nutrients such as ascorbic acid and so the antioxidant activity of the guava leather. Therefore, it is advisable to treat HMF as a nutritional quality indicator in guava leather and to lay down a permissible limit as a part of implementation of food standards.

CONCLUSION

This study revealed the effects of different sugars (Fructose, glucose, sucrose and sorbitol) and their role in non-enzymatic browning and antioxidant activity in guava leather. The application of different sugars during the product development affected the colour (*L*, a*, b*, C*, h°*, Browning Index), total free amino acids, ascorbic acid, total phenols, antioxidant activity, NEB, furfural and hydroxymethylfurfural. Highest losses in nutritional attributes such as total free amino acids, ascorbic acid, total phenol and antioxidant activity was found in guava leather incorporated with fructose and glucose and the least in sorbitol which wasfollowed by sucrose. While, the colour values *i.e.*, highest *L**and *h°,* lowest *a* b**, and *C**values, lowest browning index and lowest NEB were found superior in sorbitol and sucrose followed by fructose and glucose. Among the biochemical markers for NEB, HMF was found to be predominant than FUR and was found in high level in fructose followed by glucose, sucrose and sorbitol. Therefore, from this study it was evident that sugar composition and its concentration

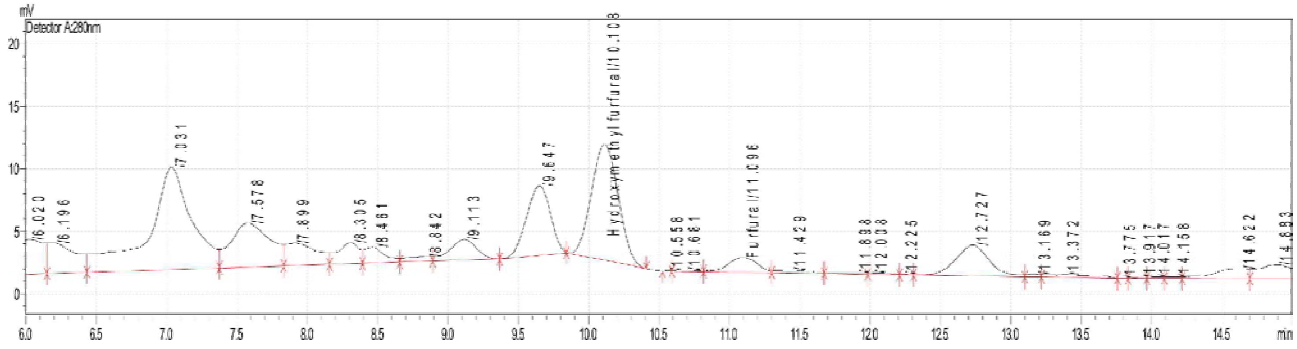

Fig. 1. Chromatogram showing hydroxymethylfurfural (10.32) and furfural (11.38) as biochemical markers in NEB in guava leather

in guava leather play a significant role in non-enzymatic browning. Use of optimal non reducing sugar, least reducing sugar and their combinations will aid in minimizing the browning and preserving functional attributes of dehydrated product.

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Conflict of Interest

The authors have declared no conflicts of interest for this article.

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Original Research Paper

Effect of modified atmosphere package on physico-chemical properties of pomegranate (*Punica granatum* L.) fruits

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ABSTRACT

Pomegranate is an important table and processed fruit owing to its nutritional quality. Extending the fruit life of the plant is very much limited owing to its metabolic activities *viz*., respiration, transpiration and microbial infection. An experiment was conducted to investigate the effect of different packaging materials on physico-chemical properties of pomegranate fruits during storage. Fruits were harvested with stalk and washed with sodium hypochlorite, air dried and graded. Fruits were stored under modified atmospheric packaging conditions using different packaging materials *viz*., polyethylene bag, polypropylene bag, Xtend® bag and silver nano bag Hima Fresh®. Fruits without package served as controls. Fruits were stored at low temperature 7±2 °C and 90±5 % RH. MAP treated fruits had higher quality parameters across all packaging treatments. PLW and respiration rate increased while, moisture content, colour, texture and acidity decreased with prolonged storage, but the rate of decrease was highest in unpacked fruits. MAP maintained the quality of pomegranate fruits upto 100 days compared to unpackaged fruits (40 days). Shelf life of stored fruit at ambient condition was 4 to 5 days. Fruit decay was 12 % in polyethylene whereas it was 6 % in Xtend® bag at the end of 100 day of storage.

Keywords: Decay percentage, MAP, pomegranate, shelf life and storage life

INTRODUCTION

Pomegranate (*Punica granatum* L.), one of the most favorite table fruits is native to Persia. The crop is very hardy and thus thrives well under arid and semi-arid climatic conditions. During the last two decades, the area under pomegranate cultivation is increasing substantially and many growers have taken up it as commercial farming due to the fact that the fruit satisfies the nutritional and medicinal needs of the consumer as the fruits have potent anti-mutagenic, anti-hypertensive, anti-inflammatory properties and ability to reduce liver injury (Holland *et al.,* 2009). In spite of several benefits, the fruit consumption is not to the expected consumption and the availability of pomegranate fruit in the market are largely restricted to the harvesting season due to a high demand and lack of appropriate post-harvest technology to extend the storage life and maintain fruit quality (Erkan and Kader, 2011). Pomegranate being a non-climacteric fruit can be stored for few days under ambient conditions, but has potentiality to be stored for longer duration. But, long-term storage of pomegranate fruit has often been limited by weight loss, decay development, husk scald, loss of aril quality and taste (Porat *et al*., 2016). However, modified atmosphere packaging (MAP) has been found to be successful in reducing water loss, visible shriveling symptoms, husk scald and decay of pomegranate fruit during cold storage, but improper use of MAP will have negative impact (Artés *et al*., 2000; Selcuk and Erkan, 2015). MAP bags have been widely used for pomegranate storage and shipping in

pomegranate exporting countries. MAP is most widely used technology to alter the gas composition in package in passive approach. This is achieved by the interaction between the respiration rate of the produce and the transfer of gases through the packaging material (Mahajan *et al*., 2007). In MAP, respiration rate is reduced by increasing CO_2 and decreasing O_2 concentration. MAP for pomegranates has been shown to reduce weight loss, shrinkage, scald development, decay, delay senescence and maintain post-harvest fruit quality of pomegranates (Selcuk and Erkan, 2014). The present study aims at extending the storage life of pomegranate fruits by application of modified atmosphere and humidity using different packaging materials.

MATERIAL AND METHODS

The pomegranate fruits *cv*. Bhagwa were hand-harvested at ripe stage with 0.5 cm stalk intact and were graded based on the uniformity. Fruits were washed in 150 ppm sodium hypochlorite. Later, the fruits were washed in running tap water and air dried to remove surface water. Fifteen to twenty uniform fruits weighing 4-5 kg were packed in modified atmosphere package bags *viz.,* polyethylene bag (T_1), polypropylene bag (T_2), Xtend® bag (T_3) and silver nano bag Hima Fresh® (T_4) along with control unpack (T_5) and kept in corrugated fiberboard boxes as per treatment and stored at low temperature 7±2° C and 90 ± 5 % relative humidity (Fig.1). Experiment was carried out using completely randomized design with five treatments and five replications.

Data were recorded till the termination of experiment through numbering for all non-destructive parameters. The weight loss was recorded by using 10 mg precision electronic weighing balance (Make: Sartorius GmbH, Gottingen, Germany, Model: GE812). The PLW was calculated using standard formula and expressed as per cent. Fruit colour was measured using an instrument portable colorimeter spectrophotometer (Lovibond LC 100, Model RM200, The Tintometer Ltd, Salisbury, UK). Fruit firmness evaluation was carried out by piercing 5 mm cylindrical probe at a speed of 2 mm/s with automatic return. The downward penetration at 5 mm is pre-test speed and post-test speed were1 and 10 mm/s, respectively using texture analyzer (Model TA HD plus; Make Stable Microsystems, UK) equipped with

Fig. 1. Effect of MAP bag showing MA/MH condition during storage of pomegranate fruits

a 50 kg load cell. Finally, the data were analyzed statistically. The respiration rate was measured by piercing the probe of an auto oxygen/ carbon di-oxide analyzer (Make: Quantek, Model: 902D Dual track) and was calculated using the following formula.

$$\text{Respiration rate (mg } CO_2 \text{ kg}^{-1} \text{ h}^{-1}) = \frac{\%\ CO_2 \times \text{Container volume (ml)} \times 60}{\text{Fruit weight (kg)} \times \text{Enclosing time (min)} \times 100}$$

The titratable acidity of pomegranate fruits sample was determined by visual titration method following the protocol (Ranganna, 1986). The fruit decay incidence was visually assessed by counting the total number of rotten fruits. For both external and internal decay, percentage of discarded fruits was calculated using the following formula.

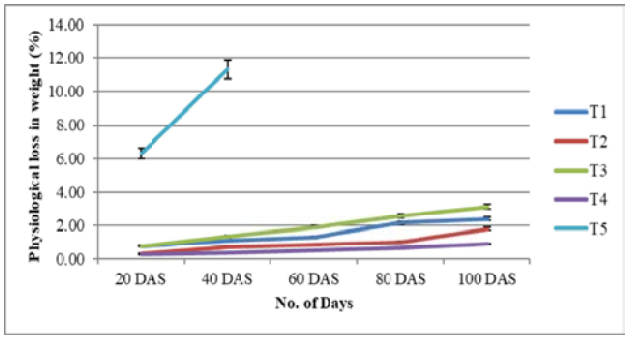
$$\text{Decay incidence (\%)} = \frac{\text{Number of discarded fruits at each sampling date}}{\text{Total number of fruits in treatment}} \times 100$$

The number of days in which the fruits were in acceptable condition was taken as the storage life or keeping quality of fruits. The fruits were removed from

bags and kept in ambient condition at 25±5° C to stimulate the commercial handling operations and to determine the shelf life.

RESULTS AND DISCUSSION

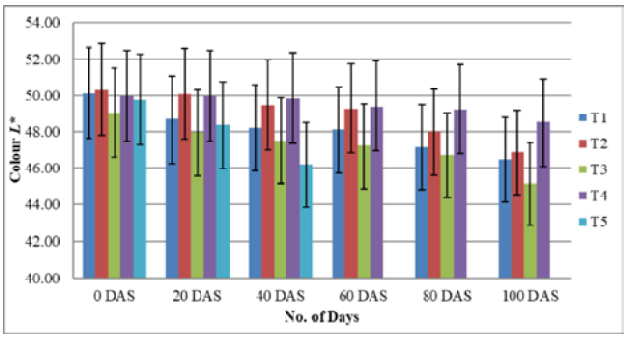
The PLW significantly increased with prolonged storage in all the treatments (Fig. 2). However, PLW was maximum in the control fruits as there was 11.32 per cent loss in weight at 40 days of storing while, the PLW was 0.87 and 3.12 % in the fruits packed in silver nano bag Hima Fresh® and Xtend® bag, respectively even after 100 days of storage. It can be assumed that the packaging materials act as barriers to moisture loss by way of establishing a micro-environment with high relative humidity similar to fruit moisture content 80-85 per cent causing a very low vapour pressure difference between fruits and external environment. All these factors help in slowing down the respiration and transpiration rates. The present results are in confirmation with the previous findings of Nanda *et al.* (2001) and Porat *et al.* (2016).



T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

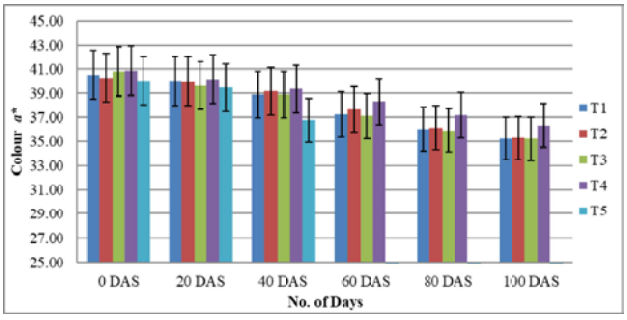
Fig. 2. Effect of modified atmosphere package on physiological loss in weight (%) of pomegranate fruits under low temperature storage (7±2° C)

Pomegranate fruits at the final stage of senescence become less intense and this characteristic visual change seems to be associated with a gradual decrease in the parameters: *L**, *a** and *b** (Fig. 3a, 3b and 3c). Decrease in *L**, *a** and *b** values was found to be more prominent in control unpacked fruits indicating the change in colour of pomegranate fruits from red to brown colour. Minimum colour change was observed in fruits packed in silver nano bag while, highest color change was observed in control. Minimum colour change in MAP fruits might be attributed to minimum moisture loss and lesser rate



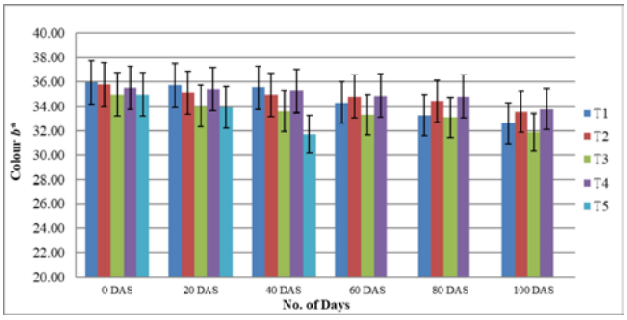
T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

Fig. 3a. Effect of modified atmosphere package on colour value (*L**) of pomegranate fruits under low temperature storage (7±2° C)



T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

Fig. 3b. Effect of modified atmosphere package on colour value (*a**) of pomegranate fruits under low temperature storage (7±2° C)

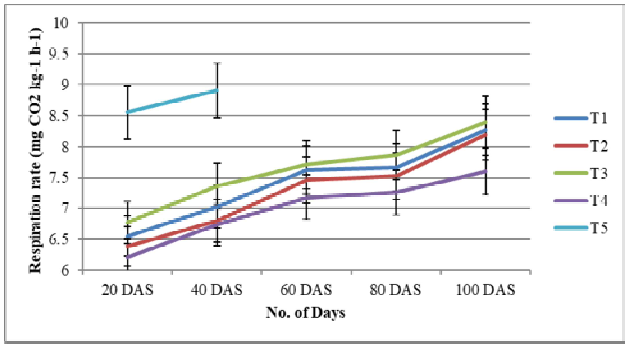


T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

Fig. 3c. Effect of modified atmosphere package on colour value (*b**) of pomegranate fruits under low temperature storage (7±2° C)

of respiration delaying senescence in pomegranate fruits. Naik *et al.* (2017) also reported that the Hunter color (L*, a* and b*) values of pomegranate fruits gradually decreased with each successive storage period.

From the data (Fig.4), it was observed that the respiration rate of pomegranate fruits packed in modified atmosphere packaging materials decreased initially after harvest but increased gradually with

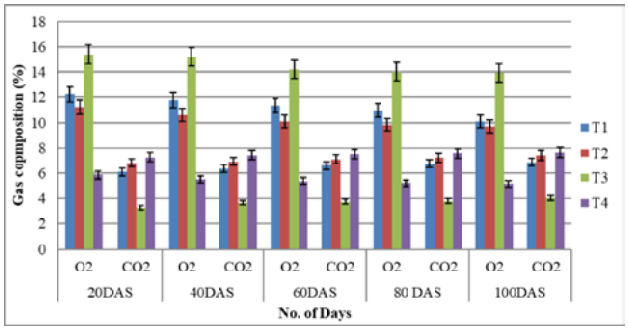


T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage; Initial respiration rate: 18.17 mg CO_2 kg^{-1} h^{-1}

Fig. 4. Effect of modified atmosphere package on respiration rate (mg CO_2 kg^{-1} h^{-1}) of pomegranate fruits under low temperature storage (7±2° C)

prolonged storage. Significant difference was recorded with respect to respiration rate in the control fruits at 20 and 40 days after storage while, no difference was noticed at 60, 80, and 100 days among MAP packed fruits. The increase in respiration rate of fruits during storage could be an indication of increase in stress including presence of physiological disorders and metabolic reactions. Low respiration in MAP bag fruits may be due to lower moisture loss, lower stress and low availability of O_2 inside the package and maximum accumulation of CO_2 in the bags. The above ûndings agree with Meighani *et al*. (2014) and Mphahlele *et al.* (2016).

The results on changes in headspace oxygen and carbon di-oxide concentration as influenced by modified atmosphere packing of pomegranate fruits during storage are presented in Fig. 5. As the storage period progressed, the oxygen concentration was significantly decreased and carbon dioxide increased due to respiration. The highest oxygen (13.92 %) and

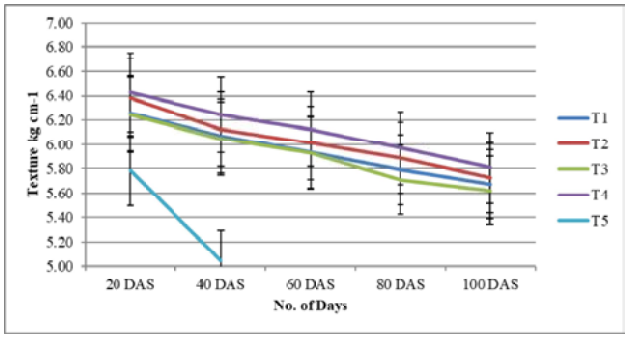


T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

Fig. 5. Effect of modified atmosphere package on gas composition inside MAP bags of pomegranate fruits under low temperature storage (7±2° C)

lowest carbon dioxide (4.04 %) concentration was recorded in Xtend® bag while, the lowest oxygen (5.18 %) and highest carbon dioxide (7.68 %) were recorded in silver nano bag at 100 days after storage compared to other treatments. This might be due to the lower permeability to gases by the silver nano bag compared to Xtend® bag which had micro-perforation resulting in optimum water vapor and gas transmission rate. Our results agree with Mphahlele *et al*. (2016) and Mshraky *et al*. (2017).

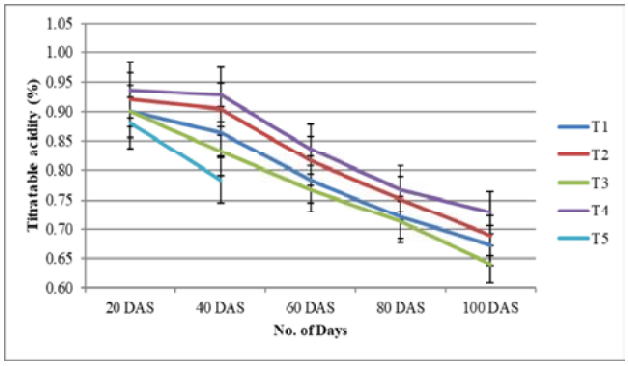
The firmness of pomegranate fruits decreased significantly with prolonged storage period (Fig. 6). However, the pomegranate fruits which were packed by silver nano bag recorded highest firmness (6.24 kg cm^{-1}) on day 40 than other treatments while, control recorded lower firmness (5.04 kg cm^{-1}) on the same day of storage. With prolonged storage, the firmness of the fruit was decreased. Silver nano bag maintained higher firmness (5.81 cm^{-1}) while, lower firmness (5.62 kg cm^{-1}) was observed in Xtend® bag after 100 days of storage. This could be attributed to slow degradational changes during initial period and also less moisture loss in MAP fruits maintaining better firmness compared to control. These results agree with Mshraky *et al.* (2016) and Kumar *et al.* (2013).



T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack)
DAS: days after storage; Initial texture: 6.51 kg cm^{-1}

Fig. 6. Effect of modified atmosphere package on texture (kg cm^{-1}) of pomegranate fruits under low temperature storage (7±2° C)

Data pertaining to the effect of different treatments on titratable acidity are presented in Fig. 7. Significant difference was recorded among treatments at 40 days after storage. The least (0.78 %) acidity was recorded in unpacked control fruits whereas maximum (0.93 %) acidity was recorded in silver nano bag. Thereafter, no difference was observed till 100 days indicating constant titratable acidity in MAP pomegranate fruits which might be due to reduction in metabolic

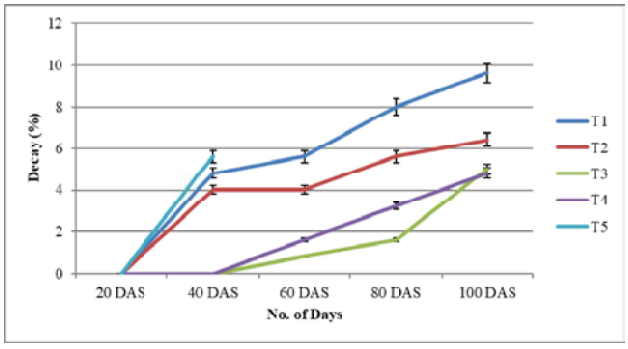


T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage; Initial titratable acidity: 0.95 per cent

Fig. 7. Effect of modified atmosphere package on titratable acidity percentage of pomegranate fruits under low temperature storage (7±2° C)

changes of organic acid into carbon dioxide and water as reported by Arendse *et al.* (2014) Nanda *et al.* (2001).

The decay percentage was significantly increased with extended storage as depicted in the Fig. 8.



T_1- Polyethylene bag; T_2- Polypropylene bag; T_3- Xtend® bag; T_4- Silver nano bag; T_5- Control (unpack) DAS: days after storage

Fig. 8. Effect of modified atmosphere package on decay percentage of pomegranate fruits under low temperature storage (7±2° C)

There was no microbial decay of fruits at 20 days after storage irrespective of treatments. The highest decay (7.00 %) was noticed in unpacked fruits while there was no decay in silver nano bag and Xtend® bag fruits at 40 days of storage. Thereafter, control fruits were terminated. Later, minimum decay of 1, 2 and 6 per cent was recorded in fruits packed in Xtend® bag at 60, 80and 100 days after storage, respectively whereas maximum per cent decay of 7, 10 and 12 was observed in fruits packed in polyethylene bag at 60, 80and 100 days of storage, respectively. This high decay incidence might be due to high availability of moisture inside the package providing congenial environment for the growth of micro-organisms. Xtend® bag reduced decay on account of the modified atmosphere and modified humidity which might be due to moisture vapor and gas transmission preventing accumulation of moisture. Our results are in confirmation with that of Samar *et al.* (2017).

The pomegranate fruits packed with different packaging materials delayed metabolic activity and increased shelf life. The pomegranate fruits packed in MAP bags had 100 days of storage life compared to unpacked fruits which recorded only 40 days (Table 1). Shelf-life simulation of stored fruits (7±2° C) at ambient condition (25±5° C) was 4 to 5 days. The increase in storage life might be due to reduced metabolic activity and optimum gas and water vapor transmission rate in MAP bags creating optimum conditions along with low temperature storage for fruits.

Table 1. Effect of modified atmosphere package on storage and shelf life of pomegranate fruits under low temperature storage (7±2° C)

Treatment	Storage life (Days) at low temperature (7±2° C)	Shelf life (Days) at ambient condition (25±5° C)		
		60 DAS	80 DAS	100 DAS
T_1- Polyethylene bag	100	5.00	4.50	4.00
T_2- Polypropylene bag	100	5.00	5.00	4.00
T_3- Xtend® bag 100	5.00	4.50	4.00	
T_4- Silver nano bag	100	5.00	5.00	4.00
T_5- Control (unpack)	40	—	—	—

DAS: Days after storage —: End of storage life

CONCLUSION

Pomegranate fruits packed with different MAP bags and stored in low temperature (7±2° C and relative humidity of 90±5 %) were found to have prolonged the storage and shelf life. The MAP bags such as polyethylene, polypropylene, Xtend® bag and silver nano bag (Hima fresh®) had shown to increase the storage life upto 100 days with minimum losses of (6 %) microbial decay in case of Xtend® bag whereas, unpacked (control) fruits had a storage life of only 40 days.

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Original Research Paper

The use of brick-walled evaporative cooler for storage of tomato

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ABSTRACT

A cost-effective alternative to cold storage is the brick-walled evaporative cooler (BEC). The effects of BEC on mature green and breaker 'Diamante Max' tomatoes were assessed. Two trials were carried out at ambient conditions with (i) 27.13±0.78 °C and 80.89±4.47%RH; (ii) 26.93±0.87 °C and 80.05±5.20% RH and with BEC (i) 25.49±0.58 °C and 99.90±0.10% RH; (ii) 25.42±0.90 °C and 97.75±3.25% RH. BEC-stored tomatoes showed 10.36% lesser weight loss, lesser decay incidence, redder color and better visual quality compared to control fruit. The higher L* and hue of around 90 in ambient-stored tomatoes indicated a lighter color as fruit turned to yellow compared to a lower L* and hue in BEC indicating a darker red color. An increased chroma was recorded as fruit turned from green or breaker to yellow, orange, or light red while the values of a* became negative. The BEC maintained the firmness and total soluble solids, especially in mature green tomatoes. After 49 days of storage, 61.8% of the fruit stored in the BEC were marketable compared to 23.3% in ambient conditions. The BEC system showed 27.16% higher annual benefit over cost than the ambient storage conditions. Thus, the BEC system can potentially maintain the quality of 'Diamante Max' tomatoes.

Keywords: Brick-walled evaporative cooler (BEC), color, quality, *Solanum lycopersicum* and storage

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important and widely grown crop in the Philippines (Philippine Statistics Authority, 2019). Tomato fruit is a climacteric fruit, and its stages of maturity or ripeness are measurable through its color from green mature to red stage (Quinet *et al*., 2019). Ripening of tomato is also associated with the fruit maturity stage and physico-chemical properties such as firmness, fresh weight loss (Tilahun *et al*., 2017a), polyphenol content, and antioxidant scavenging activity (Anton *et al*., 2017). Maintaining the good postharvest quality of tomatoes during storage is a big problem in developing tropical countries. Tomato fruit metabolizes faster at high temperatures during the postharvest stage leading to shortened shelf life (Liberty *et al*., 2017).

The evaporative cooling system uses a process that can maintain a low temperature and higher relative humidity storage conditions as heat is removed from the ambient environment with evaporation (Vanndy *et al*., 2008). This storage system has been tested on tomato varieties in Combodia and Laos (Vanndy *et al*., 2008); and sweet pepper in the Philippines (Bayogan *et al*., 2017; Majomot *et al*., 2019). The above studies have demonstrated the promising effects of the evaporative cooling storage system on the maintenance of the postharvest qualities of some crops. The brick-walled evaporative cooler (BEC) is a simple type of evaporative cooling system. The BEC is made up of a double wall of clay bricks with a moistened jute sack-covered wooden frame over the structure (Vanndy *et al*., 2008). The double-walled BEC has a 10-20 cm space between the walls filled with sand or sawdust being kept moist during use. This brick-walled evaporative cooling technology is known to be cost-efficient and easy to construct. The study aimed to evaluate the quality of tomatoes using the brick-walled evaporative cooler as a storage system and to determine the cost and benefit of its use relative to ambient storage.

