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JOURNAL OF HORTICULTURAL SCIENCES

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Short Communications

In this issue…

Horticultural Sciences ensure the nutritional security in the society. Any development in the horticultural sciences directly or indirectly makes an impact in the society. As in the earlier issues of this journal, this one also carries the reasearch articles based on the recent scientific work in different disciplines with respect to horticultural crops.

This issue bears eleven Original Research Papers and two Short Communications. Curry leaf plant (Murraya koenigii) has not been given scientific importance that it deserves so far. A review on diversity and distribution of this species in India by ***Raghu B.R*** *highlights the diversity, distribution, nutraceutical and medicinal values, industrial use and commercial cultivation with respect to this plant. Another review is on the groundwater depletion; how it affects the perennial horticultural crops and the policy measures needed to address the issue that has been written by* ***Ganeshamurthy et al.***

Fruit production has seen tremendous increase in last five years in India. To achieve the more crop per drop, water utilization is important. ***Manjunath et al.*** *report that it is sufficient to irrigate papaya to meet only 30 per cent of soil volume with 1.5 m x 1.5m spacing to enhance water use efficiency.* ***Rashmi et al.*** *used soil and plant analysis and diagnosed the micronutrient imbalance in lime and sapota orchards in tablelands of Chambal Ravine region of India. Identifying the deficiencies of micronutrients timely and application of balanced fertilizers at correct time can enhance crop production and quality of fruits. Studies on fruit development in wax apple Syzygium samarangense (pink and white types) by* ***Priya Devi et al.*** *indicated that the fruit development followed a sigmoid growth pattern with peak in growth rate between 21 and 28 days after anthesis for fruit weight.*

Vegetable crop improvement is a priority area of research. ***Jessymol et al.*** *validated the usefulness of two DNA markers genetically linked to S-Cytoplasm and restoration-of-fertility (Rf) loci in hot pepper (Capsicum annuum L.) and found that they can be used in marker aided selection in chillies.* ***Susmita et al.*** *have identified lines of garden pea (Pisum sativum L.) that have high temperature tolerance that are suitable for off-season cultivation.* ***Singh et al.*** *studied the nature and magnitude of genetic diversity in long day onion germplasm by using the principal component analysis and single linkage cluster analysis an experiment among 34 onion genotypes and report the diversity among the onion genotypes. Similarly* ***Thangam et al.*** *also studied the variability and genetic divergence in vegetable cowpea germplasm in Goa and report that divergent lines can be used in selection of parents in breeding programmes.*

Demands of floriculture industry drives the research programmes in ornamental crops. ***Aswath*** *and* ***Rajiv Kumar*** *have come out with two novel gerbera (Gerbera jamesonii) hybrids with good flower quality traits for polyhouse cultivation.* ***Jadhav et al.*** *evaluated hybrids and cultivars of*

single type tuberose (Polianthes tuberosa) and found that Arka Prajwal and genotype LP14 were superior in many traits. ***Chandran et al.*** *identified resistant gene analogues in rose genotype IIHR13-4 which is resistant to powdery mildew and these RGAs will be useful in mapping and characterization of R genes in rose.*

To obtain nutritional security carotenoid content in fruits and vegetables has to be high. Measuring carotenoid content is challenging in selection of breeding lines. ***Shilpa et al.*** *validated a non-destructive method of measuring carotenoid content in cherry tomatoes correlated to the color space values L*, a*, b* that can be used in many other studies.*

The Editorial team of Journal of Horticultural Sciences is striving hard to do its best in reporting the recent development in horticultural sciences. The editorial team acknowledges the support and encouragement from all the members of Society for Promotion of Horticulture and subscribers of the journal.

S. Sriram
Editor in Chief

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Review

Diversity and Distribution of Curry Leaf in India

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ABSTRACT

Curry leaf is an aromatic tropical and sub-tropical plant originated from India. Besides its culinary purpose, curry leaf is known for its medicinal and industrial applications. Based on ethno-botanical reports and other floral distribution studies, the germplasm rich regions of curry leaf in India could be identified into six zones as Foot hills of Himalaya, North-East region, Middle India, Eastern Ghats, Western Ghats and Andaman – Nicobar Islands. With respect to color and size of leaves, habitat and flavor, the curry leaf plant is classified as brown/ gamthi, regular and dwarf morphotypes. Four genetically diverse chemotypes of curry leaves such as β-pinene, α-pinene, β-carophyllene and β-phellandrene exist in India. Due to its nutritional value, increasing export potential, less input, lower cost of cultivation, assured income, perennial nature and constant demand in local, national and international markets, the curry leaf is being cultivated on commercial scale in recent years in India.

Key words: Curry leaves, Distribution, Diversity, Germplasm and Morphotypes

INTRODUCTION

Curry leaf (*Murraya koenigii* (L.) Spreng) is an aromatic, tropical and sub-tropical plant with several culinary, nutraceutical, medicinal, therapeutic and industrial values (Reddy *et al*., 2018). It is an important and indispensible part of Indian cuisine (Verghese, 1989). The ethno-botanical use of curry leaf for medicinal purposes is known since centuries. Carbazole alkaloids present abundantly in curry leaves have anticancer, anti-diabetic and anti-oxidant properties (Igara *et al.,* 2016). The essential oils extracted from curry leaf plant have several industrial applications in the manufacturing of soaps, perfumes, cosmetics, food processing and many others. As curry leaf is a rich source of bio-available calcium and other essential nutrients, it makes an important component of Indian diet and imparts several direct and indirect health benefits to its consumers. As curry leaf is a perennial leafy vegetable, it provides assured income to small and marginal farmers if cultivated on commercial scale and earns considerable amount of foreign returns through export particularly to Gulf and European Unions (Joseph and Peter, 1985).

Regardless of its numerous benefits and several applications, curry leaf is still an unexplored and underutilized crop. This is evident from inadequate efforts being put towards the collection, characterization, conservation and efficient utilization of plant genetic resources (PGR) for crop improvement and limited area of cultivation under this crop. However, understanding the extant of variability, distribution pattern and identification of germplasm rich regions are the prerequisites in any crop improvement program. Furthermore, accelerated use and augmentation of available PGR, identification of trait specific genotypes, mapping and introgression of economically important gene(s)/QTLs (Quantitative Trait Loci) into well adapted and elite genetic background are sign of more efficient and rapid genetic improvement program in any crop species and so with the curry leaf. Moreover, efficient breeding programmes should backup with detailed survey and frequent collaborative germplasm exploration trips to cover wide range of variability, followed by regular exchange and characterization of germplasm between

the breeders. In addition, development of various robust genomic resources and their efficient use in rapid germplasm screening and detection of elite genotypes and traits discovery. Thus, in the present article efforts are made to throw a light on diversity and distribution pattern of curry leaves and its present status of genetic improvement in India.

BOTANY

Curry leaf belongs to the family Rutaceae and the genus *Murraya* J. Koenig ex L (Satyavati *et al*., 1987). It is true diploid with chromosome number, 2n=18 (x=9) (Raghvan, 1957). It is found in various tropical and subtropical regions of the world (Smith, 1985). It is a semi-deciduous, aromatic, pubescent and unarmed shrub or small tree capable of growing up to 6 meter height with slender but strong woody stem (Figure 1). Later, the woody stem develops into densely crowded shaded crown. The leaves of curry leaf plant are alternate, estipulate, bipinnately compound, glabrous, 15-20 cm long, rarely pubescent at young and gland dotted and extremely strongly aromatic with numerous volatile compounds. The leaflets are alternate and short stalked; ovate to ovate lanceolate in shape with number varies from 9 to 12 or more per leaf. The leaves are slightly pungent, bitter and aciduous in taste. The major chemical compounds responsible for characteristic intense aroma and flavor are sabinene, pinene, cadinol, caryophyllene and cadinene (Singh *et al.,* 2014). Monoterpenoids and their oxygenated derivatives are the chief chemical constituents present in essential oil of curry leaf (Singh *et al.,* 2014). The essential oils extracted from fresh leaves collected from different parts of Western Ghats, India contains significant amount of monoterpene hydrocarbons such as sabinene (6.90-40.59%), β-phellandrene (1.39-45.89%) and α-pinene (1.93-63.66%), and a sesquiterpene hydrocarbon β-caryophyllene (6.68-18.46%) (Syamasunder *et al.,* 2012).

Curry leaf is highly self-pollinated crop. The flowers are bisexual, produced many in a terminal pedunculate inflorescence arranged compact, corymbiform and cymose panicle. The bisexual flowers are very small measuring about a cm length, white and fragrant. The flowers contain five deeply cleft and pubescent calyx, five free and spreading petals, ten freely and alternatively arranged short and long, linear and subulate stamens with small and short anthers. The style is thick, elongated, cylindrical and articulate with capitate or grooved stigma. The flowering occurs mainly in the middle of April to middle of May reaching its peak in last week of April. The curry leaf plant bears small berries, measuring 1-2 cm diameter with thin pericarp and mucilaginous pulp enclosing 1 or 2 seeds. The fruiting starts from middle of July and continue up to end of August. Upon ripening, the fruits turn red and ultimately black and seeds are non endospermic with membranous and glabrous testa bearing small embryo. Propagation occurs either through seeds or rooted suckers.

ORIGIN AND DISTRIBUTION

The curry leaf originated from Indian sub-continent including Andaman and Nicobar Islands, Sri Lanka and Bangladesh (Joseph and Peter, 1985), later expanded to different parts of the world by Indian migrants. Presently, it is grown in various tropical and subtropical regions such as, India, Sri Lanka, Bhutan, Nepal, Malaysia, Southern China, Guangdong, Southern Hainan, Southern Yunnan, Laos, Vietnam, Thailand, Mariana Islands, Vanuatu, New Caledonia, Ryuku Islands, Australia and South Africa (Smith, 1985).

Curry leaf is widely distributed throughout India except in higher altitudes of Himalayas. It is found abundantly in forests and waste lands in natural, wild and cultivated forms up to 1650 m altitude (Joseph and Peter, 1985). In southern India, it is found in homestead gardens of every household (Joseph and Peter, 1985). Based on several ethno-botanical reports and other floral distribution studies, the germplasm rich regions of curry leaf could be identified into six zones for future exploration and genetic improvement in India (Figure 2).