MATERIAL AND METHODS

Experimental materials

Newly harvested tomatoes (*cv*. Diamante Max) were procured from a commercial farm in Calinan, Davao City. Fruits of uniform and good quality at two maturity stages, mature green and breaker, were used in the experiment. Storage and quality evaluation were done at the Postharvest Biology Laboratory, University of the Philippines Mindanao (UP Mindanao) in Mintal, Tugbok District, Davao City from November 2018 to January 2019, and January 2020 to March 2020 for the first and second trials, respectively.

The brick-walled evaporative cooler (BEC) construction was done at the UP. The BEC has dimensions of 76 in L x 32 in W x 26 in H outer brick wall, 70 in L x 26 in W x 26 in H inner brick wall, and a 3 in space between the two walls which is filled with sand. The faucet connected to the pipes was slightly turned on for 30 min for twice a day to moisten the sand. The covering of BEC at the top was made of a jute sack framed with bamboo. The dimensions of the cover for BEC was 78 in L x 30 in W. The sand and jute sack covering were moistened regularly during the use of the BEC.

Two trials were conducted to assess the quality of tomato at two maturity stages, mature green and breaker, and stored in the BEC or ambient room conditions. A total of 800 medium tomatoes of uniform quality were selected. A total of 400 fruit were used for each storage condition at 200 tomatoes per maturity stage. In the second trial, 86.4 kg of medium-size tomatoes of uniform quality were used. Sample tomatoes at 945±33 g were packed in a net bag for the various data parameters. A total of 24 net bags were used for each maturity stage and stored in BEC or ambient conditions. In both trials, fruit samples were disinfected with 200 mg/L NaOCl solutions for 3 min and air-dried before holding in the BEC or ambient conditions.

Data collection

The relative humidity (RH), temperature in BEC and ambient storage conditions were recorded using digital data loggers. The digital data loggers used were TinyTag Ultra 2 TGU-4500 (Gemini Data Loggers Ltd., England) and Elitech USB Temperature Data Logger (RC-5, UK. Ltd.).

Fruit weights were taken at 0, 7, 14, 21, 35, 42, and 49 days after treatment. Weight changes were measured using a digital high precision balance (BH2-600, Fuji). Percentage weight loss was calculated using the formula:

$$Percentage\ weight\ loss = \frac{Initial\ weight - Final\ weight}{Initial\ weight}\ x100$$

The decay incidence was determined every 7 d for 49 d through a 5-point scale visual observation of the degree of decay of the sample: 1 = no visible infection; 2 = slight infection (1-10%); 3 = moderate infection (11-25%); 4 = moderately severe infection (26-50%); 5 = severe infection (>50%). The fruit was non-marketable when it reached a of decay rating of 3. The value was further expressed as the accumulated percentage of the total number of fruit decayed divided by the initial number of fruit stored (Arthur *et al*., 2015).

$$Percentage\ decay = \frac{Total\ number\ of\ fruit\ with\ decay\ rating\ of\ 3}{Initial\ number\ of\ fruit\ stored}\ x100$$

The visual quality rating (VQR) of tomato was measured using a 5-point scale (1 = excellent, fresh appearance, 2 = very good, slight defects, 3 = good, defects progressing, limit of saleability, 4 = fair, useable but not saleable, and 5 = poor). The fruit was non-marketable when it reached VQR of 3.

The changes in color of the tomatoes were evaluated based on the maturity stages of the fruit from 1 to 6 (1 = mature green, 2 = breaker, 3 = turning, 4 = pink, 5 = light red, 6 = red). In the second trial, Nix Pro Color Sensor (Nix Sensor Ltd., Ontario, Canada) was used to measure the L* a* b*, hue and chroma. The L* value corresponds to the brightness or luminosity; a* value shows the redness (+a*) or greenness (- a*); b* value indicates the yellow (+b*) or blue (- b*). The chroma corresponds to the saturation of the color while hue indicates the red (0 or 360), yellow (90), green (180) or blue (270) (Barreiro *et al*., 1997). The firmness of fruit was measured using a fruit penetrometer (Fruit Tester FT 327 Pressure Tester, Wagner Instruments, USA). A digital refractometer (Atago PAL-1 (3810) Digital Hand-held Pocket Refractometer, Atago Co., Ltd. Japan) was used to measure the total soluble solids (TSS).The costs and benefits of the use of the BEC and ambient conditions were identified and quantified (Rolfe, 2014).

Experimental Design and Statistical Analysis

A sample size of 50 pieces was used per replication in each maturity stage at each storage condition in the first trial. Each treatment was replicated four times. A sample of 3.8 kg of tomato per maturity stage and storage condition in the second experiment. Each experiment was laid out in a Completely Randomized Design. Data were analyzed using two-way ANOVA and treatment means were compared using LSD at $P<0.05$.

RESULTS AND DISCUSSION

Temperature and relative humidity (RH)

Throughout the storage period in Trial 1, a temperature of 27.13±0.78^{0}C and relative humidity (RH) of 80.89±4.47% were recorded in ambient storage while 25.49±0.58^{0}C and 99.90±0.10% RH were recorded in the brick-walled evaporative cooler, BEC (Fig. 1). The temperature and RH differences between the two storage conditions were 1.64^{O}C and 19.01%, respectively. In the second trial, 26.93±0.87 ! and 80.05±5.20% RH were recorded in ambient storage while 25.42±0.78^{O}C and 97.75±3.25% RH were recorded in BEC (Fig. 2). BEC showed a slightly lower temperature by 1.51^{0}c and higher RH by 18.06% and 17.7% in the second trial.

A lower temperature (0.41^{O}C) difference from ambient conditions was reported during the storage of sweet pepper in a cabinet evaporative cooler, yet it allowed

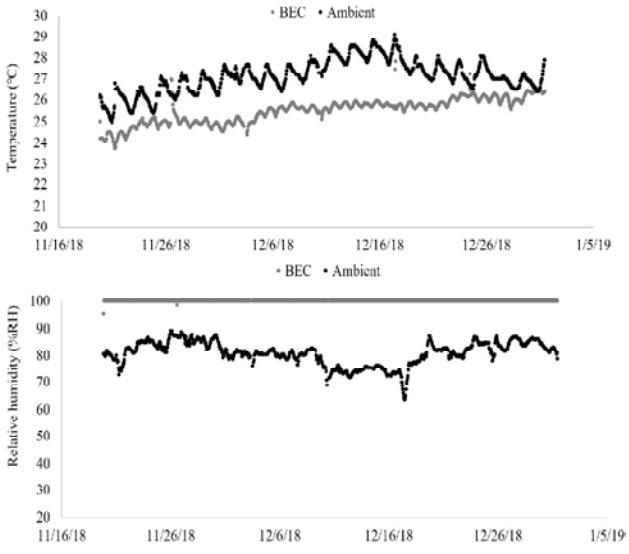
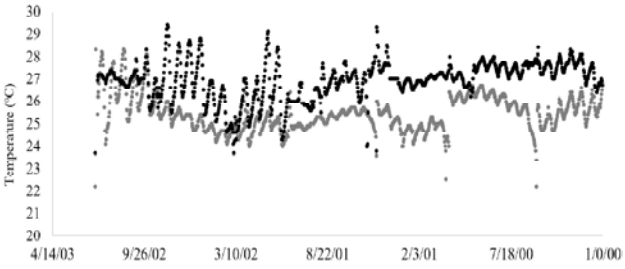


Fig. 1. Temperature and relative humidity in ambient and brick-walled evaporative cooler (BEC) conditions during November 2018 to January 2019.



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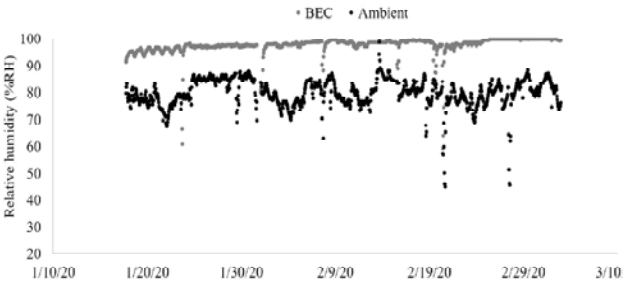

Fig. 2. Temperature and relative humidity in ambient and brick-walled evaporative cooler (BEC) conditions during January 2020 to March 2020.

maintenance of fruit quality longer due to the relatively higher RH (Majomot *et al*., 2019). In South Sulawesi, Indonesia, an underground zero-energy cool chamber made of bricks (without produce in it) registered a relatively lower temperature (26.2°C) and higher RH (87.2%) compared to the outside conditions of the chamber (Dirpan *et al*., 2017). However, the BEC used in the present study provided a slightly lower temperature (25.49±0.58°C and 25.42±0.78°C) and higher RH (99.90±0.10% and 97.75±3.25%) compared with the zero-energy cool chamber used in the previous study (Dirpan *et al*., 2017).

Percentage weight loss

Weight loss of tomato in BEC was consistently lower at 2.36 % (Figure 3A) and 5.63% at the end of the storage period for the first and second trials, respectively, compared to ambient conditions. Weight loss was 10% lower in tomatoes stored in BEC than those in ambient room conditions. At 42 days of storage, weight loss in tomatoes stored at the breaker stage was higher relative to tomatoes stored at the mature green stage (Fig. 3A).

Decay incidence

BEC storage conditions reduced the decay incidence among stored tomatoes by 29.5% (Figure 3B).

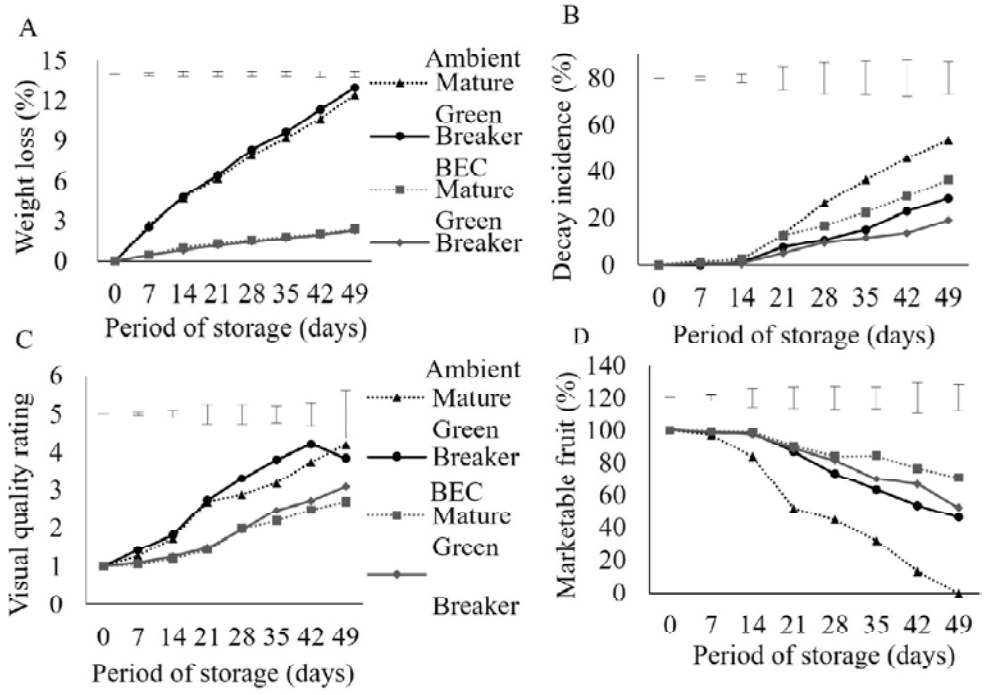

Fig. 3. Weight loss, visual quality, incidence of decay and percentage of marketable fruit of mature green and breaker 'Diamante Max' tomatoes stored at ambient or in brick-walled evaporative cooler (BEC) conditions. Bars refer to LSD values at $P < 0.05$.

At 49 days after storage, the cumulative decay incidence in samples stored in BEC ranged from 19 to 28.5% only compared to 36.5 to 53.5% in ambient conditions. Tomatoes stored at the breaker stage showed a higher percentage of fruit decay than fruit stored at the mature green stage.

As the fruit ripens, metabolic activity and fruit degradation tend to escalate (Quinet *et al*., 2019) and makes the fruit more prone to decay as obsorved in fruit stored at the advanced maturity stage. Further, the higher temperature(i.e., 21 to 30°C) in ambient conditions is a favorable condition for microorganism growth and development (da Cruz Cabral *et al*., 2019). However, sweet pepper stored in the cabinet evaporative cooler system showed higher decay due to excessive surface moisture (Majomot *et al*., 2019). Fluctuations in temperature and relative humidity cause condensation or surface moisture (Islam *et al*., 2019). Given that the temperature and RH recorded in the BEC have been relatively stable, especially in the first experiment, surface moisture was relatively low resulting in a lower incidence of decay.

Visual quality and shelf life

Regardless of maturity stage, samples stored in BEC had better appearance due to lower decay and shriveling than fruit stored in ambient conditions (Figure 3C). A lower visual quality rating of fruit in ambient conditions indicated a higher degree of fruit deterioration. Fruit stored in the BEC showed a longer shelf life and was highly marketable for an extended duration both in the first (Figure 3D) and second trials (data not shown).

The lower temperature and higher RH in the evaporative cooler extended the shelf life of sweet pepper (Majomot *et al*., 2019). Likewise, in the present study, the conditions in the BEC with lower temperature and higher RH helped maintain better quality of tomato during storage compared to ambient conditions. The storage conditions slowed down the respiration and transpiration that preserved the quality of tomatoes (da Cruz Cabral *et al*., 2019).

Peel color

In the first trial, tomatoes stored in BEC developed color faster than those in ambient storage starting at day 14 with a peel color of 4.75 (pink with some starting to turn light red), while tomatoes stored in ambient conditions had the mean color of 4.47 (pink) (Table 1). Tomatoes in BEC were redder than fruit held in ambient conditions that were more yellow-orange.

Table 1. Peel color of 'Diamante Max' tomatoes stored at mature green and breaker stages under ambient and brick-walled evaporative cooler (BEC) conditions.

Treatment	Peel color index[z]						
	Time of storage (day)						
	7	14	21	28	35	42	49
Storage							
Ambient	4.26[a]	4.47[b]	4.71[b]	4.66[b]	4.68[b]	4.75[b]	4.77[b]
Brick-walled EC	3.59[b]	4.75[a]	4.98[a]	4.99[a]	5.02[a]	5.05[a]	5.03[a]
Maturity Stage							
Mature Green	3.36[b]	4.50[b]	4.78[b]	4.76[b]	4.79[b]	4.89[a]	4.90[a]
Breaker	4.49[a]	4.72[a]	4.91[a]	4.88[a]	4.92[a]	4.91[a]	4.91[a]

Per storage period, means in a column with a common letter are not significantly different using LSD at 5% level of significance.

The color change was confirmed in the second trial as indicated by the L*, a* b*, hue and chroma values (Figure 4). The results showed that tomatoes were redder in the storage condition where the temperature was slightly lower (i.e., in the BEC), compared to yellow and lighter pink fruit color in ambient room conditions. The L* was consistently higher in ambient-stored fruit indicating a lighter color as fruit turned yellow compared to lower L* in fruit stored in BEC (Figures 4A). A redder color of tomato stored in BEC was indicated by higher positive a* values compared to fruit in ambient conditions (Figure 4B). The higher b* (Figure 4C) and the hue of around 90 (Figure 4D) indicated fruit were more yellow when stored in ambient than in BEC. The vividness of fruit color increased as shown by increasing chroma (Figure 4E).

Climacteric fruits like tomatoes continue to mature even after being removed from the main plant. As the fruit continues to mature, its color changes from green to red due to the degradation of chlorophyll and the synthesis of lycopene (Tilahun *et al*., 2017a; He *et al*., 2019). However, inhibition of lycopene synthesis was reported at temperatures below 12°C and above 30°C, which favored other carotenoids responsible for the yellow to the orange color of fruit (Tilahun *et al*., 2017b). The present agreed with the previous study wherein the use of the BEC for the storage of tomatoes resulted in a more uniform bright red color compared to fruit stored in ambient conditions (Babarinsa and Omodara, 2016).

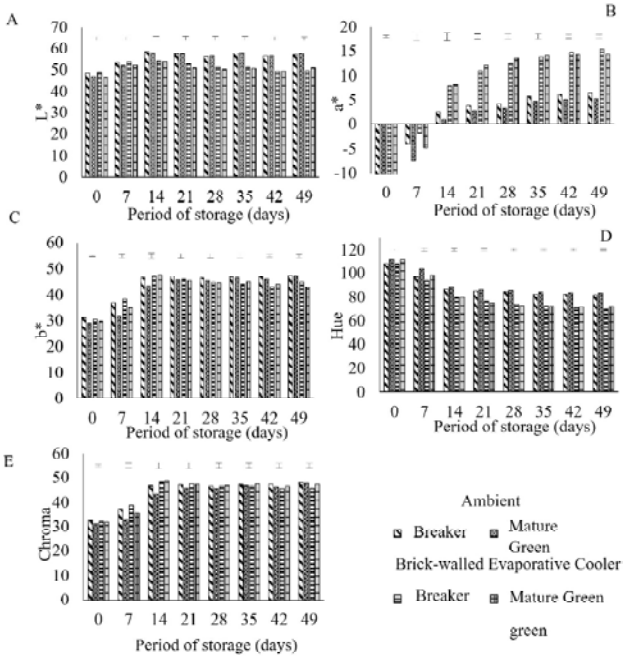

Fig. 4. Color changes, L*, a*, b*, hue and chroma, of mature green and breaker 'Diamante Max' tomatoes stored at ambient or in brick-walled evaporative conditions. Bars refer to LSD values at $P < 0.05$.

Firmness and total soluble solids (TSS)

Regardless of maturity stage, tomatoes stored in BEC were firmer compared to fruit stored under ambient conditions (Figure 5A). On the other hand, mature green tomatoes stored in BEC had lower TSS than fruit stored in ambient conditions (Figure 5B). Regardless of the maturity stage, TSS in fruit stored in ambient conditions were higher than those tomatoes

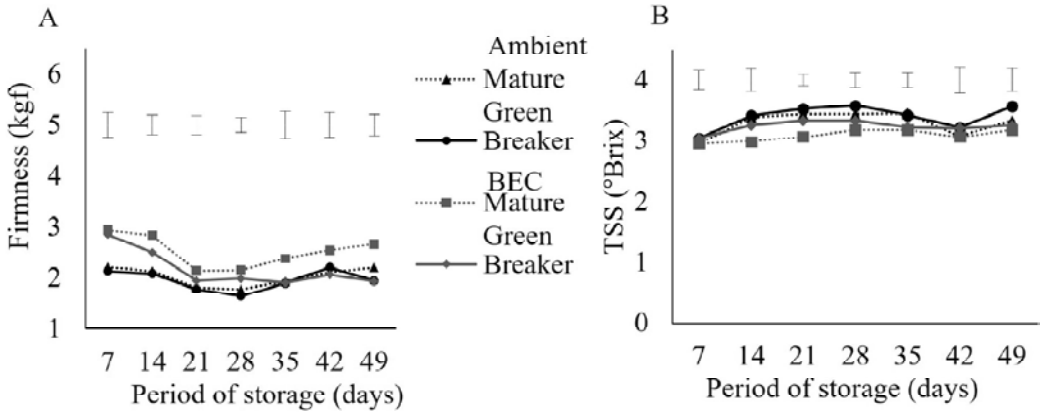

Fig. 5. Firmness and total soluble solids content of mature green and breaker 'Diamante Max' tomatoes stored at ambient or in brick-walled evaporative cooler (BEC) conditions. Bars refer to LSD values at $P < 0.05$.

stored in BEC at 28 days. The low temperature in BEC could have delayed the ripening in mature green tomatoes

The temperature has been reported to affect the firmness of tomatoes in which storage at lower temperature delayed the reduction of firmness while a sharp decrease was observed after transfer in room conditions (Najat *et al*., 2018). Changes in fruit firmness or softening during postharvest occur due to moisture loss and ripening-related cell wall metabolism or cell wall-modifying enzymes (Lara *et al*., 2019). The present results validate the previous finding that tomatoes stored in an evaporative cooler were firmer than those stored in ambient conditions (Adekanye *et al*., 2019; Manyozo *et al*., 2018).

TSS in fruit is associated with starch degradation into sugar as the fruit ripens (Adjouman *et al*., 2018). Hence, there was higher TSS in tomatoes at the breaker stage than fruit stored at the mature green stage. The increase in carbohydrate hydrolysis into soluble sugars at higher temperatures and reduced RH of ambient conditions resulted in a higher accumulation of TSS in tomatoes.

Cost-Benefit analysis

The cost-benefit analysis of using the brick-walled evaporative cooling (BEC) storage system showed that an estimated 168.42 kg of the stored tomatoes is expected to be marketable at the end of the storage period compared to the ambient storage with only 108.72 kg of fruit. The benefit over cost value of the BEC, assuming eight months (dry months) a year of use, was 27.16% higher than the ambient storage system (Table 2).

Moreover, monthly income from produce stored in BEC could potentially increase compared with ambient storage. The BEC system could last longer than a year, further lowering the maintenance costs. After one year of usage, the producers can earn more profit for the succeeding years since the only cost they need to pay is the water usage and disinfection of the BEC system. The evaporative cooler made of bricks, or the zero-energy brick cooler, was reported to be the cheapest evaporative cooler than other evaporative cooling technologies such as charcoal cooler and pot-in-pot cooler, hence, recommended for smallholder farmers (Manyozo *et al*., 2018).

CONCLUSION

The brick-walled evaporative cooler (BEC) recorded a lower temperature and higher relative humidity (RH) compared to ambient conditions. The mean temperature differences between the two storage conditions in the two experiments were 1.64°C and 1.51°C, while the differences in RH were 19.01% and 17.70% for the first and second trials, respectively. Percentage weight loss was consistently lower in BEC and showed 10.36% lesser weight loss compared in ambient conditions after 49 days. Decay incidence was lower in BEC and green tomatoes compared to fruit stored in ambient conditions and fruit stored in advanced stage. Fruit stored in BEC had better visual quality and longer shelf life. Fruit can be stored in the BEC for up to 49 days in which 61.8% of the initial fruit remained marketable compared to only 23.2% of fruit in the ambient storage system. Storage of fruit in BEC resulted in a redder fruit than those in

Table 2. Cost-benefit analysis of tomato storage in ambient and brick-walled evaporative cooling storage systems for one month computed for use for eight months per year.

	Quantity	Price/unit (USD)	Total (USD) for ambient storage	Total (USD) for brick-walled evaporative cooler (BEC)
Benefit				
Monthly income with marketable produce	108.72 kg[a] 168.42 kg[aa]	1.4047	152.72	236.59
Monthly benefit			152.72	236.58
Annual benefit[b]			1,221.76	1,892.64
Cost				
Sanitizer	8 set-ups	1.00/set-up	8.00	8.00
Container	8 pieces	3.01/crate	24.08	24.08
Newspaper lining	1/2 kg	0.50/kg	4.00	4.00
Construction of BEC	1,100 pcs of bricks, labor and transportation	362.17	-	362.17
Water Consumption	60L/day	0.19/ month x 8**	-	1.52
Labor Costs[c]	2-man days (MD)/200 kg x 8 set ups	6.42/MD	92.47	102.72
Total annual cost, USD [b]			128.55	502.49
Annual benefit minus annual cost, USD			1,093.21	1,390.15
Advantage of BEC over ambient, %				27.16

[a]Two hundred (200) kg of very good quality mature green or breaker tomato are stored in ambient in 8 plastic crates; After one month of storage, 40.81% were non-marketable = 118.38 kg are marketable/month; with 8.16% weight loss after 1 month= 108.72 kg/month (based on results at 28-day of storage). [aa]Two hundred (200) kg of very good quality mature green or breaker tomato are stored in BEC in 8 plastic crates; After one month of storage, 17.05% were non-marketable = 165.90 kg/month; with 1.52% weight loss after 1 month= 168.42 kg/month (based on results at 28-day of storage). [b]For 8 months/cycles of storage per year. [c]Labor costs include sorting, wiping/cleaning of tomatoes, air-drying, sanitizing of plastic crates, putting in crates, loading/unloading, monitoring of tomato quality, disposal of culls, and sanitizing of bricks for BEC. *Price of tomatoes per kg based on Philippine Statistics Authority (2018). **USD 0.007/day*28days = USD 0.19/month. Conversion rate= USD 1= PHP49.83

ambient room conditions which was confirmed by L*, a* b*, hue and chroma values. Tomatoes stored in BEC were firmer and had low total soluble solids (TSS). The higher TSS of tomatoes in ambient conditions indicated faster ripening of fruit. The benefit over cost value of the brick-walled evaporative cooling storage system was 27.17% higher than the ambient storage system showing more profit. In general, the BEC storage system maintained the quality of tomatoes better than ambient storage.

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Original Research Paper

Onion detopping machine - an emerging horticultural enterprising

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ABSTRACT

Onion is the one of the important vegetable crops cultivated in India. Multiplier type onion is one among the three major types of onions. Tamil Nadu accounts for five per cent of country's area under onion and more than 70 per cent of the area is cultivated by small onion (*Allium cepa* var. *aggregatum*). Around 90 per cent of country's multiplier onion is produced from Tamil Nadu. Detopping is one of the steps in the on-farm processing carried out after harvest. Presently this is done manually by farm women. Individual onions are picked and detopping is done by using sickle. This operation is time consuming and highly drudgery in nature. An Onion detopping machine to remove the foliage after harvest was designed and developed in the present study. This machine has a capacity of 370 kg/h against 30 kg/h manual practice and works with an efficiency of 98%. The unique design of the detopper is that it is suitable for all sizes of onions. Also, the cluster is intact after detopping, which is very important requirement. This multiplier type onion is grown in Nagamangala (Tk), Mandya (Dt.) of Karnataka State. Two farmers of this area have installed this machine and running a successful enterprising. Cost involved in using this machine is Rs. 200 per quintal against Rs. 500 per quintal by manual detopping. Owing to the higher capacity by mechanization, the farmers will be able to process the higher volume of produce within a short span of time. Hence, this will facilitate the farmers to sell the produce, get good market price and earn higher returns.

Keywords: Aggregatum onion, enterprising, mechanization, onion detopper and multiplier onion

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important commercial vegetable crops widely cultivated in India. India ranks 2nd in world's onion production after China, contributing 21% of world's onion requirement. In India, an area of 12.85 lakh ha is under onion cultivation with a production of 232.62 lakh MT of onions during 2018 (NHB, 2019) amounting to 12.50 % of area under vegetable cultivation and 12.62 % of vegetable production. Indian onion is broadly classified into three types *viz*., common onion, small common onion and multiplier onion (Fig. 1). Many varieties have been developed under each type of onion, having different traits suitable for different agro climatic zones, seasons, purposes, pest and diseases resistant etc. There are about 27 common onion varieties, two small common onion varieties and three multiplier onion varieties commercially available in India (NHRDF). The type and variety has also stake holder preference based on its pungency, which vary widely among types. All the above mentioned three types of onion have comprehensible morphological difference among them. Primarily common and small common onions are single bulbs but vary largely in size. The broad characteristics of the above three types of onion are presented in Table 1.