Region-1: This region is confined to sub-tropical forests running all along the sub-Himalayan foothills from Jammu & Kashmir, Himachal Pradesh, Uttarakhand to Terai regions of Uttar Pradesh and Bihar (Figure 2). In Himachal Pradesh, curry leaf is found in the forest ranges found between Tanda and Shahpur in Kangra district, Nalagarh and Nahan-Paonta ranges in Sirmaur district and some warmer areas of Solan, Shimla, Bilaspr, Hamirpur and Mandi districts. In Uttarakhand, sub-tropical forests in foot hills of Shivalik of Dehradun and Gharwal and Udham Singh Nagar including Rajaji national park and sub-tropical forests of Kumoan

regions of Almora, Nainital and Champavat districts are rich in curry leaf. The Terai regions of Uttar Pradesh covering 15 districts (Saharanpur, Muzaffarnagar, Bijnor , Moradabad , Rampur , Bareilly , Pilibhit, Kheri , Bahraich, Shravasti, Balrampur, Siddharth , Mahrajganj, Kushinagar, Gorakhpur) including Dudhwa national park, and West-Champaran, East-Champaran and Gopalganj districts of Bihar are rich sources of curry leaf.

Region-2: The region is confined to tropical evergreen and semi-evergreen, and tropical moist and dry deciduous forests of North-eastern states (Figure 2). This region covers from foot hills of South Sikkim, Darjeeling, Jalpaiguri and Cooch Behar districts of west Bengal. The Assam valley includes Brahmaputra valley, Barak valley, Karbi plateau and Barail hills and parts of South Kamrup, Sibsagar, North Lakhimpur, Cachar, Goalpara, Nowgoan and Darrang districts of Assam. East and West Kameng, lower & Upper Subansiri, Lohit and Tirap districts in Arunachal Pradesh. Southern and Northern slopes of Meghalaya including Northern and North-western slopes of Garo hills. Western and North-western parts of Nagaland. Jiri, Moreh, Vangoi, Tamenglong forest areas and forest areas adjacent to Myanmar in Manipur. Dharam Nagar, Kailashahr, Belonia, Amarpur, Sonamura, Udiapur and Sadar sub-division in Tripura. Northern side forest areas of Kawnpuri, Hortaki, Bhairabi, Kolarib, Vairentee and western parts of Mizoram. Besides, the curry leaf is an integral part of many tribal medicines and hence it could be seen commonly in home-stead gardens in north eastern states.

Region-3: This region is confined to central India, from Sundarban delta to Satpur range covering Chotanagpur plateau, Hazaribagh plateau, Ramgarh hills, Malayagiri, Dandakaranya and Vindhyan ranges (Figure 2). Unlike regions 1& 2, this region is loosely spread over South-western parts of West Bengal, Jharkhand, Northern parts of Odisha, Chhattisgarh, and Northern parts of Maharashtra to Madhya Pradesh including Southern Uttar Pradesh. A pursuance of review of literature of ethno-botanical use and floral and medicinal plants diversity studies of curry leaf in central India indicated that, it has been grown and used in Sonebhadra district of Uttar Pradesh, Jabalpur, Neemuch, Raisen, Rewa, Umaria, Anuppur, Nimar eco-region, Satpur plateau of Madhya Pradesh, Bhadrak, Koraput, Jharsuguda, Keonjhar districts, Rourkela, Nandan Kanan wild life sanctuary of Odisha, Mahasamund, Dantewada, Koria, Jashpur, Raipur, Surguja, Ratanpur region of Bilaspur, Raigarh area, Bhoramdeo wild life sanctuary, Kabirdham wild life sanctuary of Chhattisgarh, Dumka of Jharkhand, Bhagalpur, Banka, Buxar of Bihar and Burdwan, Hoogly, South 24 Parganas, Birbhum, West Rarrh region of West Bengal.

Region-4: This region is confined to Eastern parts of India mainly covering Eastern Ghats (Figure 2). This region starts from Southern parts of Odisha, covering Andhra Pradesh and up to Northern Tamil Nadu.

Region-5: This region is confined to Western parts of India mainly covering Western Ghats (Figure 2). This region starts from Southern parts of Gujarat, covering, Maharashtra, Goa, Karnataka and up to Kerala.

Region-6: This region is confined to Andaman and Nicobar islands mainly covering the Andaman semi-evergreen forests, Andaman moist deciduous forests and Andaman secondary moist deciduous forests (Figure 2).

NUTRITIONAL POTENTIAL OF CURRY LEAVES

The fresh and dried leaves of curry plant are used for flavoring the food stuffs. It is good sources of minerals (Calcium, Phosphorus, Iron, Zinc, Magnesium), vitamins (A, E, B, C), and are rich in carbohydrates, proteins, amino acids and fibre. Besides, leaves contains alkaloids, flavonoids, phenols, saponins, steroids, quinones, tannins and other bio-active compounds. Vyas et al. (2015) reported ascorbic acid (23.41 mg/g), total flavonoid (17.38 mg/g), and total phenol (3.21 mg/g) in curry leaves, and which are associated with higher antioxidative capacity, thus act as very good sources of dietary antioxidants. It is reported that, 100 gm of fresh leaves contains 8.7g carbohydrate, 6g protein, 1g fat, 7560 µg ß-carotene, 830mg

Calcium and 0.93mg Iron. Whereas, 100g of dehydrated leaves contains 64.31g carbohydrate, 12g protein, 5.4g fat, 5292 µg ß-carotene, 2040mg Calcium and 12mg Iron (Khatoon et al., 2011).