Among the above three types of onion, multiplier onion is known for its pungency and flavour which is commonly grown in Tamil Nadu. About 35,000 ha area is under multiplier onion cultivation in India which is about 2.7 % of area under onion production. About 3,32,500 MT of multiplier onion is being produced in India which is 1.5 % of onion production. Around 90% of country's multiplier onion is produced from Tamil Nadu and Andhra Pradesh, South Karnataka, parts of Orissa, Kerala contribute for the remaining portion. In Tamil Nadu multiplier onion is

a b c

Fig. 1. Types of onion grown in India
(a. Common onion b. Small Common onion c. Multiplier onion)

Table 1. Broad characteristic of Indian types of onion

S.No.	Type of onion	Shape	Size	Colour	TSS	Pungency	Storability
1.	Common onion	global round in shape	big in size (≈ 40-60 mm dia.)	Ranges from light to dark red	12° - 14° brix	10.07-13.0µmol/g FW	Very good to medium
2.	Common small onion (Bengaluru rose onion)	flattish round in shape size	Small in (≈ 25 – 35 mm dia	Scarlet red in colour	16°-18° brix	10.27 µmol/ g FW	Good
3.	Multiplier onion (sambar onion)	5-6 bulblets clump in shape	20 - 25 mm in size	Red in colour	8°-18° brix	10.13 µmol/g FW	Very good

Source: Saraswathi *et al.* (2017); Sabina Islam *et al.* (2019).

cultivated in an area of 30,255 ha with a production of 2,86,000 MT (www.tn.gov.in).

Detopping is one of the on-farm processing operations carried out after harvesting and it is removing of the leaves from the onion. Onion is harvested when 50% tops begin to collapse on the ground but before the foliage dries down completely (Anon, 2011). After digging, the onion is field-cured for 3-5 days, cut at the necks for separation of onion from the tops, graded, shed-cured and stored. Separation of onion bulbs from tops is called de-topping and women labourers are engaged for this operation. Individual onion is de-topped by sickle thus makes it highly drudgery that requires 12.5 woman-hours/t and time consuming (Anon, 2017.). The average weight of each clump of multiplier onion is about 60 g, thus one kg of onion contain about 15 individual onions. When huge quantity of onion needs to be harvested and processed, it needs mechanization. Onion detopping machine was designed and developed at ICAR-Indian Institute of Horticultural Research, Bengaluru. This machine has been demonstrated widely at farmers' field. This paper discusses the entrepreneurial opportunities of this machine.

MATERIALS AND METHODS

Process of onion detopping

The matured onion is harvested and left in the field for 3-5 days for field curing. Afterwards they are detopped and stored or without detopping stored in a well-ventilated storage structures or facilities. The either second category is detopped and sold to trader based on market demand.

Onion detopping machine

The prototype onion de-topper consists of i) Feeding chute for feeding onion crop, ii) de-topping unit, iii) collection chutes for the de-topped onion bulbs and tops, iv) main frame and v) power transmission system (Fig. 2).

The feeding chute is for feeding of the onion crop to the onion de-topping unit. This was fabricated out of MS sheet of 1.5 mm thickness sheet and had dimensions of 700 mm x 400 mm X 200 mm (L x W

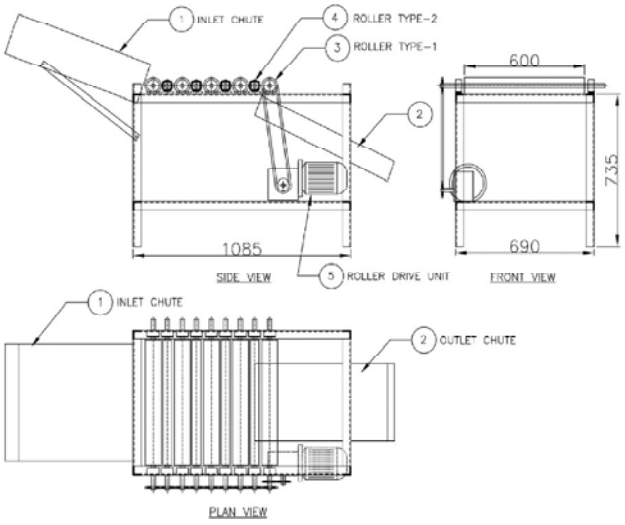

Fig. 2. Diagram of onion detopping machine all dimensions in mm

x H). The de-topping unit is the key component of the de-topping machine which should detop the onion tops efficiently without damaging the onion bulbs. The de-topping unit consists of i) set of de-topping rollers, ii) main frame and iii) power transmission system. The de-topping unit is a set of rollers comprising one roller having cutting edge along its length and the other one a plain roller. The cutting roller is fabricated out of M.S. square shaft having 35 mm cross section and 600 mm length. The plain roller is a G.I. Pipe having 600 mm length, 50 mm diameter and 3 mm thickness. Four such set of rollers were fabricated and mounted on a main frame. A clearance of 2 mm is provided between the rollers. The frame is fabricated out of M.S angle section of 40x40x5 mm having 1085 mm length and 690 mm width. The rollers were driven by sprocket and chain system and were counter rotated.

Collection chute was fabricated out of M.S sheet of 1 mm thickness having trapezoidal shape for directing the de-topped onion bulbs into the crates. The collection chute had a dimension of 530 mm width at the upper end, 150 mm at the lower end, 680 mm length and 200 mm height. This was fitted to the main frame at angle of 74^0 in order to have the free fall of the de-topped onion bulbs. A tray to collect the detopped onion leaves was fabricated and fitted below the rollers. The main frame of the machine was fabricated out of M.S. angle section of 40×40×5 mm. The feeding chute, de-topping unit, collection chute for de-topped onion bulbs, collection tray for onion tops, power transmission systems and electric motor were fitted to the main frame. A three phase, 1440 rpm, 2 hp electric motor was mounted on the main frame with necessary supports and gearbox of 1:30 reduction was fitted to the motor.

RESULTS AND DISCUSSION

Operation details

The cured onion crop was fed by the feeding conveyor to the de-topping unit. Due to counter rotating of the plain and cutting rollers, the onion tops were drawn in between the rollers and made an orientation of tops down position. The sharp edges of the shearing rollers further de-tops the onion tops and the onion tops were dropped down. Due to plurality of the rollers, the rollers conveyed the de-topped onion bulbs further for delivery. The plurality of rollers also ensured higher chances for de-topping the onion tops before the onion crop reaches the delivery. The de-topped onion tops were collected in the collection tray provided below the onion de-topping unit and de-topped onion bulbs were collected in crates which were guided by collection chute. The performance parameters of onion detopping machine is presented in the Table 2.

Table 2. Performance parameters of onion de-topping machine

Sl.No.	Performance parameters	
1.	De-topping efficiency (%)	95.20 ± 1.42
2.	Effectiveness of de-topping (%)	97.80 ± 0.00
3.	Per cent damage (%)	2.30 ± 0.29
4.	Per cent non-detopped onion (%)	2.50±0.25
5.	Capacity (kg/h)	372.88 ± 7.22
6.	Cost Economics	37 % saving against conventional method

The machine has been widely demonstrated in farmers' field, National Exhibitions and Farmers' Fairs.

Adoption of onion detopping machine as an enterprise

Nagamangala is a Taluk in Madhya (Dt.) of Karnataka State located at 12.82° N 76.76° E and 772 m elevation. This is multiplier onion growing cluster in Karnataka. About 2000 ha area in this Taluk produces multiplier onion with a production of about 17,000 MT. Planting is done during January and

matured onion is harvested during March and April. The harvested onion along with top is bundled, and are stored in a well-ventilated facilities for a period of six months (Fig. 3). These kind of structures are also funded by Government of Karnataka. The stored onion is detopped and supplied to the market as per market demand. During lean season, the stored onion is detopped manually by the farm women and supplied to the market. However, during peak demand period, when the farmers' desired to get better returns, the supply gets hampered due to limited detopping capacity in the existing manual practice and also availability of resources. It is also to be noted that towards the end of storage period, stored onions start sprouting. Hence, all the stored onion need to be detopped and supplied to the market. The manual detopping capacity is about 20 - 30 kg/h. One entrepreneur charged half of the cost meant for manual detopping and yet could gain a profit. While another entrepreneur changed Rs.200 per quintal & while manual stopping costs Rs. 500 per quintal. He obtained B: C ratio of 2.01:1 for 3 years. Hence, farmers are able to take the advantage of market price and sell the produce when the price is at its peak. Due to this farmers are benfitted in both ways i.e cost involved in detopping practice and peak market rate (Fig. 4). This machine also ensured timeliness of operation thus helped in tapping higher returns during peak market demand period.

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Fig. 3. Multiplier Onion storge

Fig. 4. ICAR-IIHR onion detopping machine installed at Nagamangala as entreprise

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Original Research Paper

Constraints in dry chilli cultivation practices and mechanization of harvesting in Southern India

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ABSTRACT

Dry chilli production in India condition faces many challenges apart from adverse weather conditions, labor-intensive production practices and higher overall production costs are limiting profitable dry chilli cultivation. Therefore, a study was carried to know the key constraints in current chilli production practices in eight major production districts of three states. A systematic research and development approach is essential to know the range of constraints and farmers preferences over technological options for field operations. The harvesting operation alone demands 43% of labour 360.5 man-days/ha. So, red chilli harvesting mechanization is a definite immediate requirement to reduce labour input. Farmers (47%) prefer small size self-propelled chilli harvester over tractor operated equipment. In the production catchments, farmer also inferred to change the cultivation practices to mechanize chilli production operations, but 18% of farmers hesitant to adopt one-time-harvesting chilli varieties due to suspicion about the yield potentials.

Keywords: Chilli harvester, mechanization and self-propelled machine.

INTRODUCTION

India is the world's largest producer, consumer and exporter of dry chilli. Dry chillies are widely cultivated in tropical and sub-tropical countries like India, Japan, Mexico, Turkey, United States of America and African countries (Asati and Yadav, 2004). Chilli (*Capsicum annuum L.*) is one of the important commercial crops of India, which belongs to the Solanaceae family. It is grown almost all agro-ecological sub regions of the country. The dry chillies are used in a number of culinary purposes such as vegetables, spice, condiments, sauce, pickles and chutneys (Patel *et al*., 2015). According to third advanced estimate of 2019-20, that in the country the area, production and productivity of dry chilli was reported to be 7.03 lakh ha, 17.52 lakh tones and 2.49 tones/ha, respectively. Among the states in India, Andhra Pradesh, Telangana, Karnataka, Madhya Pradesh, West Bengal and Orissa account for more than 75% of the area in cultivation and total dry chilli production (Spice Board of India, 2019-20). Red chilli cultivation plays an important role in economic conditions of farmers, especially marginal and small farmers at one side and help to meet out the nutritional requirements of the people on the others side.

India's agriculture produce market has entered into the ages of unlimited competition due to global market opening and increased competition of such as DDA agreement, FTA (Free trade agreement) expansion, a block economy. Although spice such as red chilli remained competitive in the market, but the production of this category of spice has been decreasing significantly due to many challenges, such as rapid reduction of labor force due to industrial and house hold products manufacture and ageing society, increased labour cost and particular growing condition. Considering the current agricultural system, we need to develop new mechanization technologies for chilli crops cultivation and engineering technology for production of high-quality products in order to win the international competition.

The present study aims to analyze the constraints in the conventional production system and to assess the mechanization needs in dry chilli harvesting. The mechanization urgency also arises partly due to new breeding hybrids which are in development stage to obtain synchronized fruiting for once-over harvesting operation to combat labour shortage in harvesting the produce.

MATERIALS AND METHODS

Andhra Pradesh state in India appeared as the largest regional dry chilli production site, followed by Karnataka and Telangana states. Present study was carried out during 2019 *kharif* season. The current status of dry chilli cultivation practices and constraints faced by the farmers was carried in these states covering total eight districts and a total of 100 farmers (Table 1). Farms producing chilli for self-consumption were excluded and farms that are producing for commercial purposes were included. The data collected pertaining to the production zones in the districts, where relatively large areas are in chilli cultivation. The data in Table1 represents the states, districts and the number of surveyed farmers in each district. We collected data and analyzed the information related to the cultivation practices, labour requirement for critical operations, possession of agriculture machinery, production cost and preference of prioritized new machines development. The farm categories were distinguished based on area in chilli production by each farmer i.e., into less than 0.5 ha, 0.5 ha-1 ha, and greater than 1 ha and analyzed them separately (Choi, *et al.,* 2010).

RESULTS AND DISCUSSION

Regional cultivation patterns and Overview of farms

In Guntur, Prakasham and Kurnool districts of Andhra Pradesh the Chilli is seeded or seedlings were transplanted in a row, mostly ridges and furrow pattern. Large farmers make beds and carryout planting in single and double row. Row spacing maintained for chilli cultivation are 60, 80, 90 and 100 cm and plant to plant spacing maintained are 30, 45 and 60 cm were common depending on soil type and its fertility status, irrigation facility and rainfed cultivation. Similar cultivation practices, row spacing and plant to plant spacings were followed in Karnataka and Telangana states also depending on farmers resources base. The data presented in Table 2 illustrates the summary of the various regions 'cultivation patterns for chilli Cultivation..

The data on land holding and percent cropped area of dry chilli growing farmers were recorded. Cultivation area < 0.5 ha was the most common and constituted 41.50% of chilli growers, whereas, area ranging 0.5-1 ha area contributes 31%. However, > 1.0 ha contributed 27.30% of chilli growers. The average cultivation area was about 0.86 ha/farmer.

The various machineries have been adopted by farmers for chilli production. The tractor, power tiller, mini tiller, trailers and cleaner equipments consume 49.50%, 105, 5.5%, 30% and 4%, respectively. More than 50% of the growers have basic tillage, seed bed preparation and to some extent interculture and weeding implements. However, none of the growers had postharvest machines like dryers, cleaner, grader etc. A small 4% of farmers reported that they were using winnower fans to clean the produce after drying. The operations viz., transplanting and harvesting of chilli are appeared as highly labour-intensive operations in dry chilli production.

Though semi-automatic indigenously developed seedlings transplanters are available, farmers are not adopting due to high initial cost of machine and lack of awareness. But farmers opined that, red chilli harvester is need of the hour, since farmers need to engage the labour multiple times for timely harvesting. Therefore, to create a design direction for chilli

Table 1. State and district wise distribution of the surveyed farms

Districts	Andhra Pradesh			Karnataka		Telangana		
	Guntur	Prakasham	Kurnool	Bellary	Raichur	Warangal	Khammam	Mahaboob Nagar
No. of farmers surveyed	20	10	10	15	10	15	10	10

Table 2. State and district wise cultivation patterns for Chilli

State / Districts	Districts	Cultivar	Pattern	Row to Row spacing (cm)	Plant to Plant space (cm)	Bed width (cm)
Andhra Pradesh	Guntur	Tejaswini, Bydagi and Syngenta 2043	Line sowing/planting, Ridge-furrow and planting on beds (Single and double row planting)	60 and 90	20, 30, 45 and 60 (Planting on bed only)	70 and 80
	Prakasham	Tejaswini, Aparna, Indo-5, LCA-206	Line sowing/planting, Ridge-furrow and planting on beds	60, 90 and 100		
	Kurnool	Aparna, super 10 and Tejaswini	Line sowing/planting, Ridge-furrow and planting on beds (Single and double row planting)	45, 60 and 90		
Karnataka	Bellary	Bydagi, Demon F_1 (East and West)	Line sowing/planting, Ridge-furrow and planting on beds (Single and double row planting)	45, 60 and 80	20, 30 and 45	70 and 80
	Raichur	Tejaswini, Syngenta 5531 and Bydagi	Line sowing/planting, Ridge- furrow and planting on beds	60, 80 and 90		
Telangana	Warangal	Tejaswini, Super-10 and Indo-5	Planting on beds, Ridge- Furrow method	80 and 90	30, 45 and 60	70 and 80
	Khammam	Tejaswini, Aparna and Superb-10	Line sowing/planting, Ridge-furrow method	80 and 90		
	Mahaboob Nagar	Bydagi, Tejswini, Demon F_1 (East West company)	Ridge-furrow, Line sowing/planting	80 and 90		

harvester, development of chilli harvesting machine matching to tractor or medium size power source is preferred over self-propelled imported pepper harvester, because it is easier to apply to India's chilli cultivation practices. It is deemed better developing the self-propelled chilli harvester machine as an add-on to considering the power requirement and sloppy field conditions.

HARVESTING PROCESS

The chilli production process was categorized into different unit operations and labor man days required for each operation wise accounted such as to raise seedlings, field preparation, transplanting, fertilizer and pesticide application, harvesting, and post harvesting processes etc.

Harvesting is a labor-intensive process that requires large amount of labor force, because it is a difficult for laborers to work continuously in a hot, humid confined space for a long period of time. In red chilli production practices, cultivar planted, number of runs the produce picked and yield potential of cultivar were the major cost determining factors in harvesting operation. Generally, in the surveyed production catchments of Andhra Pradesh, Karnataka and

Telangana States, Tejaswini and Byadagi predominantly grown hybrids. Normally recorded yields of Tejaswini and Byadagi hybrids were 7.41 to 9.88 tones ha^{-1} on dry chilli produce basis. The labor cost in harvesting season ranged from Rs. 250 –300/ day per person.

The data in Table 3 indicates the number of picking times, average yield per picking and harvesting labor costs as recorded by farmers. The number of picking times reported by majority of growers were three and the proportion of average produce picked 20%, 55% and 25%, respectively in first, second and third

Table 3. Picking wise chilli yield and harvesting costs

Picking 1st	2nd	3rd	Total	
Chilli yield (t/ha)	1.74	4.78	2.17	8.70
Yield in per cent	20%	55%	25%	—-
Harvesting cost (Rs./ha)	7,440	20,460	9,300	37,200

pickings. The data on cost of cultivation indicates that, the total production cost worked out was Rs. 1,39,668/ -ha^{-1} and harvesting alone single largest operation accounted 23% of input cost. The agriculture inputs namely, fertilizers and crop protection chemicals accounted Rs 41,708/-ha^{-1}. After the inputs cost, the harvesting cost was the second highest investment in dry chilli production and due to partial mechanization, the tillage and planting bed preparation come down to about Rs 13,000/-ha^{-1}

Manual labour involved and difficulties faced in hiring chilli cultivation practices

The data presented in Table 4 shows the comparison and analysis of drudgery felt and difficulties faced by the chilli crop growers in labor hiring at different stages of production operations as reported by the farmers in selected surveyed regions. The total average labour utilized was about 360.5 man-days/ha. Among various unit operations, the harvesting requires highest labour (43%), followed by crop management (29.54%)

Table 4. Drudgery proneness and labour deficiency (%) in chilli cultivation operations

Operation	Per cent labour engaged	Drudgery proneness of operation	Difficulty in labour hiring
Transplanting	7.62	22	6
Plant protection	Crop management	33	6
Fertilizing	29.54	14	6
Harvesting	43.0	94	82

and Transplanting (7.62%), respectively. Therefore, it is important to mechanize the harvesting operation and the second aspect is to change the present practice of individually raising seedling to community-based raising practice or implement plug seedling raising system to reduce the amount of labor force for dry chilli production. Harvesting operation was the most drudgery prone and difficult to get sufficient labour as opined by 94% and 82% farmers, respectively.

The data in Table 5 infers the survey result of farmers' preference for adoption of new technologies, adopting one-time-harvest variety of crop and development of chilli fruit harvesting machine. Majority of the farmers preferred mechanization with small power sources and matching machines such as power tiller (34%), rotary tiller (13%) and about 24% with tractor operated larger machines. About 78% of farmers responded positively to change cultivation practices towards more mechanized activities and 38% expressed willingness to adopt new cultivars and developed harvesting machinery. Other 44% of farmers answered to consider the issues after observing the efficiencies of the technologies in other farmers fields in two to three seasons. In contrast to Table 6, the farmers responded that, harvester (85.70%) was the most prioritized new machine for mechanization, followed by pest control devices, stakes installation and remover devices, dried chilli cleaner and seedlings transplanter. Therefore,

Table 5. Farmers preference for mechanization of red chilli cultivation

Operation and power sources preference		%
Major machines for mechanization	Power tiller	34
	Tractor	24
	Rotary tiller	13
	Other modes	29
Changes of cultivation method for mechanization	Yes	78
	No	22
Adoption of new one-time-harvest cultivar of chilli and developing machinery	Yes	38
	Consider	44
	No	18

Table 6. Priority and preference of developing new technologies (%)

Harvester	Plant protection	Staking remover	Staking installer	Cleaner	Transplanter
85.70	21.20	12.10	9.10	6	3

mechanized harvesting is the most prioritized developmental activity to reduce the amount of labor effort and production cost.

CONCLUSIONS

Dry chilli is one of the widely grown spice crops in the country. So, an investigation and analysis were carried out to know the labour requirement and farmers preferential pattern in mechanization of various operations for competitive production and reduce cost of cultivation. The investigation covered contiguous production catchments in eight districts in southern India. The cultivation area per farmer under chilli production was 0.83 ha in the catchments and most of the growers satisfied with the existing implements and machines in tillage, weeding and intercultural operations. Total labour required to meet all the field operations in chilli cultivation was 360.50 man-days/ha and 43% demanded by harvesting operation alone. In the surveyed districts 47% farmers preferred promotion of small farm mechanization and development of low horse power sources matching machines. Therefore, design of chilli harvester and development of self-propelled harvester were prioritized activities in production operations mechanization. About 85.70% of farmers responded positively to change the present manual picking to mechanized harvesting practices and 44% expressed willingness to adopt new one-time-harvest variety of chilli cultivars.

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Original Research Paper

Biochemical characterization of defense responses in rose genotypes in response to artificial inoculation with black spot pathogen *Diplocarpon rosae*

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ABSTRACT

Resistance responses in the leaves of eight rose genotypes, Knock Out (highly resistant), Arka Nishkant (moderately resistant), *R. multiflora* (highly susceptible), Arka Swadesh (highly susceptible), IIHRR 13-4 (susceptible), Arka Parimala (susceptible), *R. indica* (susceptible) and IIHRR 4-15-12 (moderately susceptible), exhibiting varied levels of resistance against black spot were investigated post artificial inoculation with black spot pathogen, *Diplocarpon rosae*. There was consistent increase in the activities of defense related enzymes such as catalase, peroxidase, polyphenol oxidase, superoxide dismutase and phenylalanine ammonia lyase and other defense related secondary metabolites like phenols and flavonoids at different phases of black spot progression and increase was high in resistant genotypes Knock Out and Arka Nishkant. The peak activity of defense enzymes and high concentration of other metabolites was witnessed during early stages of infection in the resistant genotypes while it was during later phase in the susceptible genotypes. These results suggested that the faster and stronger activation of defense system is associated with the resistance against black spot in the rose genotypes.

Keywords: Artificial inoculation, diplocarpon rosae, enzymes, flavonoids, phenols, resistance and rose

INTRODUCTION

Black spot, caused by *Diplocarpon rosae* is the major destructive and dominant disease among different fungal diseases in rose. Owing to the demand and popularity of roses in current flower trade and landscape gardening, breeding for the resistant varieties or developing the varieties that require less care in terms of management against the destructive diseases is the need of the hour. Plants protect themselves through various means of defenses through accumulation of several defense related biochemical compounds following infection by pathogens. Reactive Oxygen Species (ROS) that are produced as initial cellular responses following successful pathogen recognition (Ashry and Mohamed, 2011) have major roles in cell signaling and these are the secondary messengers for activation of genes that encode for protective proteins (Lamb and Dixon, 1997 and Mendoza, 2011). However, the increased ROS production causes cellular damage through peroxidation of membrane fatty acids (Lamb and Dixon, 1997) and plants defend against this with up regulation of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Mittler *et al.,* 2004). Phenolics are toxic to microbes in nature and increase the physical and mechanical strength of the host cell wall. The oxidation of these toxic phenolic compounds by polyphenol oxidase (PPO) produces quinones (antimicrobial compounds) that are highly toxic to invading fungi thereby offering resistance against a wide range of pathogens (Cahill and Mccomb, 1992). Phenylalanine ammonia lyase (PAL), one of the key enzymes in the phenyl propanoid pathway, has a role in synthesis of phytoalexin and salicylic acid. Increase in the PAL activity subsequently increases the phenolic contents offering disease resistance to plants (Klessig and Malamy, 1994).

Understanding of plant resistance mechanism against pathogens at various levels provides new opportunities to breed improved cultivars with better resistance to the diseases. There were not many studies in this line on black spot disease infection in rose. Thus, the study was conducted to investigate the role of various defense related enzymes and compounds at different progression periods post infection by black spot pathogen in different rose genotypes possessing differential resistance.

MATERIAL AND METHODS

Location and climate of experimental site

The present investigation was carried out during 2016 at ICAR-IIHR, Bengaluru, which is geographically located at 130 58' N Latitude, 780 E Longitude and at an elevation of 890 m above mean sea level with an average annual rainfall of about 890 mm.

Plant material

The rose genotypes evaluated in the present study were part of rose breeding program at ICAR-Indian Institute of Horticultural Research, Bengaluru. A total of eight rose genotypes were used for the study in completely randamized design (CRD). The plants were provided with all inputs as per the package of practices for rose cultivation except for fungicidal sprays during the period of investigation. Young and healthy leaves from 4th to 6th node from apex of the shoot were collected (Dong, 2014) ran damly from three plants of the selectedfrom the selected genotypes *viz., R. multiflora* (highly susceptible), Arka Swadesh (highly susceptible), IIHRR 13-4 (susceptible), Arka Parimala (susceptible), *R. indica* (susceptible), IIHRR 4-15-12 (moderately susceptible), Arka Nishkant (moderately resistant) and Knock Out (highly resistant) in three replications. The collected leaves were cleaned with deionized sterile water and wiped with sterile tissue paper.

Preparation of conidia suspension

Rose leaves, severely infected with black spot, preferably with yellow halo around the spots were collected and surface cleaned with sterile tissue paper. Later, the infected leaf portions were cut and submerged in deionized sterile distilled water in the sterile tubes. The tubes were kept in orbital shaker for one minute after adding two drops of Tween-20. The suspension was filtered and the concentration of conidia in the filtrate was adjusted to 2×10^4 conidia/ml (Leus, 2005) using haemocytometer. This filtrate was used for inoculation of leaves. The spores from pure culture of the pathogen maintained on detached leaves was used for further inoculation of healthy leaves in the present study.