The nutritive value of the curry leaf is comparable to other vegetables. Without realizing the nutritive value of curry leaves, it is usually not consumed along with food, but considered merely as a flavouring agent. The leaves can retain original flavour and other qualities even after drying. Curry leaves contain some important free amino acids like glycine, asparagin, serine, aspartic acid, theonine, alamine, proline, glutamic acid, tryptophan, leucine, tyrosine, alpha amino butyric acid, phenylalanine, isoleucine, lysine, arginine and histidine and Vitamin A (Wealth of India, 1962). Although curry leaf is a rich source of calcium, its bioavailability is affected due to the presence of high oxalic acid (1.35% total oxalates; 1.15% soluble oxalates). Thus, identification of low oxalate and low oxalic acid genotypes will be an important step towards the effective utilization of curry leaves as leafy vegetable. The comparative nutrient value of curry leaf with some common vegetables as per ICMR (Aykroyd, 1996) is given below.

Parameters	Curry leaf	Cauli flower	Cabbage	Radish	Carrot	Dry Pea	Tomato	Dry Chilli
Protein (g/100g)	6.1	2.6	1.8	0.6	0.9	19.7	0.9	15.9
Carbohydrates (g/100g)	18.7	4.0	4.6	6.8	10.6	56.5	3.6	31.6
Fat (g/100g)	1.0	0.4	0.1	0.3	0.2	1.1	0.2	6.2
Calcium (mg/100g)	830	626	39	50	80	75	48	160
Phosphorus (mg/100g)	57	107	44	20	530	298	20	370
Iron (mg/100g)	7.0	40	0.8	0.5	22	5.1	0.4	2.3
Vitamin-A (I.U./100g)	12600	51	2000	5	3150	66	585	576
Vitamin-C (mg/100g)	4	56	124	17	3.0	0	27	50

MEDICINAL PROPERTIES OF CURRY LEAVES

The different parts of curry leaf plant have many applications in Ayurveda and other traditional medicine system (Karthikar and Basu, 1935). Curry leaves are richest source of carbazole alkaloids, which have bioactive functions like anti-oxidant, anticancer, antidiabetic, and antiulcer (Igara *et al.,* 2016). The carbazole alkaloids, mahanimbine and koenigine present in these leaves showed higher anti-oxidant activities (Ganesan *et al*. 2013). The extracts from roots, bark and leaves the are used in aboriginal medicine as tonic, anthelmintic, stomachic, analgesic, and as appetizing, carminative and stimulative agents for treating influenza, piles, itching, fever, dropsy, asthma, bronchial eruptions, diarrhoea, body aches, kidney pains, vomiting, fresh cuts and bites of poisonous animals (Rana *et al.* 2004). The green leaves eaten raw for cure of dysentery (Dryry, 1978). The pulped bark and root of curry leaf plant are externally applied to cure eruptions and bites of poisonous animals (Dastur, 1970).

INDUSTRIAL USE OF CURRY LEAVES

Fresh leaves on steam distillation under pressure yields 1.6-3.7 mL volatile oils called curry leaf oil per kilogram (kg) of biomass (Syamasunder *et al.,* 2012). The leaf oil is characterized by specific gravity of 0.9748 at 25^0c, saponification value of 5.2 and acid

value of 3.8 (Wealth of India, 1962). Similarly, the mature fruits also yield a yellow colour volatile oil with specific gravity of 0.872 at 13^0c and boiling point of 173.74^0C, having neroli-like odour and pepper like taste accompanied by agreeable sensation of coolness on the tongue. Similarly, yellow colour oil extracted from the seeds is known as limbolee oil (Drury, 1978). The leaf oil is used as a fixative for a heavy type of soap Perfumes. The essential oils extracted from other parts are also used in cosmetics industry and aromatherapy. Besides, the extracts from berries of curry leaf plant are used to prevent oxidative damage of meat and meat products (Yogesh *et al.,* 2012). The curry leaves are incorporated in functional poultry meat finger sticks to improve lipid stability and antimicrobial quality of the products. This indicate that the effective use of curry leaves as an alternative to synthetic food preservatives in functional food industries (Aswathi *et al.,* 2014). Besides, the curry leaf oil is also exported from India (Salikutty and Peter, 2001). The age of the leaves influences the oil composition, with advancing maturity there is gradual decrease in volatile oils and oleoresin (acetone extracted). The specific gravity of curry leaf oil is 0.97 while refrective index is 1.50 and optical rotation is +48 at (25^0C). The saponification value of this oil is 5.2 and after acetalation it is 54.6

COMMERCIAL CULTIVATION OF CURRY LEAVES IN INDIA

Besides, naturalized forest and homestead gardens habitation, the curry leaves are also being cultivated in large commercial scale in several districts of Southern India due to increasing export potential, low inputs for cost of cultivation, adoptability to small and marginal lands, assured income, perennial nature, and constant domestic demand in local markets. The large scale commercial cultivation of curry leaves is seen in Guntur, Nellore, Anantapur and Krishna districts of Andhra Pradesh, Sanga Reddy, Medak, Siddipet, Kama Reddy and Nizamabad districts of Telangana and Coimbatore, Tiruppur, Selem and Thoothukudi districts of Tamil Nadu (Mohan, 2012) (Fig. 2).