Artificial inoculation of leaves

The inoculation of excised leaves with *D. rosae* was performed as described by Debener *et al*. (1998). The cleaned healthy leaves were placed on moist blotting paper, with their petioles wrapped in moist cotton plugs in glass petri plates to maintain 100 per cent humidity. On each leaflet surface, 4-6 droplets of 10µL conidial suspension (2×10^4 conidia/ml) was pipetted out in laminar air flow to avoid contamination. The inoculated leaves were then incubated at ~25ºC under 10 h photoperiod for two weeks (Dong, 2014).

Enzyme assays:

The infected and control leaves were analyzed for the activities of following defense related biochemical compounds on every third day *i.e.,* on 0, 3, 6, 9, 12 and 15 days after inoculation (DAI).

POX activity (EC 1.11.1.7) (Enzyme units/ g fresh weight -EU/g FW): The POX activity was determined by following same method described by Chander (1990) and enzyme activity was expressed as enzyme units g/ FW.

PPO activity (EC 1.10.3.1) (EU/g FW): The PPO activity was determined following the method of Selvaraj and Kumar (1989) without any modifications and the enzyme activity was expressed as EU/g FW.

CAT activity (EC 1.11.1.6) (EU/mg FW): The CAT activity was determined by following the same procedure as per Masia (1998) and activity was expressed as EU/mg FW.

PAL activity (EC 4.3.1.24) (EU/g FW): The PAL activity was estimated as per the same procedure followed by Hodgins (1971) and activity was expressed as EU/g FW.

SOD activity (EC 1.15.1.1) (EU/mg FW): The SOD activity was estimated as per the same procedure followed by Du and Bramlage (1994) and activity was expressed as EU/mg FW.

Total phenols (mg/ g fresh weight): Total phenol content was estimated by same procedure followed by Singleton and Rossi (1965) by spectrophotometric method using Folin-Ciocalteau's Phenol Reagent (FCR) and the phenol content was expressed as mg/g FW.

Total flavonoids (mg/ g fresh weight): Total flavonoid content was estimated by the spectrophotometric method using same procedure as followed by Chun *et al.* (2003) and total flavonoid content was expressed as mg/g FW.

RESULTS AND DISCUSSION

a) POX activity

Analysis of data revealed that POX activity increased significantly in inoculated leaves of all genotypes after infection with the pathogen (Fig S1, S2). Among the inoculated leaves (I_2) of all genotypes, highest peroxidase activity (1.84 EU/ g FW) was observed in highly resistant variety Knock Out, on 6th day after inoculation (G_8D_3) which was almost 1.4 times to the maximum activity found in highly susceptible genotype, *R. multiflora* (1.29 EU/ g FW) that was observed on 9th day after inoculation (G_1D_4) (Table 1). In the variety Arka Nishkant, which was moderately resistant, peak enzyme activity was observed (1.76 EU/ g FW) on 12th day (G_7D_5). No significant changes in enzyme activity were found in un-inoculated leaves (I_1) of all genotypes during entire observation period (Fig. S1, S2) (data not presented). Peroxidase is involved in biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989). Peroxidase mediates the oxidation of phenols to oxidized phenols that are highly toxic to the pathogen (Sequeira, 1983). Thus, the increased activity of peroxidase in infected tissues contributes to resistance by inhibiting the pathogen growth.

In the present study, resistant genotypes have recorded quick and high peroxidase activity compared to susceptible ones. Due to the increased activity of peroxidase at early stages of infection, the pathogen growth was hindered and thus offered resistance against black spot. Similar increased activity of peroxidase in resistant genotypes in response to pathogen infection has been reported in *Fusarium* infection in melon (Hanifei *et al.*, 2013), *Botrytis* infection in faba bean (El-Komy, 2014) and brown rust infection in wheat (Riaz *et al.*, 2014).

b) PPO activity

PPO activity increased in inoculated leaves of all genotypes in response to the pathogen inoculation (Fig. S3, S4). At a given time period on 3rd (D_2) and 6th (D_3), highest PPO activity (2.62 EU/ g FW and 3.13 EU/ g FW respectively) was found in highly resistant variety Knock Out (G_8D_2 and G_8D_3) respectively) whereas the lowest activity (0.85 EU/ g FW and 1.84

Table 1. POX activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	POX activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.47	0.68	1.22	1.29	1.18	0.71
2	Arka Swadesh (G_2)	0.38	0.69	1.25	1.38	1.23	0.8
3	IIHRR 13-4 (G_3)	0.46	0.71	1.3	1.38	1.22	1.02
4	Arka Parimala (G_4)	0.55	0.93	1.32	1.56	1.39	1.04
5	*R. indica* (G_5)	0.47	1.01	1.44	1.52	1.62	1.65
6	IIHRR 4-15-12 (G_6)	0.49	0.78	1.31	1.7	1.59	1.65
7	Arka Nishkant (G_7)	0.44	1.48	1.69	1.68	1.76	1.71
8	Knockout (G_8)	0.46	1.81	1.84	1.74	1.71	1.64
	S.Em ±	0.06	-	-	-	-	-
	C.D. @ 5%	0.18	-	-	-	-	-

EU/ g FW respectively) was recorded in highly susceptible genotype *R. multiflora* (G_1D_2 and G_1D_3 respectively) (Table 2). This revealed that the enzyme activity in resistant genotype increased immediately in response to the pathogen infection whereas the enzyme activity increased slowly and gradually in the susceptible genotype. Among all genotypes, highest activity of PPO in inoculated leaves (I_2) (3.64 EU/ g FW) was recorded in moderately resistant genotype Arka Nishkant on 9th day after inoculation (G_7D_4). No significant changes in enzyme activities were found in un-inoculated leaves (I_1) of all genotypes during entire observation period (Fig. S3, S4) (data not presented). PPO catalyzes the oxidation of phenols released due to membrane damage (Siddique *et al.*, 2014) during microbial invasion into oxidized phenols *i.e.*, quinones that are more reactive and highly toxic (Batsa, 2004) which creates toxic environment for pathogen development (Jockusch, 1966 and Mohamed *et al.*, 2012). Thus increase in PPO activity is associated with resistance. In present study, PPO activity increased quickly with pathogen inoculation in resistant genotypes whereas the increase in susceptible genotypes was slow and less. This early increase in activity of PPO in resistant genotypes inhibited the fungal growth and thereby contributed for resistance in resistant genotype. These results are in conformity with the findings of Khatun *et al.* (2009) who reported increased activity of PPO in black spot (*Alternaria tenuis*) infected resistant rose leaf tissues during progression of disease. Highest activity of PPO was also reported in *Fusarium* infected resistant melon genotypes compared to susceptible ones (Hanifei *et al.*, 2013).

c) CAT activity

The inoculated leaves of all genotypes showed significantly higher levels of CAT activity during the period of observation than those of un-inoculated controls (Fig. S5, S6). In highly resistant genotype Knock Out, the CAT activity increased sharply and reached its peak (19.83 EU/ mg FW) on 12th day after inoculation (G_8D_5), whereas in *R. multiflora* which was highly susceptible, the CAT activity increased comparatively at a slower pace and reached its peak (8.71 EU/ mg FW) on 9th day (G_1D_4), followed by a decrease by 15th day (7.15 EU/ mg FW) (G_1D_6). When the CAT activity of all genotypes was compared on third day (D_2) immediately after pathogen inoculation, the highest activity (16.06 EU/ mg FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest activity (7.64 EU/ mg FW) was found in Arka Swadesh (G_2D_2) which was highly susceptible to the disease. No significant changes were detected in control leaves (I_1) throughout the observation period (Fig. S5, S6) (data not presented).

CAT is one of the important H_2O_2 scavenging enzymes that eliminate the toxic effects of H_2O_2 through a mechanism known as Halliwell–Asada–Foyer pathway

Table 2. PPO activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	PPO activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.59	0.85	1.84	2.65	2.45	2.19
2	Arka Swadesh (G_2)	0.53	0.99	2.05	2.82	2.68	2.35
3	IIHRR 13-4 (G_3)	0.57	1.02	2.16	2.30	2.52	2.44
4	Arka Parimala (G_4)	0.57	1.53	2.41	3.09	3.24	3.33
5	*R. indica* (G_5)	0.69	1.13	2.32	2.88	2.60	2.46
6	IIHRR 4-15-12 (G_6)	0.74	2.11	2.82	3.35	3.47	3.54
7	Arka Nishkant (G_7)	0.74	2.27	2.94	3.64	3.44	3.40
8	Knockout (G_8)	0.70	2.62	3.13	3.29	3.39	3.42
	S.Em ±	0.07	-	-	-	-	-
	C.D. @ 5%	0.18	-	-	-	-	-

(Hanifei *et al.*, 2013). It protects the plant cells from oxidative damage caused by ROS (Gill and Tuteja, 2010). The results of present study revealed that inoculated leaves of all genotypes showed sig-nificantly higher levels of CAT activity than those of un-inoculated controls. In inoculated leaves of both resistant and susceptible genotypes, the CAT activity increased during the progress of infection. However, induced levels of CAT were significantly higher during progression of infection in the inoculated leaves of resistant genotypes compared to those of susceptible genotypes. These differences in CAT activity in present study suggested that the low enzyme activity in susceptible genotypes made them less efficient in reducing the high levels of H_2O_2 produced during *D. rosae* infection. These results are in agreement with the findings of El-Komy (2014) who reported increased activity of CAT in resistant genotypes over susceptible ones after inoculation with chocolate spot pathogen of faba bean. Mandal *et al.* (2008) have also reported that a less efficient enzymatic ROS scavenging system, mainly a decrease in CAT activity caused high level of damage caused by *F. oxysporum* f. sp. *Lycopersici*, in tomato. (El-Komy, 2014).

d) PAL activity

The inoculated leaves of all genotypes showed significantly higher levels of PAL activity than those of un-inoculated controls (Fig S7, S8). PAL activity changed significantly in inoculated leaves (I_2) of all genotypes with progression of time after inoculation. In inoculated leaves of all genotypes, PAL activity increased in response to pathogen infection and reached peak by 9th day in all genotypes except in highly resistant variety Knock Out where the peak activity was observed on 6th day itself. Further, the enzyme activity got decreased slightly by 15th day in all genotypes after reaching peak (Fig. S7). In highly resistant variety Knock Out, maximum activity that was recorded on 6th day was 2.95 EU/ g FW (G_8D_3) whereas in *R. multiflora* which was highly susceptible, peak PAL activity was recorded as 1.48 EU/ g FW (G_1D_4) which was observed on 9th day. No significant changes were detected in control leaves (data not presented) throughout the observation period (Fig. S7, S8).

PAL is primary enzyme in the phenylpropanoid metabolism and plays a significant role in the synthesis of several defense-related secondary compounds such as phenols and lignin (Hemm *et al.* 2004; Tahsili *et al.* 2014). The activation of PAL and subsequent increase in phenolic content in plants is a general response associated with disease resistance (Siddique *et al.* 2014). Results of present study revealed that PAL activity was high in resistant genotype compared to susceptible genotypes. This increased activity of PAL in resistant genotypes have lead to more production of defense related secondary compounds which conferred protection against disease. The increased activity of PAL in defense against fungal pathogens in resistant genotypes was also reported in case of brown rust interactions in wheat (Riaz *et al.*, 2014)

e) SOD activity

The SOD activity changed significantly in inoculated leaves of all genotypes with progression in days after inoculation (Fig S9, S10). At a given time period on third day (D_2) immediately after inoculation, highest SOD activity (2.99 EU/ mg FW) was found in moderately resistant genotype Arka Nishkant (G_7D_2) where the lowest activity (1.50 EU/ mg FW respectively) was recorded in highly susceptible genotype *R. multiflora* (G_1D_2). The highly resistant genotype Knock Out (G_8) recorded SOD activity equivalent to 2.91 EU/ mg FW on third day (G_8D_2). On sixth day (D_3) after inoculation, highest SOD activity among all genotypes (3.76 EU/ mg FW) was found in highly resistant genotype Knock Out (G_8D_3) where the lowest activity (2.08 EU/ mg FW) was recorded in highly susceptible genotype *R. multiflora* (G_1D_3). This revealed that the enzyme activity in resistant genotype increased immediately in response to the pathogen infection whereas the enzyme activity increased gradually at a slower pace in susceptible genotype. The SOD activity in highly resistant genotype Knock Out reached its peak on 6th day (3.76 EU/ mg FW) (G_8D_3) after inoculation and thereafter decreased by 15th day (2.21 EU/ mg FW) (G_8D_6) whereas in highly susceptible genotype *R. multiflora*, the activity remained increasing throughout the observation period and reached peak on 15th day (2.60 EU/ mg FW) (G_1D_6). No significant changes in enzyme activity were detected in control leaves throughout the observation period (Fig S9, S10) (data not presented).

SOD is one of the important reactive oxygen species scavenging enzymes which catalyzes the dismutation of superoxide anion radicals (O^{2-}) into H_2O_2 and O_2

(Smirnoff, 1993; Khan and Panda, 2008). H_2O_2 generation in infected plants is considered one of the important defense strategies of plants against the invading necrotrophic pathogen (Hanifei *et al.*, 2013). Results of present study revealed that increased SOD activity was observed in both resistant and susceptible genotypes but the increase was more and quick in resistant ones, in response to pathogen inoculation. In case of susceptible genotypes, though there was increase in enzyme activity, it may not be adequate and quick enough to counter pathogen development, making them susceptible to the disease. Similar results of higher SOD activity in resistant cultivar over susceptible cultivar, after pathogen inoculation were reported in case of chocolate spot disease of faba bean (El-Komy, 2014) and *Mycosphaerella fragariae* infection in strawberry (Ehsani-Moghaddam *et al.* 2006).

f) Total phenols

The inoculated leaves of all genotypes showed significantly higher levels of total phenols during the period of observation than those of un-inoculated controls (Fig S11, S12). In inoculated leaves (I_2) of all genotypes, total phenols changed significantly with progression in time period after inoculation and reached their peak on 9th day inoculation (D_4) and thereby decreased by 15th day (D_6) (Table 6). In highly resistant genotype Knock Out, the total phenols increased sharply and reached peak (81.94 mg/g FW) on 9th day (G_8D_4) whereas in *R. multiflora* which was highly susceptible, total phenols increased comparatively at a slower rate and reached its peak (49.84 mg/g FW) on 9th day (G_1D_4). When the total phenols content of all genotypes was compared on third day (D_2) immediately after pathogen inoculation, highest accumulation (71.94mg/g FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest accumulation (31.59 mg/g FW) was found in IIHRR 13-4 (G_3D_2) which was susceptible to the disease. No significant changes in enzyme activity were detected in control leaves throughout the observation period (Fig S11& S12) (data not presented).

Phenols enhance the mechanical strength of host cell walls by synthesis of lignin and suberin which are involved in the formation of physical barriers that can block the spread of pathogens (Ngadze *et al.* 2012; Singh *et al.* 2014). Further, Khatun *et al.*, 2009 reported that the phenols are fungitoxic in nature. In the present study, the amount of total phenols was significantly higher in inoculated leaves of resistant genotypes, while it was significantly lower in susceptible genotypes. Thus, high accumulation of phenols in resistant genotypes may be playing role in eliciting resistance response against black spot pathogen. The increased phenolic content in resistant genotypes after pathogen inoculation was also reported in case of chocolate spot disease of faba bean (El-Komy, 2014) and in cotton interaction with cotton leaf curl Burewala virus (Siddique *et al.*, 2014).

g) Total flavonoids

The results revealed that inoculated leaves of all genotypes showed significantly higher levels of total flavonoids during the period of observation than those of un-inoculated controls (Fig. S13, S14). Total flavonoids changed significantly in inoculated leaves (I_2) of all genotypes with increase in number of days after inoculation and showing their peak on 9th day after inoculation (D_4) and further decreased by 15th day (D_6) (Table 7). In highly resistant Knock Out genotype, total flavonoids increased sharply and reached peak (35.11 mg/g FW) on 9th day after inoculation (G_8D_4) whereas in *R. multiflora* which was highly susceptible, total flavonoids increased comparatively at a slower rate and reached peak (18.33 mg/g FW) on 9th day (G_1D_4). When the total flavonoids of all genotypes were compared on third day (D_2) immediately after pathogen inoculation, highest accumulation (28.73 mg/g FW) was observed in highly resistant genotype Knock Out (G_8D_2) whereas lowest accumulation (10.57 mg/g FW) was found in IIHRR 13-4 (G_3D_2) which is susceptible to the disease. No significant changes were detected in control leaves throughout the observation period (Fig. S13, S14) (data not presented).

Flavonoids are very important in plant resistance against pathogenic bacteria and fungi. Antipathogenic properties of flavonoids can be non-specific in nature and partly could be the result of their antioxidative properties. Flavonoid compounds are transported to the site of infection and induce the hypersensitivity reaction, which is the earliest defense mechanism employed by the infected plants and programmed cell death (Mierziak *et al.*, 2014) thus restrict the spread

Table 3. CAT activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	CAT activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	6.78	8.10	8.10	8.71	7.73	7.15
2	Arka Swadesh (G_2)	5.08	7.64	9.65	10.71	9.44	8.91
3	IIHRR 13-4 (G_3)	5.38	8.65	7.87	6.54	5.68	5.88
4	Arka Parimala (G_4)	6.60	9.40	10.18	10.63	9.61	8.70
5	*R. indica* (G_5)	5.58	9.83	8.39	7.65	6.10	5.83
6	IIHRR 4-15-12 (G_6)	6.69	12.08	15.02	16.77	17.44	18.28
7	Arka Nishkant (G_7)	6.55	13.12	16.27	17.86	18.88	17.39
8	Knockout (G_8)	4.50	16.06	18.01	18.48	19.83	17.21
	S.Em ±	0.18	-	-	-	-	-
	C.D. @ 5%	0.51	-	-	-	-	-

Table 4. PAL activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	PAL activity (EU/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	0.60	0.86	1.36	1.48	1.41	1.33
2	Arka Swadesh (G_2)	0.71	1.18	1.51	1.80	1.68	1.48
3	IIHRR 13-4 (G_3)	0.88	1.41	1.60	1.91	1.73	1.46
4	Arka Parimala (G_4)	0.85	1.51	1.79	2.07	1.98	1.68
5	*R. indica* (G_5)	0.85	1.40	1.76	1.86	1.70	1.59
6	IIHRR 4-15-12 (G_6)	0.57	1.87	2.03	2.22	2.14	2.10
7	Arka Nishkant (G_7)	0.63	1.94	2.25	2.36	2.24	2.21
8	Knockout (G_8)	0.69	2.48	2.95	2.91	2.71	2.51
	S.Em ±	0.02	-	-	-	-	-
	C.D. @ 5%	0.06	-	-	-	-	-

Table 5. SOD activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	SOD activity (EU/mg FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	1.18	1.50	2.08	2.31	2.41	2.60
2	Arka Swadesh (G_2)	1.20	1.75	2.35	2.27	2.08	1.62
3	IIHRR 13-4 (G_3)	1.23	1.86	2.44	2.53	2.60	2.57
4	Arka Parimala (G_4)	1.18	1.97	2.53	2.76	2.59	2.63
5	*R. indica* (G_5)	1.09	2.02	2.67	2.82	2.81	2.68
6	IIHRR 4-15-12 (G_6)	1.32	2.80	3.30	3.48	3.55	3.58
7	Arka Nishkant (G_7)	1.17	2.99	3.42	3.60	3.30	3.43
8	Knockout (G_8)	1.19	2.91	3.76	3.10	2.61	2.21
	S.Em ±	0.03	-	-	-	-	-
	C.D. @ 5%	0.07	-	-	-	-	-

Table 6. Total phenols (mg/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	Total phenols (mg/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	35.78	42.27	47.07	49.84	45.72	39.14
2	Arka Swadesh (G_2)	28.45	36.40	40.48	43.36	41.33	36.17
3	IIHRR 13-4 (G_3)	26.84	31.59	37.27	46.66	41.25	37.03
4	Arka Parimala (G_4)	34.31	44.79	52.81	56.63	51.49	45.72
5	*R. indica* (G_5)	28.38	41.27	46.46	52.84	49.78	41.42
6	IIHRR 4-15-12 (G_6)	35.20	54.72	67.07	74.04	68.35	57.41
7	Arka Nishkant (G_7)	26.99	54.27	58.09	62.84	59.61	49.93
8	Knockout (G_8)	33.15	71.94	76.77	81.94	71.11	65.91
	S.Em ±	0.66	-	-	-	-	-
	C.D. @ 5%	1.84	-	-	-	-	-

Table 7. Total flavonoids (mg/g FW) in *D. rosae* inoculated leaves (I_2) of rose genotypes (G) at different intervals after inoculation (D)

Sl.No.	Genotypes (G)	Total flavonoids (mg/g FW) in *D. rosae* inoculated leaves (I_2) at different days interval after inoculation (D)					
		Day 0 (D_1)	Day 3 (D_2)	Day 6 (D_3)	Day 9 (D_4)	Day 12 (D_5)	Day 15 (D_6)
1	*R. multiflora* (G_1)	11.86	14.48	17.10	18.33	17.62	15.41
2	Arka Swadesh (G_2)	7.45	12.93	14.11	16.34	14.19	12.96
3	IIHRR 13-4 (G_3)	4.46	10.57	14.46	15.36	12.82	11.11
4	Arka Parimala (G_4)	13.92	20.73	24.89	26.38	24.91	19.48
5	*R. indica* (G_5)	10.85	18.81	24.63	27.45	24.12	19.41
6	IIHRR 4-15-12 (G_6)	13.13	22.87	27.10	29.55	28.89	21.14
7	Arka Nishkant (G_7)	4.60	18.45	21.57	23.85	20.94	18.85
8	Knockout (G_8)	12.38	28.73	32.63	35.11	29.54	19.95
	S.Em ±	0.25	-	-	-	-	-
	C.D. @ 5%	0.68	-	-	-	-	-

of pathogen. In the present study, the amount of total flavonoids was significantly higher in inoculated leaves of resistant genotypes, while it was significantly lower in susceptible genotypes. This high accumulation of flavonoids in resistant genotypes might have contributed for the resistance. Resistance against the fungal infection due to increased accumulation of flavonoids in leaves was also reported in interaction of cedar-apple rust pathogen and apple trees (Lu *et al.*, 2017).

CONCLUSION

The changes in activity of defense related enzymes like CAT, POX, PPO, SOD and PAL and accumulation of plant defense related secondary compounds like phenols and flavonoids were distinguished clearly in inoculated leaves compared to un-inoculated leaves. Further, the trend of either increase or decrease in activity of defense related biochemical compounds was more prominent and varied significantly among the studied genotypes with progression in time period of black spot disease. All studied defense related biochemical compounds increased drastically faster in higher quantities in resistant genotypes compared to susceptible genotypes during disease progression contributing for resistance.

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Original Research Paper

Cropping duration and non-rhizomorphic mycelial phenotype of *Pleurotus djamor* woody1 co-segregate in the hybrid progenies

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ABSTRACT

Crop duration of the cultivated *Pleurotus* spp. is 45 to 50 days. *P. djamor* isolate woody-1 was collected as natural selection and was found to be short cropping duration variety with total cropping duration of 30 days but it is less palatable. It produced very thin, loose and non-rhizomorphic mycelia appearing light white color. Whereas, other commercial *Pleurotus* varieties such as *P. florida* and *P. djamor* MDU1 are long crop duration varieties and palatable producing thick, compact and rhizomorphic mycelia with bright white color. Co-segregation of non-rhizomorphic mycelial phenotype and short cropping duration trait of *P. djamor w*oody-1 in hybrid progenies was evaluated. Hybrid strains *viz*., H2W12 and H2W14 have thin, loose and non-rhizomorphic mycelium and they produced primordia in 9-10 days after spawning with total cropping duration of 29-32 days. Whereas, hybrid strain namely Pf1W2 has thick, compact and rhizomorphic mycelial phenotype and it produced primordia in 20 days after spawning with the total cropping duration of 47 days. This study indicated that genes governing short cropping duration and non-rhizomorphic mycelial pattern were tightly linked and co-segregated in the progenies. Thus, non-rhizomorphic mycelial phenotype of *P. djamor* woody1 can be used as a phenotypic marker for selection of hybrid cultivar having short cropping duration with other desired agronomic traits in future breeding strategy.

Keywords: Basidiocarp, hybridization, mycelium and *Pleurotus*

INTRODUCTION

Mushrooms have been identified as an excellent food source to alleviate malnutrition in developing countries. Oyster mushroom has proteins, carbohydrates, vitamins, calcium, and iron (Hilal *et al.*, 2012). It is a good dietary supplements which can lower cholesterol (Khatun *et al.*, 2007). Globally, *Pleurotus* is the second largest cultivated mushroom after Shiitake (Royse *et al.*, 2017). *Pleurotus* species are popular and widely cultivated throughout the world especially in Asia, America and Europe because of their simple, low-cost production technology and high biological efficiency. *Pleurotus* species are efficient lignin degraders which can grow on a wide variety of agricultural wastes and acclimatize a wide range of temperatures.

The productivity and quality of cultivated edible mushrooms mainly depend on the genetic makeup of the strain (Kaur and Sodhi 2012). The improvement of *Pleurotus* mushroom production primarily utilizes natural selection. The productivity of the mushroom strain can be improved to some extent by manipulating the environmental and physiological conditions during cultivation. However, genetic manipulation of the ruling mushroom variety can enhance the productivity and quality of the mushroom. Genetic manipulation of mushroom can be done by hybridization, protoplast fusion and genetic engineering for strain improvement. Selection and mating of genetically diverse parents is an important approach to exploit heterosis through hybridization. The objectives of mushroom breeding are to obtain *Pleurotus* strain with desirable agronomic traits such as high yield, wider substrate utilisation, spore lessness, wide temperature tolerance, good

palatability, good texture of the fruiting body and resistance to pest and diseases.

Recently, *P. djamor* isolate woody1 was collected as natural selection process and it has short crop duration (30 days) and high biological efficiency. However, it is leathery in sensory while eating, contains less plectenchymatous tissue in the pileus. Thus, it is less palatable (Praveen *et al.,* 2017). But several ruling *Pleurotus* spp. including *P. florida* and *P. djamor* MDU1 are long crop duration varieties with good palatability. In order to transfer the short cropping duration trait into the commercially ruling mushroom cultivar, *P. djamor* woody1 need to be crossed with any of the ruling oyster mushroom and the suitable hybrid possessing short cropping duration along with desired agronomic traits such as good palatability and high yielding potential has to be selected. There are some sequential steps followed for carrying out successful breeding process (hybridization) between two *Pleurotus* spp. starting with collection and culturing of basidiospores; crossing monokaryotic mycelia and evaluation of successful crosses and finally analysis of the hybrid strain for desired agronomic traits in comparison with parental strains (Barh *et al*., 2019). *Pleurotus* has tetrapolar / bifactorial mating system and requires two compatible mating type for dikaryotic mycelial formation and basidiocarp initiation and need to carry out several crosses to get the dikaryons for obtaining hybrid with desired agronomic traits (Raper and Raper, 1966; Casselton and Olesnicky, 1998).

Several crosses need to be made to find a hybrid having short crop duration trait with desired agronomic trait or a hybrid with several desirable traits. Thus, it would be wise to have additional phenotypic marker that could co-segregate with any one of the desirable trait for screening the hybrid having other desired traits. In our previous studies conducted during 2018 and 2020 on breeding between *P. djamor* woody1 and *P. djamor* MDU1 or *P. florida* resulted in several hybrids having both short cropping duration and long cropping duration (Reihana *et al*., 2018; Samundeeswari, 2020). We speculated that short cropping duration and non-rhizomorphic mycelial phenotypes could co-segregate in the hybrid progenies. Keeping these points in mind, cropping duration and mycelial phenotypic characters of three selected hybrid progenies (H2W12, H2W14 and Pf1W2) of *P. djamor* woody1 were analysed upon crossing with *P. florida*. In this study it was found that non-rhizomorphic phenotypic character co-segregate with the short cropping duration in hybrid progenies.

MATERIAL AND METHODS

Pleurotus culture and growth medium

Dikaryotic mycelia isolated from the basidiocarps of different *Pleurotus* spp. *viz*., *P. djamor* woody1, *P. florida*, *P. djamor* MDU1 and hybrids strains *viz*., H2W12, H2W14 and Pf1W2 (obtained upon crossing between *P. djamor* woody1 and *P. florida*) were used in this study. Mycelial cultures were cultured on PDA medium. Spawn production was carried out on sorghum/paddy grains. Mushroom cultivation for analysing the primordial formation and cropping duration was carried out on paddy straw.

Somatic hybridization of different *Pleurotus* spp.

Collection of basidiospores was carried out by placing healthy pileus in sterilized Petri plate in such a way that gills were facing down the bottom plate for an hour to allow shedding of basidiospore from the pileus. Then, the basidiospores were collected by adding 10 ml of sterilized water and counted using haemocytometer. The basidiospore stock suspension was serially diluted to the concentration of 300 spores /ml and about 30 to 100 basidiospores were spread plated using sterilized glass L rod and incubated at 28! for 4 to 6 days or until individual small white mycelial colonies appear with the diameter of 3-5 mm. Markedly fast growing monokaryotic colonies with typical radial growth were identified and sub cultured on fresh PDA plate in a grid form at equi-distance.

Somatic hybridization was carried out between *P. florida* and *P. djamor* isolate woody 1. Dual culture technique was employed for pairing monokaryons of two parental strains at possible combinations. Small discs of monokaryons from two parental strains were cut and inoculated at two centimeters apart from each other on PDA medium at the center of the plate and incubated at 28^{o}C The plates were incubated until two monokaryotic mycelia grow towards each other and the hyphae of two monokaryons were interwoven or fused with each other. A compatible mating consisted of formation of fluffy and vigorous mycelia (with thick and bright white color) at the confrontation zone/merger region of anastomosis/ junction point of two monokaryotic mycelia. From this junction point, the fluffy putative dikaryotic mycelium was taken and

sub-cultured onto the new PDA plate and incubated for five days. Dikaryotic mycelia (crossed/hybrid/ paired mycelia) were further confirmed by the presence of clamp connection under light microscope.

Assessment of mycelial growth of different *Pleurotus* spp.

To assess the radial growth of mycelium and mycelial growth pattern, the dikaryotic mycelia of *P. djamor* woody1, *P. florida*, *P. djamor* MDU1 and hybrid strains *viz*., H2W12, H2W14 and Pf1W2 were inoculated onto the PDA medium. The cultures were incubated at 28°C. Three replications were maintained for each culture. The radial growth of the mycelium was recorded when anyone of the isolates completely covered the Petri plate. Mycelial growth patterns such as fluffiness, color and rhizomorphic pattern were noted.

Spawn preparation

The spawn preparation was carried out as described by Krishnamoorthy *et al*. (2005). The paddy or sorghum grains were washed in water thoroughly to remove chaffy and damaged grains. The grains were cooked in vessel for 30 minutes just to soften them. Then, the excess water from the cooked grains was drained off and grains were spread evenly over a hessian cloth on a platform to remove the excess water. At 50% moisture level, calcium carbonate ($CaCO_3$) was applied on the grains (dried grains) @ 40 g /kg of grains. Then, the grains were filled in polythene bags up to 3/4th height (approximately 300-330 g / bag), PVC ring was inserted, edges were folded down and the mouth of the bag was plugged tightly with non-absorbent cotton. After plugging with cotton plug, the bag was covered with a piece of paper and tied tightly around the neck with a jute thread or a rubber band. The bags were arranged inside an autoclave and sterilized at 20 lbs for 2 hours. Then, the bags were taken after cooling and kept inside the laminar air flow chamber for inoculation.

The mycelial culture (10 mm diameter disc) of *Pleurotus* spp. was cut and transferred to a bag. The inoculated bags were incubated in a clean room under room temperature (28±2°C). The spawn running period was recorded.

Bed preparation

Paddy straw was used as a substrate for the bed preparation. The substrate was cut into 5 cm long bits, soaked in cold water for 4 hours and pasteurized in hot water for 30 min at 80°C. The transparent polythene bags (30 x 60 cm length and 80-gauge thickness) were used for the cultivation of oyster mushroom. Initially, hands were thoroughly washed with alcohol. The bottom end of the bag was tied with a thread and the bag was turned inside out. Then, the dried straw was mixed thoroughly to get a uniform moisture level in all areas. The well-grown bed spawn was taken out, squeezed thoroughly and divided into two halves. (Two beds are prepared from the single spawn bag). Bits of chopped straw (5 cm long) were placed at the bottom of polythene bag to make a layer (10 cm height) on which 40 g of spawn was sprinkled. Second layer of straw to a height of 10 cm was placed and 40 g of spawn was sprinkled on top of the second layer. In the same way, five layers of straw and four layers of spawn were kept in the polythene bag and finally the bag was tied at the top. Six ventilation holes of one-cm diameter were made at random in the polythene bag. Then, these beds were kept in spawn running room where the temperature was maintained at 28°C. The fully spawn run beds were taken to the cropping room in which the temperature was maintained at 25±2°C and RH- > 80% for initiation of basidiocarp (Krishnamoorthy *et al.*, 2005).

Primordia formation and cropping duration assessment

The days required for the primordia formation were recorded after spawning and the days required for the harvest of the first, second and third flushes and total cropping duration of each variety were recorded. The yield and biological efficiency were recorded.

RESULTS AND DISCUSSION

Assessment of mycelial growth pattern of different *Pleurotus* spp.

To assess which *Pleurotus* spp. grow actively on the culture media, six isolates of *Pleurotus* spp. *viz*., *P. djamor* woody1, *P. florida*, *P. djamor* MDU1 and hybrid strains *viz*., H2W12, H2W14 and Pf1W2 were cultured on PDA medium. Among the various *Pleurotus* spp. tested, *P. djamor* isolate woody1 grew to the maximum level of 88.67 mm followed by the hybrid strains Pf1W2 (86.33), H2W12 (85.67 mm), H2W14 (85.33 mm) and *P. djamor* isolate MDU1 (77.67 mm). Whereas, the minimum mycelial growth was recorded by *P. florida* on PDA medium (75 mm) (Fig. 1 and Table 1).

Table 1: Phenotypic characters of different *Pleurotus* spp.

Pleurotus strains	Radial mycelial growth (mm)*	Mycelial growth pattern
P. djamor woody1	88.67[a]	Thin, loose and non-rhizomorphic growth; dull white in color
Hybrid - H2W12	85.67[c]	Thin, loose and non-rhizomorphic growth; dull white in color
Hybrid - H2W14	85.33[e]	Thin, loose and non-rhizomorphic growth; dull white in color
Hybrid Pf1W2	86.33[b]	Thick, compact and rhizomorphic growth; bright white in color
P. florida	75.00[d]	Thick, compact and rhizomorphic growth; bright white in color
P. djamor MDU1	77.67[e]	Thick, compact and rhizomorphic growth; bright white in color

*Mean of three replications

In the column, mean values followed by a common letter are not significantly different (pd"0.05, DMRT analysis).

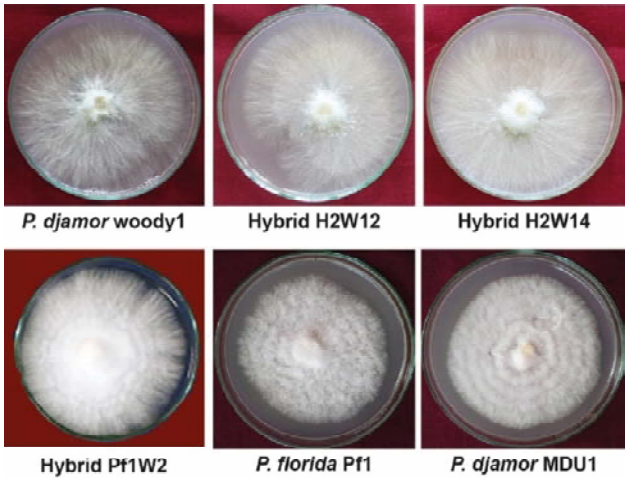

Fig. 1. Colony characters of different *Pleurotus* spp.

Mycelia of *P. djamor* woody1 (one of the parental strains used for hybridization) appeared as thin, loose and non-rhizomorphic filament and light white in color. Similarly, the hybrid strains such as H2W12 and H2W14 also produced thin, loose and non-rhizomorphic filament and light white in color. Whereas, hybrid strain namely Pf1W2 produced thick, compact and rhizomorphic mycelium and appeared bright white in color as that of other parental strain *P. florida* (Fig. 1 and Table 1). Mostly, the mycelial growth phenotype of *Pleurotus* appears as rhizomorphic like radial growth with thick and white in color. But, mycelial growth characters of *P. djamor* woody1 and some of its hybrid progeny appeared as loose, thin and non-rhizomorphic type. This is the important phenotypic and distinguishing character for the identification of this isolate during culturing time. Similar type of varied mycelial phenotypic characters in different *Pleurotus* spp. was noticed in different *Pleurotus* spp. Mycelia of *P. sajor-caju* and *P. platypus* were compact. Whereas, mycelia of *P. citrinopileatus* were highly fluffy. Similarly, mycelial pattern in *P. fossulatus, P. flabellatus, P. sapidus* and *P. ostreatus* was slightly fluffy (Mishra *et al.*, 2015).

Spawn running period and mycelial pattern

Days required for spawn development was analysed for different *Pleurotus* cultivars such as *viz.*, *P. djamor* woody1, *P. florida*, *P. djamor* MDU1 and hybrid strains *viz.*, H2W12, H2W14 and Pf1W2. Days required for spawn development for *P. djamor* woody1 and hybrid strains *viz.*, H2W12 and H2W14 were ranged from12 to 15 days and that for hybrid strain namely Pf1W2 and *P. florida* were 16 to 17 days. Mycelial growth pattern of *P. djamor* woody1 and hybrid strains such as H2W12 and H2W14 appeared as thin, loose and non-rhizomorphic filament and light white in color on spawn substrates (sorghum/paddy grains) as observed on PDA medium. Whereas, hybrid strain namely Pf1W2 and other parental strain namely. *florida* produced thick, compact and rhizomorphic mycelium and appeared bright white in color on spawn substrates as grown on PDA medium (Table 2). In other studies, it was reported that *P. eous* covered the spawn within 7 to 20 days on different grains used as spawn substrate (Sahu *et al.*, 2014). Blue oyster mushroom took spawn 18.5 days for the spawn production when paddy grain was used as a substrate (Sumi and Geetha, 2017).

Days required for primordia formation

Days required for primordia formation was analysed for different *Pleurotus* cultivars such as *P. djamor* woody1, *P. florida*, *P. djamor* MDU1 and hybrid strains *viz.*, H2W12, H2W14 and Pf1W2. Days required for primordia formation for

Table 2. Agronomic traits for the different *Pleurotus* spp.

Pleurotus strains	Spawn running period (days)*	Days for primordia formation (days)*	Days for harvest			Yield (g/ bed) *	Biological Efficiency (%)*	Pileus characters
			1st harvest (days)*	2nd harvest (days)*	3rd/Crop duration (days)*			
P. djamor woody1	12.67^{a}	9.33^{a}	13.00^{a}	19.00^{a}	30.00^{a}	475.00^{ab}	95.00^{ab}	Wavy margin
Hybrid - H2W12	14.33^{b}	10.67^{ab}	14.33^{ab}	21.67^{b}	32.67^{b}	456.67^{bc}	91.33^{bc}	Slightly wavy margin
Hybrid - H2W14	14.67^{b}	11.67^{b}	15.33^{b}	22.00^{b}	32.00^{b}	499.33^{a}	99.87^{a}	Wavy margin
Hybrid Pf1W2	16.33^{c}	20.00^{c}	24.67^{c}	36.33^{c}	47.00^{c}	450.00^{bc}	90.00^{bc}	Smooth margin
P. florida	16.00^{c}	21.00^{c}	25.00^{c}	36.00^{c}	48.00^{c}	433.00^{c}	86.60^{c}	Smooth margin
P. djamor MDU1	17.00^{c}	20.00^{c}	24.00^{c}	35.00^{c}	47.67^{c}	440.00^{c}	88.00^{c}	Smooth margin

*Mean of three replications

In the column, mean values followed by a common letter are not significantly different (pd"0.05, DMRT analysis).

P. djamor woody1 and that for hybrid strains *viz*., H2W12 and H2W14 were ranged between 9 to 12 days and that for hybrid strain Pf1W2, parental strain *P. florida* were 20 to 21 days. In general, primordia (pin head formation) formation of *Pleurotus* spp. occurs at 20 days after spawning (Table 2). Ahmed (1998) reported that pinhead formation of oyster mushroom occurred between 23 and 27 days from spawning in different substrates. Fan *et al*. (2000) observed that pinhead formation took place between 20-23 days. Patra and Pani (1995) also recorded 20-24 days taken for the pinhead formation on paddy straw substrate.

Days required for basidiocarp production and total crop duration

Days required for the first flush basidiocarp production for *P. djamor* woody1 and hybrid strains *viz*., H2W12 and H2W14 were between 13 to 15 days and that for hybrid strain Pf1W2 and parental strain *P. florida* was 24 to 25 days after spawning. Similarly, days required for the second flush basidiocarp production for *P. djamor* woody1 and hybrid strains *viz*., H2W12 and H2W14 ranged between 19 to 23 days and that for hybrid strain Pf1W2, parental strains *viz*., *P. florida* and *P. djamor* MDU1 was 35 to 36 days. Total cropping duration for the *P. djamor* woody 1 and hybrid strains *viz*., H2W12 and H2W14 ranged between 30 to 33 days and that for *P. florida* and *P. djamor* MDU1 was 47 to 48 days. Margin and outer surface of basidiocarps of *P. djamor* woody1 and hybrid H2W14 appeared wavy and the hybrid H2W12 appeared slightly wavy. Whereas, margin and outer surface of basidiocarps of hybrid Pf1W2, *P. florida* and *P. djamor* MDU1 appeared smooth. The hybrid H2W14 gave the highest yield of 499.33 g with biological efficiency of 99.87 % followed by *P. djamor* isolate woody 1 (475.00 g and 95 %),

Fig. 2. Basidiocarp phenotypic characters of different *Pleurotus* spp.

hybrids H2W12 (456.67 g and 91.33 %), Pf1W2 (450.00g and 90.00 %) and *P. djamor* var MDU 1 (440.0 g and 88.00 %) and *P. florida* (433.00 g and 86.60 %). (Fig. 2; Table 2). Baral *et al*. (2017) developed an intraspecific hybrid of *P. flabellatus* showing better nutritional quality, earliness in production and higher yield compared to their parental strains. Interspecific hybrids *viz.,* P1xC9 and P3xC8, obtained by crossing between *P. citrinopileatus* and *P. pulmonarius*, showed desirable traits such as higher productivity and biological efficiency and less offensive aroma compared to their parental strains (Rosnina *et al*., 2016).

Thus, this study clearly showed that hybrid strains *viz*., H2W12 and H2W14 are short crop duration varieties with non-rhizomorphic mycelial type. Whereas, hybrid strain Pf1W2 is long cropping duration variety with rhizomorphic mycelial type. The present study clearly showed that short crop duration phenotype and non-rhizomorphic phenotype co-segregate together in the hybrid strains. Thus, from this study, it was concluded that non-rhizomorphic mycelium character can be used as a phenotypic marker to screen and select the short duration hybrid strains with additional desirable agronomic traits in the breeding program. *Pleurotus* breeding program involves five steps such as 1. collection of basidiospores 2. culturing of individual basidiospore to form monokaryotic mycelium. 3. crossing/ mating between monokaryotic mycelia of two *Pleurotus* spp. 4. evaluation of successful cross/dikaryon by checking clamp connection and 5. analysis of the hybrid strain for desired agronomic traits by mushroom cultivation (Petersen and Ridley, 1996). This study showed that only putative dikaryotic crosses/ hybrids having non-rhizomorphic phenotype at the fourth step of breeding program could be further evaluated in the fifth step for screeding/analysis of the hybrid progenies having short crop duration with other desired agronomic traits. Thus, co-segregating phenotypic marker saves the time and speed up the screening process of development of hybrid strain having short cropping duration with desired agronomic traits such as good palatability and high yielding potential. Hence, having co-segregating phenotypic marker (non-rhizomorphic phenotype) with short cropping duration traits of *P. djamor* woody1 would facilitate in speeding up breeding program with other commercially cultivated ruling *Pleurotus* cultivar.

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Original Research Paper

Morphological, physiochemical and colour characteristics of fresh and cured starch in potato varieties

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ABSTRACT

The present study was conducted to study the morphological, physicochemical and colour characteristics of potato starch extracted by control and combined methods from potato varieties *viz*., Kufri Chipsona-4, Badshah, Pushkar, Bahar and Sindhuri (fresh and cured). Among these varieties, Kufri Chipsona-4 exhibited maximum percent of small size (< 30 µm) particles (48%). Kufri Sindhuri showed highest starch purity (87.1%) but lowest whiteness (92.2%) whereas, highest whiteness (95.4%) was recorded in starch extracted from Kufri Badshah. Among starch extraction methods, combined method showed significantly lower starch moisture content (11.8%), fat (0.28%), protein (0.31%), ash (0.28%) and crude fibre (0.15%) whereas; starch purity (87.2%), percentage of small size particles (45%) and starch whiteness (96.3%) were observed higher than control methods in all varieties.

Keywords: Curing, starch purity, starch whiteness and tuber

INTRODUCTION

Potato (*Solanum tuberosum* L.), the fourth most important vegetable crop, serves as an important raw source for starch extraction and applications in food industry. Potato starch can form thick visco-elastic gel unlike millet starches due to its composition of phosphate ester groups on amylopectin, larger granule size, longer amylose and amylopectin chain length, and higher purity (Singh *et al.,*2003). Its major application in food industry is limited by properties such as low shear resistance, thermal decomposition and thermal resistance, and its higher tendency towards retrogradation (Avula and Singh 2009). These limitations can be easily overcome by modification of extracted starch using extraction methods to meet the demands of final product (Liu *et al.,*2003). Changes in methods of extraction affect yield and recovery, cost, product purity, desired physico-chemical properties, and mechanical properties of starch. Potato starch is unique compared to cereal starches (corn, wheat, rice, etc.) because of its wider granule size and purity, longer amylose and amylopectin chain length, presence of phosphate ester groups on amylopectin, ability to exchange certain cations with corresponding effects on viscosity behaviour, ability to form a thick viscoelastic gel upon heating and subsequent cooling in water, and poor thermal as well as shear stability of this gel (Singh *et al.,* 2003). Pre-treatments such as curing have also been reported to affect yield and amylose content of starch.

This investigation was thus performed out with an aim to characterize the morphological and physiochemical characteristics of potato starch extracted by control and combined method (extraction with ambient water 30^{o}C + 0.25% NaOH + 2% w/v SDS: ME + 5.25% NaOCl + 0.15% cellulase enzyme) from fresh and cured tubers of five cultivars to identify varieties of potato with highest starch content so as to aid the farmers and industry.

MATERIALS AND METHODS

Plant material

The fresh harvested potato tuber (*Solanum tuberosum* L.) of Kufri Chipsona-4 (V_1), Kufri Badshah (V_2), Kufri Pushkar (V_3), Kufri Bahar (V_4) (white flesh

varieties) and Kufri Sindhuri (V_5) (pink flesh variety) were procured from Vegetable Farm, CCS Haryana Agricultural University, Hisar.They were sorted and cured without packaging in a BOD (Biological Oxygen Demand) incubator at ~22 °C temperature and 90% relative humidity in the dark for 18 days.

Extraction of starch

Fresh and cured potato tubers were used for starch extraction. For control extraction, starch was extracted as described by Peshin (2001) with slight modifications. For combined extraction, a combined method of Phogat *et al.,* (2020) (extraction with water at 30°C +0.25% NaOH + 2% w/v SDS:ME + 5.25% NaOCl + 0.15% cellulase enzyme) was used. The starch was analysed for the following parameters:

Physico-chemical properties

Potato starch was analysed for moisture, crude protein, fat, ash, and crude fibre content by the AOAC (2006) method. Starch yield (%) or crude starch content was calculated by the following formula:

$$\text{Starch yield (\%)} = \frac{\text{Extracted starch}}{\text{Total amount of raw potato tubers}} \times 100$$

Starch purity (%) was calculated with the following formula:

Purity (%) = [100 – (moisture + fatty materials+ crude protein+ ash + crude fibre)]

Colour of starch: whiteness value [L* (whiteness or blackness), a* (redness or greenness) and b* (yellowness or blueness)] was measured by Hunter Lab Colorimeter (Colour flex, USA).

$$\text{Whiteness} = 100 - [(100-L)^2 + a^2 + b^2]^{1\backslash 2}$$

Morphological properties

The shape and size of extracted starch particles were ascertained using an inverted compound microscope (Olympus, Japan; model: CX-41with 10× magnification) equipped with a digital camera. Starch particle size was measured using calibrated ocular scale fitted on the microscope lens.

Statistical analysis

The factorial CRD was used with three replications for analysis using OPStat software (Sheoran *et al.,*1998). Means were separated by critical difference (CD) at 5% significance level. Principal component analysis (PCA) was performed with PAST-3 software.

RESULTS AND DISCUSSION

Physico-chemical properties

Varieties, curing and extraction methods had significant effect on physico-chemical properties of starch. Moisture content was varied from 11.7 to 12.6% (Table 1). Combined extraction method had lower starch moisture content.V_5 had least (11.7%) starch moisture content and it was maximum (12.6%) in V_2. The starch fat content ranged from 0.33 to 0.43% (Table 1). Combined treatment has significantly lower fat content. There was no significant difference in starch fat (%) extracted from 5 varieties, except V_5 which exhibited significantly lower fat content (Table 3). The starch protein (%) of potato varieties ranged from 0.35 to 0.48% (Table 1). Combined treatment has significantly lower protein content. It was recorded minimum (0.35%) in V_5 and maximum (0.48%) in V_4 (Table 4). For all the varieties, there was nonsignificant effect of curing on starch moisture, fat and protein content (Table 2, 3 and 4). The starch ash content ranged from of 0.32 to 0.36% (Table 1). Variety and curing did not significantly affect ash content (Table 5). The starch crude fiber content ranged from 0.15 to 0.23% (Table 1). Combined treatment extracted starch had significantly lower ash and crude fiber. It was minimum (0.15%) in V_3 and it was maximum (0.23%) in V_1 (Table 6). Curing had non-significant affect in crude fiber.

The slight difference with respect to moisture content could be the result of extraction method, varieties, and curing (Table 2). Kim and co-workers (1995) reported differences ranging from 7.2-16.70% in starch moisture contents among 42 potato varieties. Karmakar *et al.,* (2014) compared the moisture content of potato with taro and corn starch and pointed that starch moisture content also depends on the extent of drying. Similar was the observation by Abegunde *et al.,* (2013). The lower fat (Table 3) and protein content (Table 4) in starch extracted by combined treatment attributed to the action of alkali and SDS used during extraction. NaOH, an alkali solvent, can easily solubilize major proteins enclosing the starch and thus soften-up the protein-starch matrix. Kaur and co-workers (2007) observed that the Kufri Sindhuri had highest ash content and Kufri Chandarmukhi the lowest.

Starch purity

The starch purity varied between 86.0 to 87.1% (Table 1). Variety and curing did not significantly affect the starch purity (Table 7). The starch purity for all the potato varieties was observed significantly higher

Table 1. Summary statistics of starch characteristics of potato varieties.