DIVERSITY OF CURRY LEAVES IN INDIA

Curry leaf plant is native to India. A tremendous variability exists at morphological, bio-chemical and molecular level for this crop. Several chemo-types and morpho-types have been reported in curry leaves of Indian origin (Rao *et al.*, 2011; Sivakumar and Meera, 2013). There are four genetically diverse chemotypes of curry leaves such as β-pinene, α-pinene, β- caryophyllene and β-phellandrene were reported in India (Raina *et al.* 2002). Similarly, the essential oils extracted from leaves of curry plants from various locations of Western Ghats, India, were categorized into 4 chemotypes namely, β-phellandrene, sabinene, α-pinene and β-caryophyllene (Syamasunder *et al.,* 2012)

Three different morphotypes with respect to color and size of leaves, habitat of the plant, flavor of the plant as, Brown/Gamthi (GM), Regular (RE) and Dwarf (DF), are identified in India. Brown types are most fragrant slowly growing; leaves are small, thick with serrated edges and dark brown in color. Regular types are fastest growing and grow as tree, greater in look, and leaves are exstipulate, bipinnately compound and long having reticulate venation with dark in color and available throughout the country. Dwarf types grows as a shrub with moderate growth, spreading branches and appears as a bush, leaves are light green, look like a regular type but aromacity of its own. All three morphotpes differ for intensity of flavor. The radical scavenging capacity of methanolic extracts of three morpho-types was in the order of Gamthi>Dwarf> Regular, with IC_{50} values of 171, 365 and 471 (g/ml), respectively (Sivakumar and Meera, 2013). Besides, Gamthi (532.8 mg/ml) had more phenolic content over dwarf (168.2 mg/ml) and Regular (111.6 mg/ml) types and 6.01, 4.82 and 3.58 mg/ml of flavonoids were reported in GM, DF and RE, respectively (Sivakumar and Meera, 2013). Further, curry leaves exhibited huge variability with respect to chemical composition of essential oils over locations and seasons (Syamasunder *et al.,* 2012).

GENETIC IMPROVEMENT OF CURRY LEAVES IN INDIA

Genetic improvement of curry leaves interms of germplasm collection, characterization and conservations are still in nascent stage in India. Majority of curry leaf growing farmers on commercial scale have adapted locally available genotypes in which genetic potential for yield, resistance to pests and diseases and information on quality is unknown. However, two improved varieties of curry leaves

namely, DWD-1 and DWD-2 (Suwasini) are genetically distinct genotypes, released from University of Agricultural Sciences (UAS), Dharwad for commercial cultivation. Besides, a local landrace called Senkaampu is very popular in different parts of Tamil Nadu due its good aroma and high oil content. Recently, the research efforts are initiated at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru for genetic improvement of curry leaf for improved yield and quality and resistance pests and disease. Over 100 germplasm of curry leaves were collected from different parts of the country and successfully established in field gene bank at ICAR-IIHR, Bengaluru. Further, efforts are being made to characterize them and registered with national gene bank for further utilization in research programmes in India.

FUTURE THRUST

1. Area expansion under commercial cultivation: In India, the commercial cultivation of curry leaves is confined to few distracts of Tamil Nadu, Andhra Pradesh, Telangana and Karnataka covering more 90% of commercial cultivation. However, curry leaf can easily fit to other dry land parts of the country. Besides, it assures income security to small and marginal farmers in rainfed ecosystems. Thus, there is a huge scope to expand curry leaf cultivation to non-conventional arid and semi-arid regions of central, western and north-east parts of the country.

2. Conservation of PGR in Curry leaves: Curry leaf is native to India and tremendous diversity exists for this crop. To understand the extant of variability for important economic traits and identification of trait specific genotypes requires comprehensive exploration of PGR, characterization and documentation. There should be a system of regular exchange of germplasm among the researchers.

3. Development of DUS guidelines and protection of farmer's varieties: In various parts of India, the farmers are popularly growing local cultivars of curry leaves. They known to possess unique traits and have economic importance. However, there is no internationally or nationally accepted DUS test guidelines available in curry leaves; which is a mandatory requirement to register and protect farmer's varieties under PPV&FRA, New Delhi. Thus, there is an urgent need to develop

Fig. 1. Curry leaf plant (bushy) (a) and small tree (b).

internationally or nationally accepted DUS test guidelines in curry leaves.

4. Comprehensive research efforts in curry leaves: A breeding program inclusive of increased use of genomic resources in PGR management, identification of trait specific genotypes and trait discovery, successful introgression into elite genetic background is need of the hour. Further, much intensive effort towards ideotype breeding in curry leaves is required for development of ever green and bushy type with quick generation capacity after pruning.

CONCLUSION

Curry leaves is a multi-utility leafy vegetable with numerous benefits. It is known to enhance the palatability of food and is a rich source of nutrients and valuable volatile oils. It is an easily cultivable perennial crop with low input cost, being grown as a small tree in homestead gardens; intercrop with other perennial crops and also on commercial scale. Due to its perenniality, it can provide assured income for 10-12 years of planting with minimum annual expenditure. Though curry leaf is an ancient crop native to India, its nutritive and medicinal values have not been fully appreciated yet. Efforts towards characterization and conservation of genetic wealth and genetic improvement of curry leaf have not been addressed adequately. Thus, there is need to popularize and utilize this native crop with adequate research and institutional efforts.