Starch characteristics	Min	Max	S.D.	Skewness	Kurtosis	Coeff. Var
Moisture content (%)	11.70	12.60	0.34	-0.28	-0.09	2.81
Fat (%)	0.33	0.43	0.04	-1.81	3.25	10.47
Protein (%)	0.35	0.48	0.06	-0.24	-2.91	14.49
Ash (%)	0.32	0.36	0.01	0.55	0.87	4.39
Crude fibre (%)	0.15	0.23	0.03	0.61	-0.68	17.44
Purity (%)	86.00	87.10	0.45	0.38	-1.14	0.52
Whiteness (%)	92.20	95.40	1.41	0.45	-2.59	1.50
Small size particles (%)	41.00	48.00	2.86	0.31	-1.54	6.48

Table 2. Moisture content (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	13.4±0.54	12.2±0.51	12.8	10.4±0.47	12.5±0.64	11.5	11.9	12.4	12.1
Kufri Badshah (V_2)	11.7±0.68	13.3±0.78	12.5	11.4±0.41	13.9±0.76	12.7	11.6	13.6	12.6
Kufri Pushkar (V_3)	12.5±0.88	11.7±0.74	12.1	12.7±0.81	11.4±0.39	12.0	12.6	11.6	12.1
Kufri Bahar (V_4)	13.5±0.71	12.8±0.64	13.2	11.7±0.85	11.7±0.59	11.7	12.6	12.3	12.4
Kufri Sindhuri (V_5)	12.5±0.58	12.4±0.62	12.5	11.4±0.61	10.5±0.60	11.0	12.0	11.5	11.7
Mean			12.6			11.8	12.1	12.3	
CD at 5%	Varieties (V) = 0.54			Curing (C) = NS			Methods (M) = 0.35		
V×M = 0.78	V×C = 0.78			M×C = NS			V×M×C = 1.11		

Mean±SD; NS – non-significant

Table 3. Fat content (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	0.59±0.15	0.58±0.11	0.58	0.28±0.08	0.24±0.05	0.26	0.44	0.41	0.42
Kufri Badshah (V_2)	0.48±0.06	0.53±0.03	0.50	0.22±0.06	0.36±0.07	0.29	0.35	0.44	0.40
Kufri Pushkar (V_3)	0.47±0.11	0.60±0.05	0.53	0.34±0.09	0.34±0.08	0.34	0.40	0.47	0.43
Kufri Bahar (V_4)	0.56±0.06	0.57±0.16	0.56	0.29±0.04	0.30±0.09	0.29	0.43	0.43	0.43
Kufri Sindhuri (V_5)	0.47±0.05	0.43±0.09	0.45	0.17±0.05	0.25±0.07	0.21	0.32	0.34	0.33
Mean			0.53			0.28	0.39	0.42	
CD at 5%	Varieties (V) = 0.07			Curing (C) = NS			Methods (M) = 0.04		
V×M = NS	V×C = NS			M×C = NS			V×M×C = NS		

Mean±SD; NS – non-significant

Table 4. Protein content (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	0.48±0.08	0.53±0.08	0.51	0.34±0.17	0.38±0.05	0.36	0.41	0.46	0.43
Kufri Badshah (V_2)	0.55±0.15	0.61±0.11	0.58	0.35±0.18	0.36±0.19	0.35	0.45	0.48	0.47
Kufri Pushkar (V_3)	0.46±0.07	0.41±0.03	0.44	0.29±0.09	0.27±0.04	0.28	0.37	0.34	0.36
Kufri Bahar (V_4)	0.70±0.01	0.67±0.12	0.68	0.30±0.06	0.25±0.13	0.28	0.50	0.46	0.48
Kufri Sindhuri (V_5)	0.38±0.06	0.49±0.05	0.43	0.28±0.14	0.26±0.04	0.27	0.33	0.37	0.35
Mean			0.53			0.31	0.41	0.43	
CD at 5%	Varieties (V) = 0.09			Curing (C) = NS			Methods (M) = 0.06		
V×M = 0.13	V×C = NS			M×C = NS			V×M×C = NS		

Mean±SD; NS – non-significant

Table 5. Ash content (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	0.32±0.06	0.37±0.06	0.35	0.30±0.05	0.31±0.05	0.31	0.31	0.34	0.33
Kufri Badshah (V_2)	0.39±0.04	0.41±0.09	0.40	0.27±0.04	0.31±0.02	0.29	0.33	0.36	0.34
Kufri Pushkar (V_3)	0.36±0.05	0.40±0.05	0.38	0.31±0.03	0.21±0.05	0.26	0.34	0.30	0.32
Kufri Bahar (V_4)	0.42±0.03	0.39±0.06	0.41	0.26±0.02	0.28±0.04	0.27	0.34	0.33	0.34
Kufri Sindhuri (V_5)	0.36±0.03	0.47±0.07	0.42	0.34±0.06	0.26±0.03	0.30	0.35	0.37	0.36
Mean			0.39			0.28	0.33	0.34	
CD at 5%	Varieties (V) = NS			Curing (C) = NS			Methods (M) = 0.03		
V×M = NS	V×C = NS			M×C = 0.04			V×M×C = 0.08		

Mean±SD; NS – non-significant

Table 6. Crude fibre content (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	0.26±0.09	0.30±0.08	0.28	0.15±0.04	0.20±0.05	0.17	0.21	0.25	0.23
Kufri Badshah (V_2)	0.17±0.03	0.24±0.06	0.20	0.16±0.04	0.22±0.07	0.19	0.17	0.23	0.20
Kufri Pushkar (V_3)	0.19±0.04	0.15±0.04	0.17	0.11±0.04	0.13±0.04	0.12	0.15	0.14	0.15
Kufri Bahar (V_4)	0.28±0.06	0.16±0.07	0.22	0.15±0.07	0.13±0.06	0.14	0.21	0.14	0.18
Kufri Sindhuri (V_5)	0.17±0.05	0.25±0.09	0.21	0.10±0.03	0.11±0.04	0.11	0.14	0.18	0.16
Mean			0.22			0.15	0.18	0.18	
CD at 5%	Varieties (V) = 0.04			Curing (C) = NS			Methods (M) = 0.03		
V×M = NS	V×C = 0.06			M×C = NS			V×M×C = NS		

Mean±SD; NS – non-significant

Table 7. Purity (%) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	85.0±0.98	86.0±0.95	85.5	88.5±0.83	86.4±0.66	87.4	86.7	86.2	86.5
Kufri Badshah (V_2)	86.7±0.41	84.9±0.74	85.8	87.5±0.85	84.9±0.51	86.2	87.1	84.9	86.0
Kufri Pushkar (V_3)	86.0±0.79	86.7±0.73	86.4	86.3±0.39	87.7±0.82	87.0	86.2	87.2	86.7
Kufri Bahar (V_4)	84.5±0.92	85.4±0.58	85.0	87.3±0.80	87.3±0.55	87.3	85.9	86.4	86.1
Kufri Sindhuri (V_5)	86.1±0.77	85.9±0.69	86.0	87.7±0.85	88.6±0.44	88.1	86.9	87.2	87.1
Mean			85.7			87.2	86.6	86.4	
CD at 5%			Varieties (V) = NS		Curing (C) = NS		Methods (M) = 0.38		
V×M = 0.85			V×C = 0.85		M×C = NS		V×M×C = 1.20		

Mean±SD; NS – non-significant

(87.2%) when starch was extracted by combined treatment. Pure starch had lower protein, fat, and ash content. Thus, the non-significant differences observed in purity of starches from different varieties was due to the nonsignificant differences in fat and ash contents of their starches (Table 5 and 6). Abegunde and co-workers (2013) reported that starch purity was reasonably high (>91%) in sweet potato cultivars due to low starch impurities (moisture, fat, protein, ash, and crude fibre). In the present study starch purity was maximum in V_1 because it had less impurities (Table 7). Combined extraction resulted in significantly lower crude fibre, fat, protein and ash contents of starch hence combined treatment had lower impurity content in starch and thus produced starch with higher purity. Starch paste thought to be clear and did not contain any off colouration, especially if it's to be used in food application. Kordylas (1990) reported that impurities in form of moisture, fat, protein, ash and crude fibre content decrease the starch whiteness value.

Principal component analysis (PCA)

PCA was performed keeping in mind the characteristics of starch among the potato varieties. The eigenvalue, variance contribution rate of PCs and the cumulative variance are presented in Table 10. The first three PCs with eigen values >1.0 accounted for 92.71 % of variation among potato varieties. Other PCs were not interpreted since they had eigen values <1.0.The first PC, explained 56.56 % of total variation. Eigen vector of the first principal component had high loading values for starch moisture content (0.41), protein content (0.41), purity (-43) and whiteness (0.38). Second principal component which represented 21.47 % of total variation mainly represented the starch ash (0.56), fat (-44) content and starch small size particles (-0.53). Third principal component explained mainly crude fibre (0.64). The biplot between PC1 and PC2 (Fig 2) compares the potato varieties based on their starch characteristics.

Starch whiteness

Starch whiteness ranged from 92.2 to 95.4 (Table 1). Varieties, extraction methods and curing had significantly affected starch whiteness value. It was minimum (92.2) in V_5 and it was maximum (95.4) in V_2. For all the varieties, combined extraction method had significantly higher starch whiteness value and curing of potatoes resulted in significantly lower whiteness value of extracted starch (Table 8).

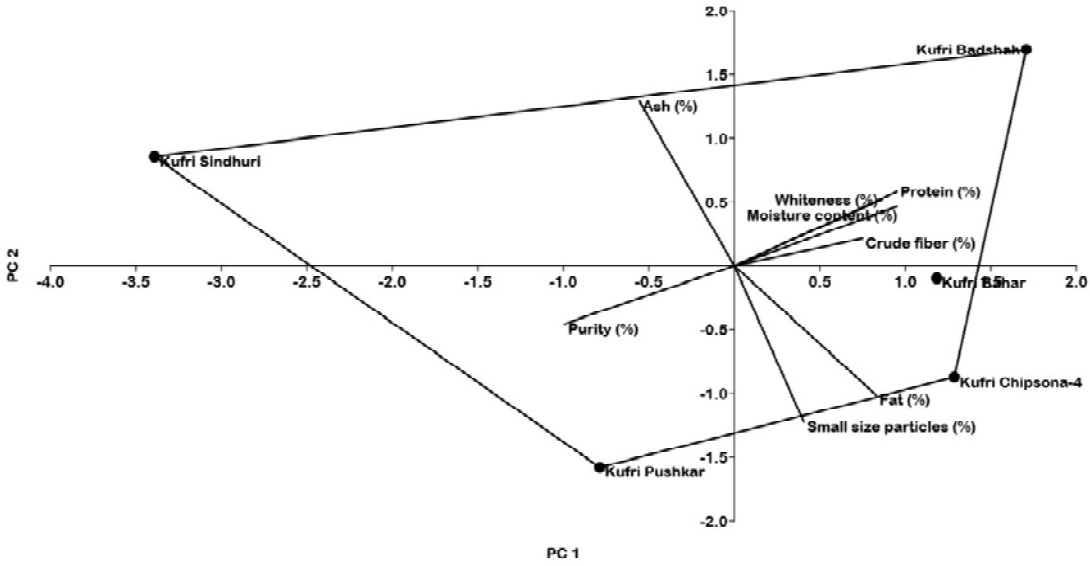

Fig. 2. Segregation of the potato varieties based on their respective starch traits as determined by PCA.

Combined method extracted starch had significantly higher starch whiteness because of bleaching action of chemicals or by decreased moisture content, protein, fat, ash, and crude fibre contents which act as impurity. Colour is an important criterion for starch quality, especially for use in various types of food products. Minimum whiteness value was recorded in starch extracted from Kufri Sindhuri (92.2) due to its pink flesh and maximum (95.4) in V_2 (Table 8). Curing resulted in lower starch whiteness. Abegunde and co-workers (2013) also reported different whiteness values of starches extracted from varieties of sweet potato using multiple extraction methods to remove pigments from starch. This is in agreement with the reports of Hu and co-workers. (2011) who observed that starch colour isolated from two-day old root was slightly grey.

Morphological properties

The percentage of small size particles (< 30 µm) in different potato varieties ranged from 41% to 48%. Curing and method of extraction non significantly affected the percentage of small size particles. Minimum number of small size particles was observed in V_2 (41%) and V_5 (42%) and maximum in V_1 (48%). In the present investigation, Minimum number of small size particles was observed in V2 (41%) and V5 (42%) and maximum in V1 (48%) (Table 9& Figure 1). Minimum number of small size particles was observed in V2 (41%) and V5 (42%) and maximum in V1 (48%) (Table 9). This may be attributed to difference in temperature of the locations during tubers growth. Singh and Singh (2001) documented small and large starch granules of 15-20 µm and 20-45 µm respectively, with shapes ranging from oval to irregular or cuboidal, which may be attributed to difference in tubers growth. Further, it has also been reported that starch granule size is directly proportional to the weight of a potato tuber (Liu *et al.,* 2003). During tuber development, the membranes and physical characteristics of plastids differ among potato varieties and this in turn lead to difference among shape of starch granules among varieties (Lindeboom *et al.,* 2004). Physicochemical properties of starch had been linked to difference in its granule shape and size.

Skewness and kurtosis

Skewness and kurtosis were calculated to analyse the genetic difference among potato varieties. The positive skewness was obtained for starch small size particles, yield, ash content, crude fibre, purity and whiteness whereas negative skewness was found for starch moisture content, fat and protein. The starch fat and ash content showed platykurtic distribution (positive) pattern. Leptokurtic distribution (negative) was followed by starch small size particles, crude fibre, purity, whiteness, peak viscosity, moisture, and protein content (Table 1).

Table 8. Colour value (whiteness) of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
Kufri Chipsona-4 (V_1)	94.4±0.51	90.9±0.64	92.6	97.4±0.67	97.0±0.86	97.2	95.9	93.9	94.9
Kufri Badshah (V_2)	95.8±0.64	91.1±0.78	93.5	97.9±0.53	96.8±0.51	97.4	96.9	94.0	95.4
Kufri Pushkar (V_3)	90.8±0.87	88.7±0.77	89.7	95.3±0.72	96.0±0.84	95.7	93.0	92.4	92.7
Kufri Bahar (V_4)	91.8±0.84	90.1±0.49	91.0	95.2±0.77	95.4±0.65	95.3	93.5	92.8	93.1
Kufri Sindhuri (V_5)	88.6±0.79	88.2±0.58	88.4	95.8±0.60	96.1±0.61	95.9	92.2	92.1	92.2
Mean			91.0			96.3	94.3	93.0	
CD at 5%	Varieties (V) = 0.56			Curing (C) = 0.36			Methods (M) = 0.36		
V×M = 0.80	V×C = 0.80			M×C = 0.50			V×M×C = NS		

Mean±SD; NS – non-significant

Table 9 Percent of small size (< 30 µm) particles of starch as influenced by varieties, curing and extraction methods.

Varieties	Extraction methods						Curing		Overall mean
	Control method			Combined treatment					
	Fresh	Cured	Mean	Fresh	Cured	Mean	Fresh	Cured	
V1	45	49	47	47	51	49	46	50	48
V2	39	40	40	41	43	42	40	42	41
V3	42	43	43	44	45	45	43	44	44
V4	43	46	45	46	48	47	45	47	46
V5	40	42	41	42	44	43	41	43	42
Mean			43			45	43	45	
CD at 5%	Varieties (V) = 4			Curing (C) = NS			Methods (M) = NS		
V×M =6	V×C = 6			M×C = 4			V×M×C = 6		

Mean±SD; NS – non-significant

Table 10. Principal component (PC) loadings for quality variables of the potato starch.

Starch characteristics	PC1	PC2	PC3
Moisture content (%)	0.41	0.20	-0.36
Fat (%)	0.36	-0.44	-0.25
Protein (%)	0.41	0.25	0.00
Ash (%)	-0.24	0.56	0.23
Crude fiber (%)	0.33	0.09	0.64
Starch purity (%)	-0.43	-0.20	0.23
Starch whiteness (%)	0.38	0.23	0.28
Small size particles (%)	0.18	-0.53	0.46
Eigen value	4.52	1.72	1.17
% Variance	56.56	21.47	14.67
Cumulative variance (%)	56.56	78.04	92.71

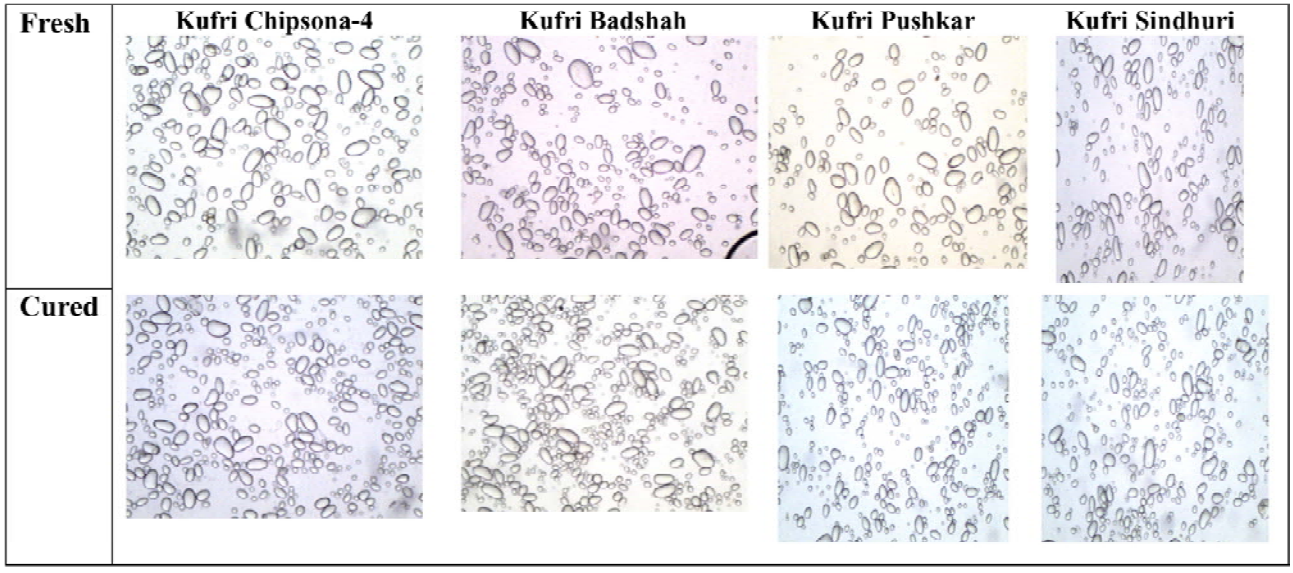

Fig. 1. Particle of starch from potato varieties as effected by curing (inverted compound microscope (Olympus, Japan; model: CX-41) equipped with digital camera facility at 10 x power lens.)

In the present study, biplot indicates that starch crude fibre, moisture, protein and starch whiteness correspond more to Kufri Badshah and Kufri Bahar whereas, starch fat (%), and small size particles values correspond more to Kufri Chipsona-4 (Fig. 2). The starch purity was more associated with the Kufri Sindhuri and Kufri Pushkar. The angle size between two or more traits in the biplot is directly proportional to correlation between those characters. A high positive correlation was discerned between the starch crude fibre, moisture, protein and starch whiteness value whereas, high negative correlation was discerned by starch purity with starch protein, moisture, crude fibre content, and starch whiteness. The biplot reflected diversity among potato varieties based on variables measured. Projection of the variables on the factors plane exhibits an independent group consisting of starch characteristics and the PCA analysis revealed several remarkable variations that exist among potato varieties. Kong *et al*., (2009) extracted four principal components (using 17 variables) that accounted for 88% of the total variance of starches properties, both physiochemical and functional, isolated from 15 amaranth grain cultivars.

CONCLUSION

Characteristics of starch extracted varied with potato variety, curing and, extraction method. Least moisture and protein content and highest starch purity was observed in Kufri Sindhuri. Kufri Sindhuri also resulted in least starch fat content and starch colour values. The percentage of small size particles was maximum in Kufri Chipsona-4 and minimum in Kufri Badshah. Starch extracted by combined method had lower starch moisture content, fat, protein, ash and crude fibre and higher starch purity, percentage of small size particles, yield, and starch colour values. Curing resulted in lower starch yield, starch whiteness value, higher peak viscosity. It can be thus concluded that it is profitable to extract starch by combined method from fresh tubers of variety Kufri Chipsona 4.

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Original Research Paper

Elicitors induced changes in essential oil constituents of turmeric (*Curcuma longa* L.) rhizome

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ABSTRACT

An experiment was conducted at IISR, Kozhikode to study the effect of foliar application of chemical elicitors, namely, chitosan (100, 200 and 500 ppm), phenylalanine (0.1, 1 and 10 mM) and salicylic acid (0.01, 0.1 and 1 mM) on volatile constituents of turmeric rhizome essential oil (EO). Three genotypes (Pragati, Rajapuri and Acc.849) which vary in growth duration and volatile profile were taken for the study in randomized block design with three replications. The highest EO content in Pragati (6%) and Acc. 849 (5.3%) was found in Phenylalanine (1 mM) treatment. No significant changes in EO content were observed in the genotype Rajapuri. Phenylalanine and salicylic acid were found to have positive influence on ar-turmerone, the major sesquiterpenoid in Pragati. Acc.849 and Rajapuri did not produce any significant changes to ar-turmerone content in elicitor treated samples. Moreover, the treatment related variation in the total monoterpenes and total sesquiterpene content was found significant among the genotypes. Multivariate analysis using partial least square discriminant analysis supported the variation observed among the treatments and variable importance in projection score identified the metabolites responsible for variation among treatments.

Keywords: Chitosan, essential oil, phenylalanine and salicylic acid

INTRODUCTION

Turmeric (*Curcuma longa* L.), revered as "Golden Spice", is a rhizomatous crop belonging to Zingiberaceae family. The crop is native to tropical Southeast Asian region (Ferreira *et al.,* 2013). India is the largest producer, consumer and exporter of this crop. The economic produce of the crop is the processed dried rhizome which varies in color from lemon yellow to dark orange. The earthy flavor of turmeric is contributed by its essential oil (EO) constituents. The turmeric EO is comprised of monoterpenes and sesquiterpenes compounds, namely, ar-turmerone, curlone, β-sesquiphellandrene, α-phellendrene, ar-curcumene, α-terpinolene, β-caryophyllene, etc. Leela *et al.* (2002) reported that EO of turmeric rhizome grown in Kerala, India contained ar-turmerone (31.1 %), curlone (10.6 %) and ar-curcumene (6.3 %) as the main components. Many factors namely genotypes/varieties, soil type, climate, altitudinal variation, etc. decides the differential accumulation of these terpenoids resulting in non-uniform flavor profile of turmeric (Anandaraj *et al.,* 2014).The turmeric rhizome EO is reported to have numerous biological activities. It is reported to have anti-oxidant, anti-hyperlipidemic, hypoglycemic, anti-diabetic, cytotoxic, anti-inflammatory, anti-arthritic, hepatoprotective, neuroprotective, anti-bacterial and anti-fungal activities. (Dosoky and Setzer, 2018).

Many studies have proven the effectiveness of elicitors like chitosan, carrageenan, sodium alginate, salicylic acid and others to improve the essential oil components in medicinal and aromatic plants (Ahmed *et al.,* 2020; Shabbir *et al.,* 2017). Due to its numerous beneficial bio-activities, the need arises to increase the bioactive essential oil constituents in turmeric rhizome. Based on the above facts, the study was conducted to test the hypothesis that the foliar application of elicitors like chitosan, salicylic acid and phenylalanine in turmeric would increase the essential oil constituents in its rhizome.

MATERIALS AND METHODS

Plant material and treatments

The experiment was conducted at ICAR - Indian Institute of Spices Research (IISR), Kozhikode, Kerala at rainfed condition in randomized block design with three replications. The soil parameters of the experimental plot were as follows: pH (4.3-4.6); organic carbon content (2.0-2.1 %) and N, P and K content in the range of 235-272 kg/ha, 10-23 kg/ha and 344-503 kg/ha, respectively. Average minimum and maximum temperatures were 23.8 and 31.9 ° C with mean annual rainfall of 2313 mm. Three different varieties/genotypes namely Pragati (a short-duration dwarf variety released from ICAR – IISR), Rajapuri (traded variety from Central Indian region) and Acc. 849 (germplasm collection from Sangli region of Maharashtra) which have inherent variation in the content of essential oil constituents were selected for the study. The experiment included the treatments viz., 1. Control, 2. C_1 - Chitosan at 100 ppm, 3. C_2 - Chitosan at 200 ppm, 4. C_3 - Chitosan at 500 ppm, 5. P_1 - Phenylalanine at 0.1 mM, 6. P_2 - Phenylalanine at 1 mM, 7. P_3 - Phenylalanine at 10 mM, 8. S_1 - Salicylic acid at 0.01 mL, 9. S_2 - Salicylic acid at 0.1 mM, 10. S_3 - Salicylic acid at 1 mM. The stock solutions of elicitors, chitosan (CHT) at 2000 ppm, salicylic acid (SA) and phenylalanine (PHE) solution at 100 mM concentration each were prepared and different dilutions were made freshly with 0.02 % Tween 20 on the day of spray. The elicitors were sprayed at rhizome development stage, i.e. 120-150 DAP depending upon the growth duration of the genotypes. Plants sprayed with 0.02 % Tween 20 served as the control. Once the above ground vegetative parts are dried, rhizomes are harvested, cleaned, cured by boiling them in hot water and dried in the sun for two weeks until the moisture content of the samples were brought down to 10-12 %.

Hydro-distillation and GC-MS analysis of volatile constituents

Hydro-distillation of essential oil from the dried and powdered rhizomes were done as per the method prescribed in AOAC, 2005. The separation and identification of EO constituents were done in Shimadzu GC/MS fitted with RTX-5 (5 % Phenyl and 95 % di-methyl polysiloxane) column with the dimension of 30 m x 0.25 mm x 0.25 µm. The temperature programming of the column was set as follows: 60° C for 5 min, then gradient increase to 110° C at the rate of 5° C min^{-1}, to 200° C at the rate of 3° C min^{-1} and finally to 240° C at the rate of 5° C min^{-1} with hold time of 3 minutes. Ion source and interface temperature was set as 220° C and 240° C, respectively. Other operational parameters include column oven temperature at 60° C, injection temperature at 250° C and helium flow rate at 1.0 mL/ min. The EO was injected in split mode (split ratio – 1:160) and ion fragments in the range of 40 – 650 m/ z were scanned with a scan speed of 1428. The mass spectra of the components were compared with the standard mass spectral library of NIST/WILEY and identified by similarity search (Adams, 2007). The identification was confirmed based on their retention indices calculated using the formula suggested by Van-den-Dool and Kratz (1963) by injecting homologous series of n-alkanes standard (C8-C40).

Statistical analysis

The data were analysed in SAS software and the treatment means (± S.E.) were compared by Duncan's multiple range test (DMRT) ($p < 0.01$ and $p < 0.05$). Multivariate analysis namely partial least square discriminant analysis (PLS-DA) was conducted on the identified metabolites using Metaboanalyst 5.0. Metabolites with significant differences among treatments were identified based on the variable importance in projection (VIP) scores (Xia and Wishart, 2011).

RESULTS AND DISCUSSION

The essential oil content of turmeric genotypes showed significant variation in response to elicitor treatment (Fig 1). In the genotype Pragati, the treatments P3, C2 and S2 showed 13, 11 and 5 % increase in EO content, respectively over control. Whereas, Rajapuri genotype did not produce any statistically significant increase in the treated plants. In the genotype Acc.849, the treatments P_3 (9 %) and S_1 (9 %) has given significant increase in EO content as compared to control. By comparing the results, variation towards elicitors influence were found among the genotypes studied. Phenylalanine and salicylic acid treatments were effective in the genotype Pragati and Acc. 849 whereas, chitosan increased the EO content in Pragati. Our results were in consonance with earlier reported results of various crops (Pirbalouti *et al.,* 2019; Poorgadhir *et al.,* 2020). Researchers all over the world tried to influence the terpenoid pathway to

enhance the volatile profiles of industrially relevant crops. The augmented production of terpenoids without transgenic approaches could be achieved in a limited extent using the application of elicitors (Hussain *et al.,* 2012; Ahmed *et al.,* 2020). The elicitors increased the content of essential oil by increasing photosynthetic carbon assimilation products as well as increasing the expression of key enzymes involved in terpenoid biosynthetic pathway (Srivastava *et al.,* 1990; Ahmed *et al.,* 2020; Vosoughi *et al.,* 2020). Few studies were available on the effect of chitosan, salicylic acid and phenylalanine on growth, physiology and curcumin content of turmeric, but our study is a first report on elicitor's effect on turmeric's volatile constituents.