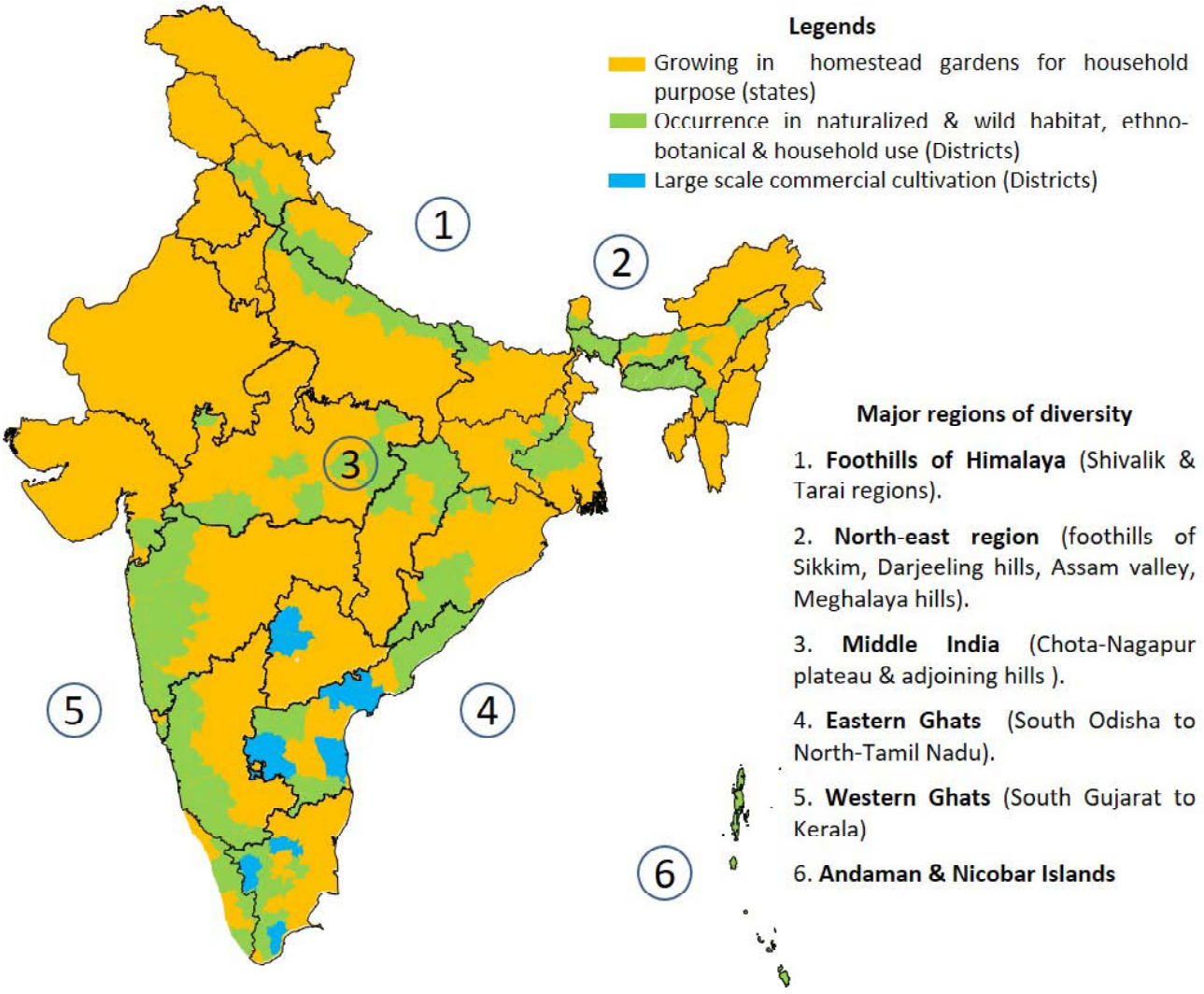

Fig. 2. Diversity map of curry leaves (*Murraya koenigii* (L.) Spreng) in India.

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Review

Groundwater Decline and Prolonged Drought Could Reduce Vigour, Enhance Vulnerability to Diseases and Pests and Kill Perennial Horticultural Crops: Needs Urgent Policy Intervention

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ABSTRACT

Perennial horticulture in India has undergone a change from rainfed system to drip fertigation systems and from isolated hedge and bund trees to high intensity orchard systems with enhanced number of trees per unit area. In several parts, particularly in the Deccan plateau, the system has now become completely dependent on water pumped from tube wells. Severe competition for water from tube wells makes farmers to devote more water for cash rich annual crops and even sell water for city dwellers nearby. As a consequence, the groundwater level in the past three decades has fallen from few feet to above thousand feet. At several places it has crossed the "peak water". Frequent and prolonged exposure of fruit trees and nuts to drought coupled with ground water depletion has led to soil profile drying leading to reduced vigour and enhanced vulnerability to diseases and pests. This has led to withering of fruit and nut trees. Perennial crops are likely to become increasingly maladapted to their environment, particularly in the earlier period of climate change they are more likely to be attacked by diseases and insects. Coconuts, areca nuts and mango trees have died in several places and the government constituted committees have recommended compensation to the farmers. As a country, we have dramatically increased our reliance on groundwater. 175 million Indians are now fed with food produced with the unsustainable use of groundwater. This increase has dried up rivers and lakes, because there is a hydrologic connection between groundwater and surface water. Yet the legal rules governing water use usually ignore the link between law and science. The issue needs thorough examination and needs policy interventions to come out of this vicious circle.