The EO constituents analyzed using GC-MS threw more light on the effect of these elicitors on major volatile aroma compounds of turmeric rhizome. The statistically analyzed full data set is available in Tables S1-S3. Major sesquiterpenoid compounds identified in the EOs of genotypes used in the study were ar-turmerone (principal aromatic sesquiterpenoid), curlone (also known as β-turmerone), β-sesquiphellandrene, ar-curcumene, germacrone and zingiberene. Among monoterpene compounds, α-phellandrene, α-terpinolene, 1,8 cineole and cymene-8-ol occupied significant share in the turmeric EO.

In the genotype Pragati, relative peak area percentage of α-terpinolene showed significant increase in C_2 (3.57 %) and C_3 (3.54 %) as compared to control (3.29 %). All other treatments showed significant reduction of this compound. Elicitor treatments increased the content of ar-turmerone with the highest content detected in S_3 (51.68 %) followed by P_1 (51.49 %). Phenylalanine and salicylic acid were found to have positive influence on ar-turmerone content. The sesquiterpenoid compounds curlone and sesquiphellandrene has showed mutual exclusivity in chitosan treatments where former showed significant reduction whereas later showed significant increase in the content (C_1 - 7.56%; C_2 - 7.13 % and C_3 - 7.24 %) as compared to control (6.93 %). Chitosan treatments also produced significant increase in zingiberene content (C_1 – 7.22 %; C_2 – 6.58 % and C_3 – 6.75 %). These two sesquiterpenoids, sesquiphellandrene and zingiberene was responsible for the modest increase in its content in chitosan treated plants (Fig. 2).

In Rajapuri genotype, major monoterpenoid compounds, α-terpinolene and 1,8-cineole did not produce significant variation among treatments. The content of ar-turmerone was also not significant among treatments. On the contrary, the content of curlone was increased in P_3 as compared to control. Salicylic acid

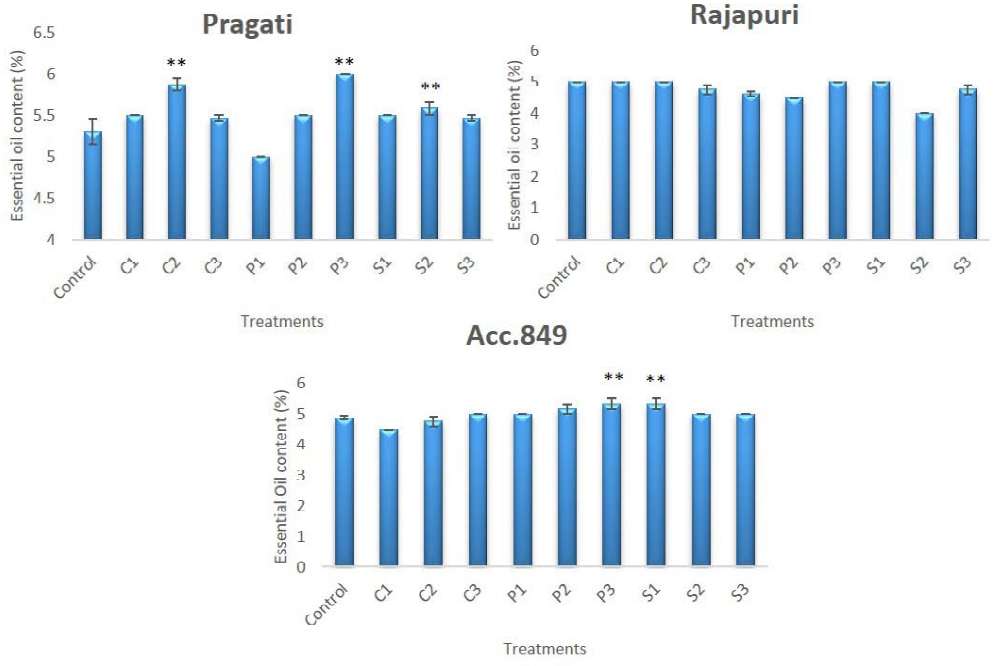

Fig. 1. Essential oil content of elicitor treated turmeric rhizomes
(** indicates significant ($p<0.01$) differences among treatments)

treatments produced some noticeable changes in the content of β-sesquiphellandrene and germacrone content as other treatments are either on par or registered lower content as compared to control. The influence of elicitors on monoterpene and sesquiterpene groups was also found negligible in this genotype (Fig. 2). Overall, influence of elicitors on volatile profile of this genotype is minimum.

In the genotype Acc.849, the main monoterpene compound α-terpinolene showed significant reduction in its content in elicitor treated plants as compared to control. The content of 1,8-cineole was the highest in salicylic acid treatment. Treatment related significant increase or decrease was not noted down in the content of ar-turmerone. Likewise, except in C_1 (6.24 %), all other treatments did not exhibit changes in the content of curlone. Another major sesquiterpene compound, β-sesquiphellandrene showed significant increase in P_1 (16.57 %) and P_2 (15.95 %) treatments over control (14.93 %). Likewise, P_2 (24.57 %) showed significant increase of zingiberene content over control (22.88 %). By comparing the results, the phenylalanine treatments had good influence on the volatile content of the genotype Acc.849. The phenylalanine treatment produced significant decrease in monoterpene content in this genotype. On the other hand, salicylic acid produced increase in total monoterpene compounds with subsequent reduction in sesquiterpenoid compounds (Fig. 2) in this genotype.

The 2D plot of PLS-DA showed more pronounced treatment related variation in the genotype Pragati and Acc.849 (Fig. 3). In the genotype Pragati, P_1 treatment group is found distinct and distant from all other group. Likewise, C_1 treatment also showed distinct grouping as compared to other groups. When this was compared with VIP score, we found that compounds like ar-curcumene, zingiberene, β-sesquiphellandrene, ar-turmerone, α-bergamotane, α-bisabolene, curlone, α-himachalane and nerolidol with score >1 are the source of variation among the treatment groups. The previous results of absence of major influence of elicitors on the volatile constituents of the genotype Rajapuri was confirmed in the PLS-DA also. The 2D score plot of this genotype showed no distinct grouping of any treatments compared to control. If sesquiterpene compounds dominated the variation caused in the genotype Pragati, the equal influence of some monoterpene and sesquiterpene compounds are observed in Acc.849. Compounds with >1 VIP score are isoborneol, α-phellandrene, 4-terpineol, β-farnasene, α-humulene, curlone, α-terpinolene, camphor, 1, 8 cineole, nerolidol, ar-curcumene and cymene. In the 2D score plot, the treatments C_1 and

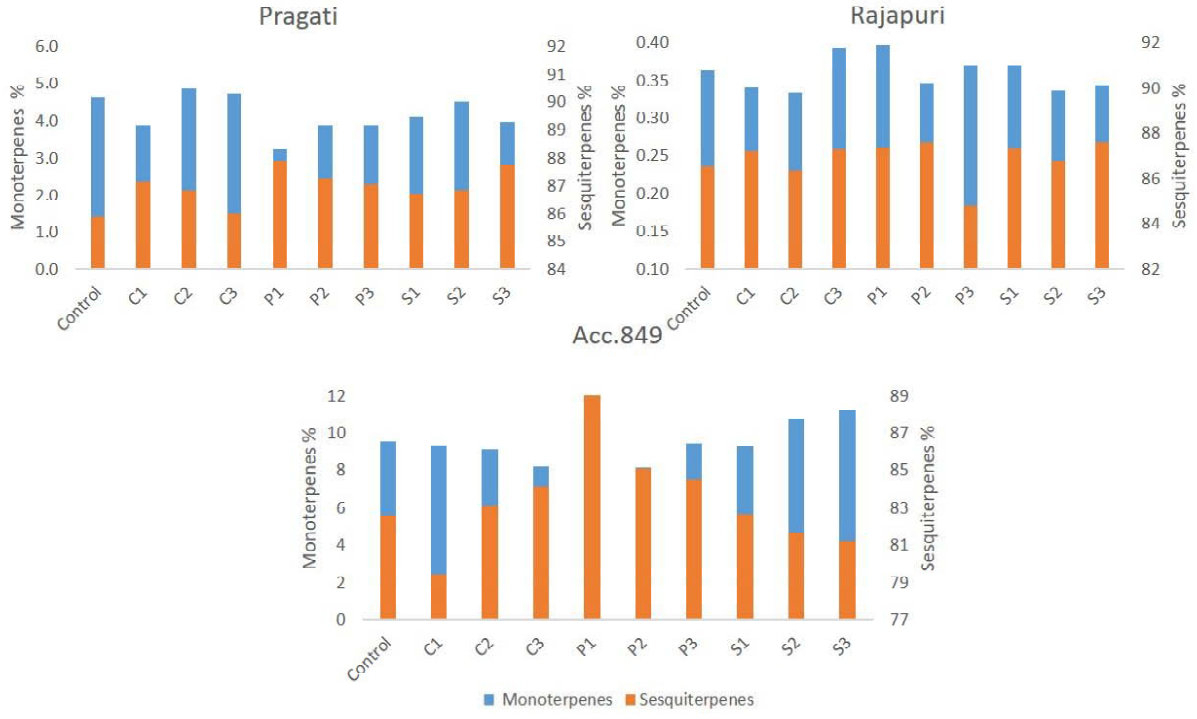

Fig. 2. Monoterpenes and sesquiterpenes content of elicitor treated turmeric rhizomes

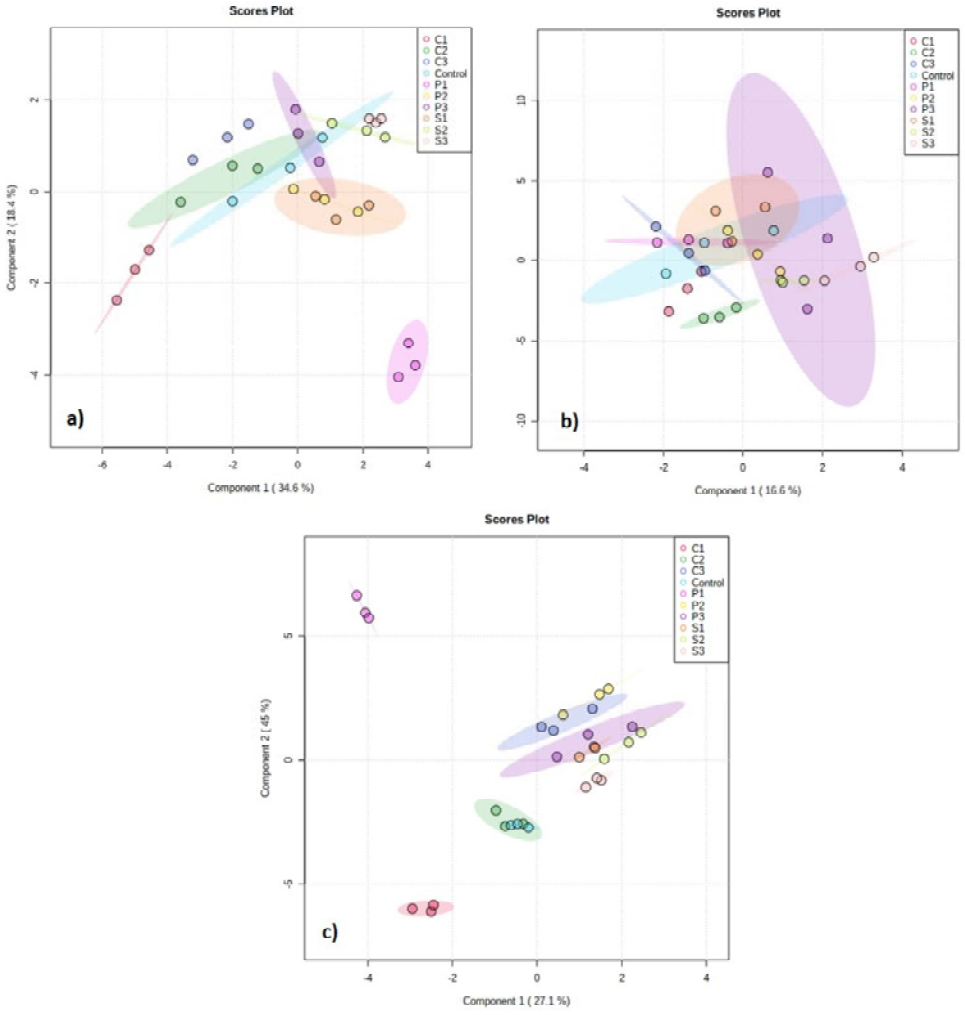

Fig. 3. 2D score plot of PLS-DA analysis of elicitor treated turmeric rhizomes a) Pragati b) Rajapuri c) Acc.849

P_1 showed distinct grouping as compared to control and other treatment groups. The results of multivariate analysis confirmed the differential influence of elicitors on volatile constituents for the three genotypes studied.

Our research finding of increased EO content in elicitor treated plants were supported by previous studies which showed that foliar application of elicitors like chitosan, salicylic acid and phenylalanine increased the quantity and quality of essential oil in different crops (Reham *et al*., 2016; Ahmed *et al.,* 2019; Garde-Cerdán *et al.,* 2018; Alizadeh *et al.,* 2020; Goudarzian *et al.,* 2020; Momeni *et al.,* 2020). Foliar application of chitosan not only enhanced EO content but also increased the concentrations of monoterpene compounds namely limonene, 1,8-cineole, β-thujone and α-humulene in sage plant (Vosoughi *et al.,* 2018). Similar results were observed in our study in the genotype Pragati.

The foliar spray of phenylalanine as growth regulator and elicitor to improve the volatile profiling of few crops were available. In grapes, foliar spray of phenylalanine increased the relative content of volatile compounds such as benzyl alcohol, total benzenoids (aromatic compounds) and total positive compounds whereas total terpenoids and hexen-1-ol were decreased as compared to control (Garde-Cerdán *et al.,* 2018). In our study also, we found that phenylalanine treatment increased the content of β-sesquiphellandrene and zingiberene in the genotype Acc.849 and increased the content of ar-turmerone in the genotype Pragati. Phenylalanine application increased not only the growth and metabolism of crops, but also the biosynthesis of secondary metabolites including terpenoids (Gonda *et al.*, 2018; Poorghadir *et al*., 2020). Elsayed *et al*. (2022) reported that foliar spray of phenylalanine increased

the monoterpene hydrocarbons in bitter fennel, which was not observed in our study. Alternately, we found increase in sesquiterpenoid hydrocarbon content in phenylalanine treatment especially in Pragati and Acc.849 genotypes.

Likewise, foliar spray of salicylic acid was reported to improve the EO yield and constituents by increasing the growth, nutrient uptake and induction of enzymes involved in terpenoid biosynthesis (Pirbalouti *et al.,* 2014; Mohammadi *et al.,* 2019). Our study also found the positive influence of salicylic acid on sesquiterpenoid in general and ar-turmerone content in particular in the genotype Pragati. Momeni *et al.* (2020) studied the effect of foliar spray of chitosan and salicylic acid on EO content and constituents of Mediterranean thyme (*Thymbra spicata* L.). They reported that the content of carvacrol, the predominant essential oil constituent is also increased in the plants sprayed with salicylic acid and chitosan.

The increase in volatile constituents like ar-turmerone, curlone and β-sesquiphellandrane observed in our study is in congruence with above mentioned literatures. We also observed increase in photosynthetic pigments and photosynthetic rate with the elicitor application (Sivaranjani *et al*., 2022) in turmeric which could have increased the supply of base carbon compounds to terpenoid biosynthesis. Being a vegetatively propagated crop, genetic improvement to increase beneficial volatile constituents in turmeric rhizome is a limiting factor which could be alleviated by elicitor application to considerable extent. Our study was first of its kind in this direction by including varied turmeric genotypes in the field experiment. Since this is an open field experiment under natural growing conditions, the concentration-dependent decrease or increase in volatile constituents were not observed in our study.

CONCLUSION

The influence of different elicitors was not uniform among different genotypes. The study concluded that the short duration turmeric genotype, Pragati has responded well with respect to EO content by elicitors application. Phenylalanine treatments increased the percentage of sesquiterpenoids in Pragati and Acc.849 genotypes. Chitosan at 200 ppm, phenylalanine at 1 mM and salicylic acid at 0.1 mM could be sprayed to increase the ar-turmerone content in these genotypes.

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Short Communication

Effect of tree age on fruit characteristics, seed emergence and seedling growth in Rambutan (*Nephelium lappaceum* L.)

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ABSTRACT

Rambutan (*Nephelium lappaceum* L.) is an important exotic fruit from Asia, which is gaining popularity due to its nutritional benefits. The objective of the study was to evaluate the effect of tree age on fruit characteristics, seed emergence and seedling growth of rambutan. The study was conducted at the CSIR-Plant Genetic Resources Research Institute, Bunso, Ghana. Fruits of rambutan were harvested from 8, 10, 25 and 40 years old trees at different locations of the field genebank. For each tree age, three trees were used as replicates. Fruits harvested from trees of different ages were assessed for total fruit weight, pulp weight, pericarp weight, seed weight, percentage seed emergence, seedling plant height and number of leaves at monthly intervals. Fruits harvested from 8, 10, 25 and 40 years old trees did not show significant difference in fruit characteristics and seed emergence. Significant ($p<0.05$) differences were observed in plant height and number of leaves at 5 and 6 months after emergence, respectively.

Keywords: Fruit characteristics, Rambutan, Seed emergence, Seedling growth, Tree age

Rambutan (*Nephelium lappaceum* L.) is a tropical fruit that belongs to the Sapindaceae family (Wall, 2006). It is closely related to several other edible tropical fruits, including the lychee, longan, pulasan, and mamoncillo (Morton, 1987). It originated in Malaysia and has been widely cultivated in South-East Asia including Thailand, Malaysia, Indonesia, Singapore the Philippines and Sri Lanka (Tindall *et al*., 1994). The rambutan tree is of medium evergreen height. Thailand is the largest producer of rambutan growing 450,000 tonnes in the world, followed by Indonesia at 100,000 tonnes, and Malaysia, 60,000 tonnes (Le Bellec, 2014).

Tree age plays an important role in fruit quality, but studies to determine its effect are rare in fruit crops. Ozeker (2000) reported that 20-year-old trees of 'Marsh' seedless grape fruit produced bigger fruit with thinner rinds compared with 34-year-old trees. Bramlage (1993) observed that Pome fruit harvested from young trees were highly susceptible to postharvest disorders. Lower quality apples were obtained from trees of old age (Smith, 2003). Khalid *et al.* (2012) in their studies reported that fruits harvested from old trees (35-year-old) had slightly inferior quality as compared to fruit produced from18-year-old trees. However, no studies have been published on the effect of tree age on fruit quality of rambutan. Thus, there is a need for comprehensive research to determine the possible variation in fruit quality in relation to tree age. The present study aimed to assess the effect of tree age on rambutan fruits characteristics, seed emergence and seedling growth.

In this study, fruits of rambutan were obtained from young and mature trees at CSIR-Plant Genetic Resources Research Institute field genebank (N 06° 17.839, W 000° 27.595, Alt 198.3 m above sea level), Bunso, Eastern region, Ghana during the harvesting season in July, 2018. The climate of the area is semi-equatorial type and the vegetation is moist-deciduous rainforest, with mean minimum annual temperature of 21.4°C and a mean maximum annual temperature of 31.3°C (Aboagye, 2005). The area experiences bi-modal rainfall pattern from April to July and from September to the middle of November. It has a mean

annual precipitation of 1455 mm; with the dry season starting from the middle of November to March.

Physiologically matured fully ripe fruits (Red cultivar) were harvested at maturity at random from trees of different ages at four locations at the same time. These comprised of 8 years old trees, 10 years old trees, 25 years old trees and 40 years old trees. For each tree age, three trees were used as replicates. Thirty fruits were sampled at random from each tree.

During seedlings establishment, insect pests such as leaf miners and ants were controlled using K-optimal insecticide (Landa-cyhalothrin 15 g l^{-1} +Acetamiprid 20 g l^{-1}: EC) at a recommended rate of 40 ml to 15 l of water at two weeks interval. Weeds were controlled using a hoe as and when necessary.

Whole fruit weight and its components (i.e. pericap, pulp, aril and seed) were determined using an electronic balance. Thirty fruits were sampled from each tree of different age for all replicates. For fruit dry mass,10 fruits were harvested and separated manually into pericarp, pulp and seed for dry mass determination. Samples were dried at 80^{O}C for 48 hours in an oven and weighed using an electronic balance.

For germination test, fresh seeds extracted from 30 fruits of each rambutan tree were sown in polybags of dimension 15.5cm x 20.5cm filled with topsoil. The completely randomised design was used with three replicates. Seeds sown were watered daily and kept under shade trees. Percentage seed emergence was computed at 21 days after sowing, as a ratio of the total number of seeds germinated to the total number of seeds sown multiplied by 100.

Growth of rambutan seedlings was assessed by the number of leaves and plant height at monthly intervals for a period of six months. Plant height was measured with a metre rule in centimetres.

Statistical analyses was conducted using SPSS Statistics 21 (IBM, Chicago, IL, USA). One-way ANOVA was used to test the effects of treatments. When a significant difference was detected, Tukey's HSD test was performed to identify significant differences among trees of different ages.

The results showed no significant differences ($p>0.05$) in total fruit weight, pericarp, seed and aril fresh weight of rambutan fruits harvested from trees of different ages. Rambutan fruits harvested from 8 to 40 years old trees were in the range of 26.26 to 29.99g in total fresh weight, 12.32 to 14.97g in pericarp weight, 11.40 to 12.86g in aril weight and 2.24 to 2.73g in seed weight while percentage seed emergence was in the range of 94.44 to 96.67%.

On rambutan fruit dry weight basis, no significant differences were observed in pericarp, seed and aril from fruits harvested from trees of different ages (Table 2). Averagely, rambutan fruit characteristics on dry weight basis were in the range of 2.29g to 3.08g for pericarp, 1.43g to 1.63g for seed and 0.36g to 0.50g for aril.

Fig. 1 shows the number of leaves per plant of rambutan seedlings recorded at monthly intervals after seed emergence. No significant difference was observed in number of leaves at 1, 2, 3, 4 and 5 MAE. At 6 MAE, the number of leaves differed significantly among seedlings established from trees of different ages. Rambutan fruits harvested from 10 years old trees had the highest number of leaves at 6 MAE, but

Table 1. Rambutan fruit characteristics and percentage seed emergence

Age	Fresh weight (g)				% Seed
(years)	Total fresh weight	Pericarp	Aril	Seed	emergence
8	26.26 (2.08)	12.32 (1.31)	11.40 (0.86)	2.53 (0.17)	94.44 (1.92)
10	28.99 (7.40)	14.97 (4.47)	11.61 (2.60)	2.41 (0.46)	95.56 (5.09)
25	28.36 (2.40)	13.46 (1.89)	12.17 (0.60)	2.73 (0.13)	95.56 (1.92)
40	27.28 (2.53)	12.49 (1.71)	12.86 (1.40)	2.24 (0.06)	96.67 (3.33)
ANOVA	n.s.	n.s.	n.s.	n.s.	n.s.

Each value is the mean of three replicates and the standard deviation is shown in parentheses. One-way ANOVA: n.s= not significant.

Table 2. Rambutan pericarp, seed and aril dry weight.

Age (years)	Dry weight (g)		
	Pericap	Seed	Aril
8	2.44 (0.26)	1.62 (0.18)	0.44 (0.26)
10	3.08 (0.83)	1.63 (0.21)	0.36 (0.17)
25	2.16 (0.10)	1.61 (0.02)	0.37 (0.03)
40	2.29 (0.28)	1.43 (0.13)	0.50 (0.07)
ANOVA	n.s.	n.s.	n.s.

Each value is the mean of three replicates and the standard deviation is shown in parentheses. One-way ANOVA: n.s= not significant.

was not significantly different from trees which were 8 and 25 years old. Rambutan seedlings from 40-year-old trees obtained the lowest number of leaves. Leaves are the principal photosynthetic organs of plants (Wright *et al*, 2004). The production of leaves represents an increase in the photosynthetic surface area for plants. Koch *et al*. (2004) and Tozer *et al.* (2015) reported that the size of leaves (e.g., leaf surface area, leaf dry mass and leaf length) profoundly affects a variety of biological processes, for instance, plant growth, survival, reproduction, and ecosystem function. In the present study, the increase in number of leaves indicates a higher photosynthetic activity in seedlings from fruits harvested from 10 years old trees. Besides, the increase in number of leaves in seedlings from 10 years old rambutan trees could also impact on plant-water relations and nutrient uptake positively.

Rambutan seedlings plant height at monthly intervals after seed emergence obtained from fruits harvested from different tree ages is shown in Fig. 2. No significant difference was observed in plant height at 1, 2, 3, 4 and 6 months after emergence. However, at 5 MAE, a significant difference ($p<0.05$) was observed. Rambutan fruits harvested from 10 years old trees had the highest plant height at 5MAE, but was not statistically different from tress which were 8 and 25 years old. Rambutan fruits sampled from 40 years old trees obtained the lowest plant height at 5MAE. The increase in seedling plant height from fruits harvested from 10 years old trees may be attributed to the increase in number of leaves observed in the present study. Similarly, Lyngdoh *et al.* (2014) indicated that seedling attributes after 12 months showed that seedlings obtained from young and

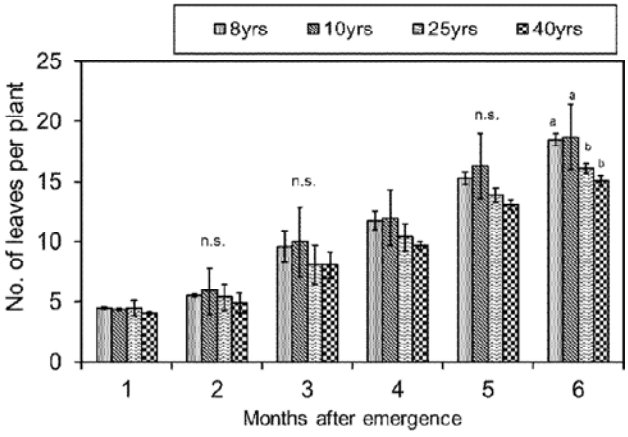

Fig. 1. Number of leaves per plant of rambutan seedlings at months after emergence from trees of different ages. Each value is the mean of three replicates and the vertical bars indicates standard error. One-way: *p<0.05, n.s.=not significant. When a significant difference was detected, Tukey's HSD test was performed to identify significant differences among the 4 treatments. Different letters above the bar indicate significant difference.

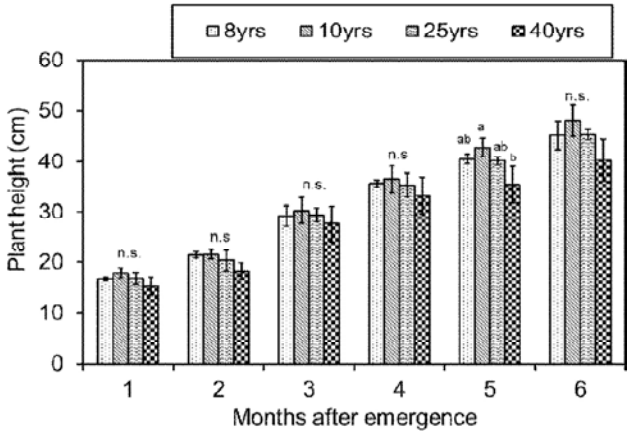

Fig. 2. Plant height of rambutan seedlings at months after emergence form trees of different ages. Each value is the mean of three replicates and the vertical bars indicates standard error. One-way: *p<0.05, n.s.=not significant. When a significant difference was detected, Tukey's HSD test was performed to identify significant differences among the 4 treatments. Different letters above the bar indicate significant difference.

middle-aged plantations of *Livistona jinkensiana* (between 18 to 45 years) performed better than those beyond 50 years. Raja *et al.* (2004) also found that seeds collected from middle-aged trees of Areca nut which were 45 years old consistently had highest shoot length, root length, number of roots, seedling dry weight and vigour index compared to seeds collected from trees aged 7,14,21 and 28 years. Mao *et al.* (2014) reported a significant effect on relative height growth rate by altering their biomass allocation among *Pinus thunbergia* seedlings obtained from different age classes.

Tree age had no significant effect on rambutan fruit characteristics and seed emergence. However, seedlings established from fruits harvested from trees of different ages showed significant differences in number of leaves per plant and plant height. Fruits harvested from 10 years old trees exhibited better seedling growth. Seedlings obtained from rambutan fruits from middle-aged trees can be considered for nursery establishment.

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Short Communication

Occurrence of algal stem blotch in ber (*Ziziphus mauritiana*) under coastal Odisha conditions in India

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ABSTRACT

The investigation was carried out during 2017-18 to identify and document the emerging diseases of Indian Jujube or ber (*Ziziphus mauritiana* Lamk.) in Odisha state located in Eastern part of India. Periodical visit and subsequent investigations revealed the occurrence of a new kind of stem blotch disease in ber caused by alga. Symptoms were observed on bark of the stem and branches as bright red velvety blotch colonies during July- September 2017. However dull grey blotches were visible throughout the year. Leaves and fruits were left unaffected. The algal stem blotch occurrence was assessed during the year 2018 and disease severity ranged from 9.4-14.8 per cent. The green alga was identified and confirmed as *Trentepohlia arborum* (Agardh) Hariot based on key morphological characters. The stem blotches lead to death of young twigs measured between 3 to 8 mm thickness on primary and secondary branches wherein thickness of branches was more than 10 mm, algal blotches caused cracking of bark. Present study highlights the causal agent of stem blotch of ber, its symptomatology, impact of disease and suggested management practices.

Keywords: Ber, Indian jujube, Odisha, Stem blotch, *Trentepohlia arborum*

INTRODUCTION

Indian jujube or ber (*Ziziphus mauritiana* Lamk.) is a spiny small tree belongs to the family *Rhamnaceae* which is native of India (Krishna *et al.,* 2014). It is also called as desert apple, jujube, chinese apple, ber etc. Although *Z. mauritiana* is extensively distributed in tropical areas of the world, India is a major place of its cultivation. In India, it is cultivated over 49,000 ha with the production of 4, 81,000 MT per year (Anon, 2017). Ber fruits are healthy as well as nutritious which contains higher quantity of vitamin C which is much higher than citrus and apple (Khera and Singh, 1976). In the current scenario, improved varieties of ber are gaining recognition among the farmers in many parts of the country because of its adaptability to various climatic condition. In Odisha too, ber cultivation is gaining momentum during recent years and in general climatic condition prevalent in coastal Odisha is different from rest of the country. Hence knowledge on diseases hampering the productivity has to be generated to develop suitable management practices at regional level to make ber cultivation as more remunerative. The diseases like powdery mildew caused by *Oidium erysiphoides* var. *zizyphi* was reported as an economically important disease of ber, which can lead to 50-60 per cent loss in fruit yield (Jamadar and Shamarao, 2004). Other diseases like rust caused by *Phakospora zizyphi-vulgaris* (Gupta *et al.,* 1984), leaf spots and fruits spots (Gupta and Madan, 1977a; 1977b;), witches broom caused by MLOs (Khan *et al.,*2008) and leaf spots caused by *Alternaria, Cercospora, Septoria, Cladosporium, Pestalotiopsis* etc. were reported to infect ber crop in India. A kind of bright orange colour cottony stem blotches of various sizes combined with cracking were observed on bark of one and half year-old ber plants (Fig. 1A) at the experimental orchard of ICAR-IIHR - Central Horticultural Experiment Station situated in the state of Odisha during July - September 2017. In severe cases twig drying also was observed. Based on symptomatology, it was identified as plant parasitic algal infection. Similar kind of symptoms was reported on black berry crop due to plant parasitic algae *Cephaleuros virescens* and the

disease was referred as orange cane blotch (Holcomb *et al*., 1998). It has been documented as one of the important diseases of black berry grown in coastal plains experiencing warm, wet, humid environment in South Eastern United states (Browne *et al*., 2020). Hence systematic study was planned to identify the organism involved in causing stem blotch in ber, its symptomatology, impact of algal parasite on crop growth and prophylactic measures to be undertaken.

The study was carried out at the experimental farm of ICAR-IIHR-Central Horticultural Experiment Station, Odisha during 2017-2018. The experimental farm is located at 20°15' N latitude and 85°15' E longitude with an elevation of 25.5 m above MSL experiencing humid hot, tropical climate which receives average annual rainfall of 1400 mm between June to September. Disease incidence and severity was recorded during 2018 crop season at fortnight interval and required number of plants remained unsprayed for assessing the disease severity. Totally 25 plants were chosen and tagged for diseases assessment and minimum 4 stems per plant was marked with field tape and assessed for stem blotch throughout the year. Severity of algal blotch was assessed visually for the total length of stem/branch using 0-5 arbitrary scale (0- No stem blotches, 1 = trace infection (< 1 per cent of branch covered with algal blotches); 2 = Light (1-5 per cent of branch covered with algal blotches); 3 = Moderate (6–25 per cent of branch covered with algal blotches); 4 = Severe (26-50 per cent of branch covered with algal blotches but no twig drying; 5 = Very severe >50 per cent of branch covered with algal blotches accompanied with twig death). Per cent disease index (PDI) was determined using the formula, PDI=Sum of all disease rating × 100/ (Total no. of rating × maximum disease grade).

Stems (n=10) bearing algal blotch from the different ber trees grown in our experimental orchard was collected during July-September 2017 and symptoms were observed visually as well under a stereomicroscope and macroscopic features of algal thalli were noted. Microscopic features of algal thalli, features of filaments, sporangiophore and sporangia were observed under bright field microscope. Dimensions of algal structures *viz*., were measured (n=30) for each structure and the range of the values were noted and described. Algal parasite was identified based on the descriptions given by Silva *et al*. (2010). and Thomas *et al*. (2019).

Bright orange, circular blotches ranged from 2-30 mm diameter were observed during humid rainy days (Fig 1B). Macroscopic structures of algae were observed under stereo zoom microscope. The orange patches consist of cottony filaments and spore masses of algae. The algal lesions were mostly circular to irregular and were raised, velvety and were often brick-red in colour during rainy months and the rest of the year, the lesions were greyish in colour. Approximately 3 mm to 8mm size thickness twigs as well as branches were severely affected which lead to twig death and branch dieback (Fig 1C) in young twigs. On primary and secondary branches where in thickness of branches was more than 10 mm, algal blotches caused cracking and plant tissue/bark beneath blotch/ algal thalli was

Fig. 1A. Stem blotch disease caused by *T. arborum* on ber, 1B. Close up view of stem blotch symptom, 1C. Drying of young twigs

discoloured, necrotized. The bark cracking was observed from mild to deep from (Fig 2A-2D) and in extreme cases big branches died due to invasion of secondary pathogens.

All the trees in orchard were found infected with mild to severe form and per cent disease severity index were ranged from 9.4-14.8 during 2018 and maximum disease severity was recorded during second fortnight of June 2018. The voucher specimens of ber infected with algal stem blotch was sent to, Lichenology and Algology Laboratory, CSIR- National Botanical research Institute, Lucknow and it was identified as *Trentepohlia arborum* (Agardh) Hariot.

Microscopic features algae were documented by using the Olympus BX 53 microscope. The main plant body of *T. arborum* was thallus that consisted of uniseriate (arranged in single row) poorly branched, entangled filaments, tapered to the apex, branched at 90° angle; individual cells were of cylindrical in shape. Grouped sporangia (ranged from 4-8 in number) from a basal enlarged cell (or suffultory cell) observed to be unique characteristic feature. Sporangia was round to elliptical in shape, present laterally or apically on the erect axes and measuring 16-20 µm in diameter (n=25) (Fig 4A and B). The above algal descriptions are in line with Thomas *et al*. (2019) and Silva *et al*. (2010). Cribb (1958) characterized *T. arborum* by its grouped sporangia from a basal enlarged cell and the tapered filament.

The genus *Trentepohlia* includes about 40 species (Hoek *et al.,* 1995) and this genus mostly exists in tropical climatic area; however, it also exists in temperate areas (Liu *et al.,* 2012). *Trentepohlia* belongs to the phylum *Chlorophyta,* class *Ulvophyceae*, order *Trentepohliales* and family *Trentepohliaceae* (Guiry and Guiry, 2016). In the current scheme of taxonomy, *Trentepholiales* comprise of single family *Trentepohliaceae* with five genera such as *Trentepohlia, Cephaleuros, Phycopeltis, Printzina* and *Stomatochroon* (Brooks *et al*., 2015). Till now in India, the green algae, *Cephaleuros* species is well known for its parasitic nature on several plants and causes orange to reddish spots consists of sporangiophores and sporangia on stems, fruits, leaves of the many ornamental and fruit trees (Pitaloka *et al.,* 2015). Extensive survey carried out in Eastern India to document the diseases of ber by Misra *et al.* (2013) revealed the occurrence of *Cephaleuros* sp. on ber leaves.

Even though wide survey was carried out in India by number of researchers with regard to *Trentepohlia* species, (Bruhl and Biswas, 1923; Randhawa and Venkataraman, 1962; Krishnamurthy, 2000), information of *Trentepohlia* as a plant parasitic algae is limited in India. As early in 1980s, Jose and Chowdary (1980) reported a species of *Trentepohlia dusenii* from Calcutta, India. *T. aroburm* was reported from Kerala and Shillong from rocks (Panikkar and Sindhu, 1993; Kharkongor and Ramanujam, 2015).

Fig. 2A to 2 D. Stages of bark cracking due to *T. arborum* blotches on bark of ber

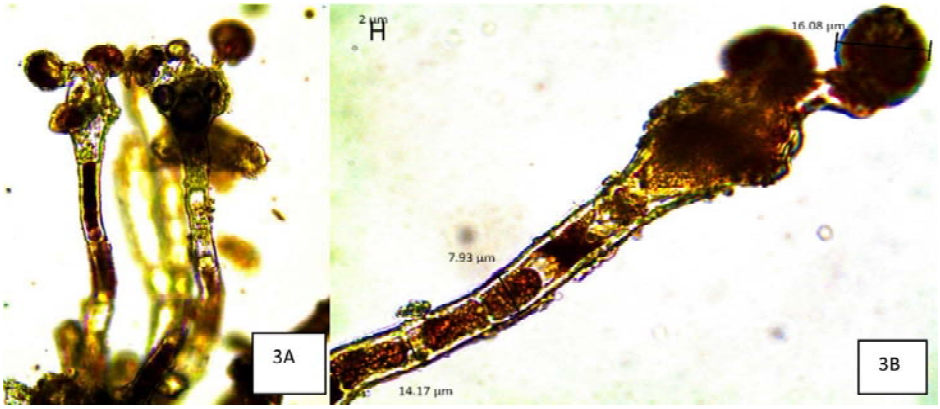

Fig. 3A and 3B. Microphotographs of *T. arborum* infecting ber

*Trentepohlia rigidula*was reported on sub-aerial habitat as greenish coating on cement walls of a temple in Bhubaneswar, Orissa (Samad and Adhikary, 2008). The ecological study of the species indicated the major occurrence of this genus on the substratum like tree bark in the tropical area. *T. rigidula* (J. Muller) Hariot was recorded from West Bengal, India from two distinct habitats (i.e.) epiphytic form on tree bark of bael (*Aegle marmelos*) and epilithic form on a concrete cement tank wall (Satpati and Pal, 2016). In a survey conducted from Indian sundarbans biosphere reserve, four *Trentepohlia* species *viz*., *T. abietina, T. sundarbanensis, T. torulosa* and *T. thevalliensis* were reported (Satpati and Pal, 2015). At Bhitarkanika National Park in Kendrapara district of Odisha, the tree species like *Avicennia alba, Avicennia officinalis, Ceriops decandra, Heritiera fomes, Rhizophora apiculata* etc were found to be the major hosts of *Trentepohlia flava* (Chakraborty *et al*., 2012) and this species were found to colonise the tree bark within the mangroves.

The present study proved the infection of *T. arborum* in *Z. mauritiana* causing stem blotches in ber which resulted in die back of young twigs and cracking of bark portion below the point of infection and has the potential to reduce the vigour of young as well as matured plants if care is not taken at right time. When blotch colony formation *i.e.,* the coverage of the stem/branch by algal blotch is limited without cracks, then this parasitic alga does not limit or have not much adverse effect on crop. But where ideal environmental conditions of warm, wet and humid environment prevail, it girdled the small stems/ branch and also paved the way for secondary infection, causing death of young branches and twigs. Similar kind of observation was made in black berry plants infected with *algae, C. virescens* which was evidenced in terms of orange lesions on stems led to girdling of canes and if favorable conditions continue in the field, algal colonization combined with secondary fungal infections could lead to dieback and death of canes (Brooks, 2004). Black berry canes with larger and more numerous blotches produced significantly lesser number of berries than canes with slight/no algal blotch (Browne *et al.,* 2020).

During the study period, it was observed that algal botch was mainly observed on ber during warm rainy weather coupled with high humidity (data not shown).The prevailing humid climate in coastal plains of Odisha accompanied by frequent rainfall and warm temperature might favoured the algal pathogen and predispose the crop to infection. The earlier studies were also revealed that the members of *Trentepohliales* have been widespread in tropical and temperate regions with humid climates (Chapman, 1984) and recurrent rains coupled with warm weather might encourage the viability of the algal parasite in the host plants (Han *et al.,* 2011; Sunpapao *et al.,* 2016). Southwest monsoon followed by sudden summer encouraged rapid infection of *Cephaleuros diffuses* in leaves of *Artocarpus* in Kerala (Thomas *et al*., 2016).

For the management of as orange cane blotch in black berry canes (woody stems) caused by parasitic algae *C. virescens*, Brannen (2018) recommended the removal of old canes and their destruction promptly after harvest, pruning to improve air movement in the canopy, strategic site drainage, proper weed control, plastic mulching combined with drip irrigation, and application of appropriate agrochemical. The present study witnessed that, from the viable algal lesions present on stem bark, algal inoculum re-emerges and

infect the crop during subsequent year; consequently, if the disease was not effectively controlled during the previous year, the succeeding crop ended up in high level of disease severity. Hence it is suggested that, the plants have to be trained and pruned with open centre system with 2-3 primary branches at a height of 50-60 cm. In addition, pruning has to be done every year to remove weak and diseased branches to obtain healthy tree growth and profitable crop. Under Odisha condition, pruning during February-March (after fruit harvest) followed by spray of copper hydroxide (2.0 g/l)) or copper oxychloride (3.0 g/l) at fortnight interval provided efficient control of stem blotch disease of ber. As the ber cultivation is gaining momentum among the farmers in the state of Odisha, more studies are warranted to know the detailed role of epidemiological factors with regard to stem blotch disease severity especially under coastal Odisha condition.

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Short Communication

Occurrence of *in vitro* flowering in coconut (*Cocos nucifera* L.)

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ABSTRACT

Immature inflorescence with outer spathe length of 5.5 cm size collected from West Coast Tall cultivar of coconut was used as the explant and rachillae bits were inoculated in Y3 media supplemented with 2, 4-D (1 mg L^{-1}). The cultures were incubated in dark for eight months and sub-cultured into the same media at monthly interval. The white shoot like outgrowths formed were sub cultured to ½ MS media fortified with 1 mg L^{-1} each of NAA and BAP and subsequently transferred to light condition. After three months, the emerging shoot like structure was transferred to Y3 media fortified with NAA and BAP. Upon developing 3 - 4 leaves, the cultures were transferred to rooting media and root initiation was observed after two months. The transition of vegetative shoot to reproductive state was accompanied by some morphological changes including rapid emergence of long and thin leaves followed by emergence of pearly white inflorescence. Unlike normal inflorescence, the inflorescence emerged was terminal and was devoid of spathe. Prolonged subculture in the same media might have resulted in pH variation and subsequent reduction in organic and inorganic constituents of the media. The chemical stress experienced by the plantlet might have induced *in vitro* flowering.

Key words: *Cocos nucifera,* immature inflorescence, hapaxanthic, prolonged subculture

The coconut palm (*Cocos nucifera* L.) is one of the most beautiful and useful trees in the world and all parts of this 'wonder palm' are useful in one way or other. Coconut, an out-breeding perennial tree, is seed propagated, exhibits great variation in morphological and agronomic characters. Vegetative multiplication of elite coconut palms is a promising possibility for producing uniform planting material with high yield and disease-resistance. Protocols for coconut micropropagation have been developed in various laboratories using different explant sources (Nguyen *et al*., 2015). Among various explants, the most extensively studied are the rachillae from inflorescence and plumule from zygotic embryos.

Flowering is a complex phenomena regulated by both internal and external factors and induction of *in vitro* flowering is very rare in most of the crops. Under natural conditions, flower formation normally commences when a plant attains maturity. Juvenile phase of a plant is genetically controlled and is species specific which means that a plant flowers only when genetic factors including photoperiodic response are congenial. However, these conditions can often be altered so that the plant can be induced to undergo an early reproductive phase. Such an attempt to induce flowering *in vitro* has been attempted in many plant systems. *In vitro* culture provides an ideal experimental system for studying the molecular mechanism of flowering. *In vitro* flowering studies has been conducted in many perennial crops e.g., bamboo (Joshi and Nadgauda, 1997), red hot pokers (Taylor *et al*., 2005), date palm (Allouche *et al*., 2010), oil palm (Nizam and Te-chato, 2012) etc. However, *in vitro* flowering in coconut has not yet been reported. Reducing duration of juvenile phase is an advantage especially in coconut with long pre-bearing period of 6-10 years. Here, in the process of establishing *in vitro* regeneration of coconut using immature inflorescence explants, strikingly, a few cases of *in vitro* flowering in coconut plantlets was observed. This paper aims

to present some observations connected with *in vitro* flowering of coconut palm and also tries to explain the possible factors involved.

The procedure followed by Shareefa *et al*. (2019) was used for immature inflorescence culture of coconut. Immature inflorescence explants with outer spathe length of 5.5 cm size were collected from 25 year old West Coast Tall variety and rachillae bits of 1 mm which were inoculated in Y3 media supplemented with 1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). The basal media also contained sucrose 40 g L^{-1}, charcoal 1 g L^{-1} and agar 6 g L^{-1}. The cultures were incubated in dark condition at 27° ± 2°C and sub cultured in same media. After eight months, cultures were transferred to ½ Murashige and Skoog (MS) medium with 1mg L^{-1} each of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). The cultures were initially kept in diffused light for one month followed by incubation in light condition for about 16 hours light (45-60 µmol m^{-2} s^{-1} PPFD) provided by white Light Emitting Diode (LED) tubes. After 4-6 months in light, the multiple shoots were separated from the parental clump and transferred for shoot regeneration to Y3 media with 1 mg L^{-1} each NAA and BAP. After developing 3-4 leaves, the cultures were transferred to rooting media containing Y3 with NAA (2 mg L^{-1}) and BAP (2 mg L^{-1}) and Indole 3-acetic acid (2 mg L^{-1}) along with sucrose 30 g L^{-1} for root initiation.

Within one month of dark incubation, the rachillae explants swelled and white outgrowths were observed in culture initiation media. The cultures when transferred to light conditions gradually turned green and developed multiple shoots which could be easily detached from parental clump. The separated shoots were transferred to shoot regeneration media for formation of well developed leaves. Root initiation was observed after two months in the rooting media.

In vitro flowering was observed in few plantlets cultured in the rooting media and such plantlets developed had four leaves and few root initials. In order to develop secondary roots, the plantlets were kept in the same media for a period of six months. The onset of *in vitro* flowering was accompanied by some morphological changes in the plantlets which include rapid emergence of long and thin leaves before the appearance of pearly white inflorescence. Unlike normal inflorescence, the emergence of inflorescence was terminal in the *in vitro* raised plantlets and the inflorescence was devoid of spathe (Figure.1).

The ability of explants to form flowers *in vitro* depends on numerous internal and external, physical and chemical factors and virtually all these factors interact in various complex ways (Compton and Vielleux, 1992). In the present study, induction of flowering was observed in plantlet cultured on Y3 media fortified with NAA (2 mg L^{-1}) and BAP (2 mg L^{-1}) and IAA (2 mg L^{-1}). The combined effect of auxin and cytokinin on *in vitro* flower induction has also described in a number of previous studies (Handro, 1983; Wang *et al*., 2002; Ammar *et al*., 1987; Jeyachandran and Bastin, 2013; Lin *et al.* 2005; Saritha and Naidu, 2007a; Sudhakaran and Sivasankari, 2002; Taylor *et al*. 2005; Thiruvengadam and Jayabalan, 2001). The role of cytokinins on *in vitro* flowering has been well documented (Wang et *al.,* 2001; Saritha and Naidu, 2007b). Cytokinins alone do not appear to be responsible for floral initiation. It is reported that cytokinins are known to interact with sucrose to cause the shift in the apical meristem from a vegetative phase to a reproductive one (Bernier *et al*. 2002; Bernier and Pe´rilleux, 2005). Sugars are primary sources known for reliable induction and development of flowers in many plant species such as rose (Vu *et al*. 2006), *Passiflora suberosa* (Scorza and Janick, 1980), *Vigna mungo* (Ignacimuthu *et al*., 1997) indicating that presence of carbon sources on the culture medium is necessary for floral stimulation.

There are many other physico-chemical factors which affected the *in vitro* flowering mechanism. Kolar and Senkova (2008) reported that reduced mineral nutrient availability accelerated *in vitro* flowering in *Arabidopsis thaliana*. The effect of Paclobutrazole, LEDs and sucrose on flowering of *Euphorbia milli* plantlets *in vitro* was studied by Dewir *et al.* (2007). In tobacco, important factors influencing in *vitro* flowering were light, growth regulators, carbohydrates and pH of the culture medium (Heylen and Vendrig, 1988).

The most essential part of plant tissue culture is the media which supplies hormones and necessary nutrients for growth and development. In the present investigation, maintaining cultures for six months in

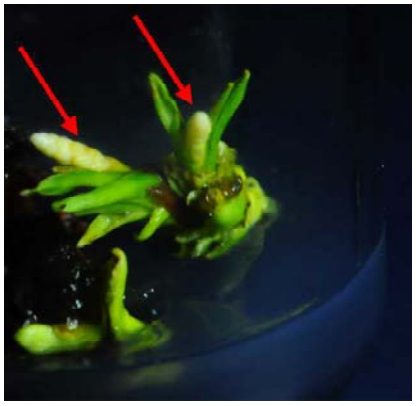

Fig.1a. Initial stage *of in vitro* flowering in coconut (arrow)

Fig. 1b. Fully emerged *in vitro* inflorescence

same media resulted in good root growth in plantlets, which also resulted in floral initiation. Prolonged culture of rooted shoots in media containing NAA and PBZ together with higher concentration of sucrose at 7% was reported to induce floral development in oil palm (Nizam and Te-chato, 2012). Delaying subculture may lead to hormone alternation and depletion of nutrients in the culture media. Therefore the altered chemical composition might have created a stress due to the increased passage time for subculturing.

It was interesting to note that *in vitro* flowering did not resemble flowering *ex vitro*, in that the inflorescences *in vitro* never matured and they subsequently senesced indicating that other factors, excluding cytokinins and a carbohydrate source, are required for continued normal development of the inflorescences. Cytokinins and sucrose therefore seem to act in the initial stages of floral initiation and development, however, full differentiation and maturation of the resulting flower bud requires involvement of other physiological factors.

The results of the current study revealed that contrary to natural flower formation, *in vitro* neoformed inflorescences were completely uncovered, *ie.*, lacking spathe. There are two types of developmental processes namely hapaxanthic and pleonanthic, in palms (Tomlinson, 1990). In hapaxanthic type, the growth of the axis of palm is determinate due to conversion of the vegetative shoot apical meristem (SAM) to the reproductive state, resulting in a short flowering phase and this phenomenon is observed only in less than 5% of palm species. The rest of the palm species are pleonanthic, with an indeterminate SAM, in which the vegetative growth continues while producing a reproductive meristem at each leaf axil. According to the Tomlinson model, under *in vivo* conditions, flowering in coconut is normally pleonanthic. However, in the present study, *in vitro* flowering was hapaxanthic as the inflorescence emergence was terminal resulting from the development of the apical bud which was devoid of any bract, which consequently gave rise to uncovered inflorescences. The flowers were malformed and never matured indicating that optimum interaction of light, temperature, plant growth regulators and nutrients are essential for flowering and normal maturation of flowers. Similarly, undersized and malformed flowers have been observed previously in other species (Ramanayake *et al.*, 2001). The malformation

occasionally observed in the flowers produced *in vitro* may have been partially due to competition and or nutritional deficiencies as reported in *Pentanema indicum* (Sivanesan and Jeong, 2007).

Summary

In the present study, prolonged subculture in the same media might have resulted in changes in the pH and reduction in concentration of organic and inorganic constituents of the media. The resulting chemical stress might have induced *in vitro* flowering in coconut. The interesting observation was that *in vitro* neoformed inflorescences were completely uncovered, lacking spathe and were terminal. The flowers were malformed and never matured indicating that optimum interaction of light, temperature, plant growth regulators and nutrients are essential for flowering and normal maturation of flowers. However, *in vitro* flowering can be efficiently used to understand the snapshots of physiological, hormonal and molecular regulation of flowering and such information gathered can be used to save time in future genetic improvement programs.

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