Keywords: Drought, Fruit trees, Groundwater depletion, Peak water, Perennial crops, Policy issue

INTRODUCTION

Perennial horticulture in arid and semi-arid regions of India was a rainfed system since beginning. With time, the area under perennial horticulture has shown enormous increase and with advancement in technology perennial horticulture along with annual horticultural crops and agriculture started receiving irrigation both through surface irrigation and through drip fertigation systems. Also, both number of trees per unit area and the intensity of cultivation have increased. Of late in arid and semiarid regions, particularly in the southern, central and north-western states, very large number of tube wells have been dug and put to use for irrigation. Such tube wells were sunk in highly unscientific way and are resulting in increase in tube well depth and deteriorating quality of water. The system now has become completely dependent on water pumped from tube wells. In the past two to three decades the groundwater levels are falling steeply. Occurrence of prolonged drought, early withdrawal of monsoon, and reduced number of rainy days spreading over short periods are exposing the trees to severe moisture stress and symptoms of declining tree vigour could be felt. In the past five years, several incidences of trees withering like coconut and areca nuts, mango,

pomegranate etc. are being reported. Government has constituted committees to look into the causes and compensations are being given to the farmers for the loss of trees. There is hence a need to scientifically look and rethink on perennial horticulture in the wake of emergence of these situations.

Impact of prolonged drought on perennial horticultural crops

Distribution of trees in arid and semi-arid lands depends mainly on rainfall, surface water, and groundwater and air moisture. Change in climate of the given region (rainfall, temperatures, wind) further affects the distribution of trees. Soil quality and extent of its deterioration decides the future of existing population and scope for further expansion. Each tree species is adapted to certain conditions and is located in its "niche". When optimal conditions are widely distributed, forests or shrubs may cover large areas. The natural distribution of vegetation has long been altered by human activities like unsustainable cropping systems. For example, by growing fruit trees, nuts and other perennial crops by exploiting the ground water in such places where its over drawing is unsustainable and can cause havoc. Conversion of forest lands to agricultural use in the past and more recently from agricultural use to unsustainable perennial systems are among the major causes of soil degradation in arid and semi-arid areas. Furthermore, global warming is expected to result in rainfall decrease throughout most of the arid and semi-arid zones, which will lead to more severe water scarcity and increased desertification risks. Some of the semi-arid regions started showing symptoms of desertification like Kolar and Chikkaballapur districts of Karnataka, Anantapur and Madanapalli in Andhra Pradesh etc.

Occasionally, fruit trees decline and often die. Diseases affecting the leaves, fruit, and twigs of fruit trees usually do not cause the trees to die, with exceptions for such diseases which causes death of trees like coconut wilt and bud rot, citrus and guava wilts and recently pomegranate wilt and nodal blight, wilt in many perennial crops. Leaf, fruit, or twig diseases weaken the tree, interrupt normal bearing, and reduce fruit quality, but the trees usually survive. The cause of death for most fruit trees is damage to the root, trunk, or the crown. Drought, flooding, crown and root diseases, and borers, winter cold, frosts, can cause injury to these parts but not lethal to the trees. Frequently, a combination of two or more of these is the cause of death. The most severity being reported and becoming more common than exceptions is the soil profile drying in arid and semiarid regions caused due to drought coupled with over exploitation of groundwater and drought alone usually will not kill healthy fruit trees, unless the drought is prolonged and severe coupled with decline in water table due to over draft of ground water. But gradual exposure of trees to drought weakens the trees which predisposes trees to insect pests and diseases.

A gradual decline in tree health as a consequence of limiting moisture is a common problem for many trees, more so, underclose spacing mono cropping, intensively managed orchard systems. Symptoms may include stunted growth, premature leaf drop, late spring leaf development, sparse foliage, light green or yellow foliage, twig and branch die-back and many other abnormal symptoms like flowering but not bearing fruits to the expected crop load. As mentioned above, usually there is no single reason for tree decline. Often, a combination of factors, linked to one another, reduces a tree's vigour. Stress on a tree can make it vulnerable to additional problems. Diseases and insects often capitalize on the tree's low vigour and accelerate its decline. Trees survive stress temporarily by using stored food and water reserves, but once these reserves are used up, symptoms of decline begin to appear. Because trees are so efficient at storing food and water reserves, it may take 2 or 3 years after a stress episode before decline symptoms appear. One of the most common causes of stress is planting orchards tree species not suited for a particular site. Many species have specific site requirements. Site characteristics that influence tree growth in limited moisture situations include soil moisture holding capacity, soil moisture availability, soil resource base erosion, mainly the organic front and drainage. All these come under land use planning which is surprisingly ignored while planning perennial crops entrepreneurships.

Declining trees as a consequence of prolonged drought and overdraft of groundwater leading to profile dryness make trees weak and susceptible to insect pests and diseases. Certain insects and diseases can cause defoliation leading to further stress. Most healthy trees can survive some defoliation, but

defoliation year after year can cause decline and even death. Mango foliage damage caused by hoppers, loppers, beetles etc. in the very initial stage of leaf emergence is a typical example in southern and western part of India. Apple scab and anthracnose of shade trees are examples of diseases that cause infected leaves to fall prematurely. The stem and root borers take the opportunity of tree weakness and overtake the tree health and intensify the attack. A typical example is the spurge in the stem borers of Arabica coffee in Coorg and mango in South India. Leaf diseases like powdery mildew and anthracnose and other diseases cause severe damage to foliage, inflorescence and fruits. Tree wilts due to Ceratocystis are emerging in mango in recent years which normally happen during trees exposed to long period of moisture stress.

Climate change effects on insects and pathogens under horticultural ecosystems

Increase in summer temperature will generally activate insect development rates. Some insects may shift from completing a generation every two years (semivoltism) to completing one generation per year (univoltism), a factor that contributes to large-scale outbreaks. Warmer winters could also lead to more successful survival. Outbreaks may also increase in entirely new areas crossing the limits of host species due to warming temperatures relative to their current distribution. The indirect effects of climate change on insects are more complex; therefore, they are more difficult to predict. Because trees are likely to become increasingly maladapted to their environment, particularly in the earlier period of climate change they are more likely to be attacked by insects.

Climate change will affect the developmental sequence of insects and their predators. Natural insect enemies of defoliator and borer species depend on climatic factors to maintain their life processes and synchronicity with their insect host and the habitat in which they live. Key parasitoids and predatory species population may dwindle due to over use of chemicals or even as a consequence of climate change and can further be a primary driver in causing the outbreaks. However, such predictions are difficult due to their complexity and variability. In general, the projected change in climate coupled with poor tree vigour as a consequence of increasing moisture deficit conditions, will promote pest and pathogen activity due to low moisture availability following prolonged drought, higher temperature and reduced mortality in winters. However, the complex interactions among hosts, pathogens and environmental conditions make scientific prediction difficult. A warmer and dry climate may change some pathogens and pests and decline in others.

Emergence of stem borers as a serious pest is a consequence of progressive exposure of intensively managed orchards to drought. Progressive foliage loss in mango due to complex insect damage may also be a consequence of this. However, short periods of hot, dry weather put severe stress on weak or injured trees and may cause them to die. Death most frequently occurs in the early summer, during or just following the first heat wave. A heat wave puts a severe strain on a weakened tree. Weak trees frequently leaf out in the spring, bloom profusely, and set a heavy crop of fruit but fail to retain the crop load. Although in mango the trees leaf out, the leaves usually are smaller than normal, are pale-green to yellowish green in colourand are severely affected by foliage pests and or diseases even before the leaves turn green from the initial copper colour.

Consequences are seen in recent years of loss of coconut trees, arecanut trees, mango trees and other perennial trees in some of the groundwater over exploited areas like Chitradurga, Tumkur, Chikkaballapur, Bangalore, Hassan, Anantapur, Madanapalli, Solapur, Theni, Virudhunagar and other districts and Arabica coffee in Coorg and Chikmagalur districts of Karnataka. Over mature trees progressively lose their resilience to climatic stress, so that a single climatic event can destroy a whole area of those species. For example, over aged coconuts and areca nuts in Southern Karnataka died few years back.

Water and land resources degradation

The United Nations Conference on Environment and Development (UNCED, 1992) defined desertification as “land degradation in arid, semi-arid and dry sub-humid areas resulting from various factors, including climatic variations and human activities”. Desertification is not an advance of existing deserts but is rather the effect of localized degradation of the land. It rapidly follows deforestation and soil exhaustion. Exposed to the sun, the wind and the rains, exhausted soils lose their organic matter and

their structure while nutrients are leached away. Fine elements are blown into dust storms and sand grains become mobile. Overexploitation of forest, tree, bush, grazing land and unsustainable cropping systems by overexploitationas of soil resources and ground water has been increasing desertification. Recent report of ICRISAT under NICRA project has shown that in the past 50 years there has been a shift from dry sub-humid climate of some region to semiarid climate and trends in semiarid regions becoming arid regions(NICRA, 2014). Fruit trees are occupying larger areas in such locations in dry sub-humid and semi-arid regions under irrigation from tube wells which are over drawn leading to a steep gradient of dry profile down from surface soil. A poor monsoon spread over short period, too low number of rainy days, early withdrawal of monsoon coupled with groundwater over draft predisposes fruit trees to wither.

Natural resources, particularly surface and ground water, are important for sustainable development and achieving higher economic growth. Efficient and scientific utilization of these resources ensures the ecological balance of an ecosystem. The contribution of natural resources to local economy is outside the market framework, which are both its strength as well as weakness. Strength in the sense of social justice, that it supports rural families. Weakness lies in unsustainable exploitation of these resources, which would result in the tragedy of these resources. Further, unsustainable exploitation leads to scarcity of resources that would then be beyond the reach of the poor.

Consequences of groundwater overexploitation

Groundwater depletion is by far the most widely debated issue in the resource economics literature.Groundwater depletion problems are related to the question of resource management and the coalition of powerful property owners protecting their interests, under a capitalist society. Overexploitation of ground water and its social consequences are the result of certain processes of development in irrigated agriculture that occurs at the cost of depletion of aquifers and sustainable farming systems (Raghupathi and Ganeshamurthy, 2013). The state intervened initially through agrarian reforms, and later by providing credit facilities and supporting marginalized groups to have irrigation facilities by implementing Million Well Schemes, Ganga KalyanYojana and politically influenced free power supply etc. All these led to rise in groundwater structures, shifting cropping pattern towards water intensive crops as well as resource abuse by overexploitation of the aquifer. The distinctive impact of irrigation, in general, and groundwater irrigation, in particular, on farming begins to emerge more clearly and recognizably where irrigation permits extension of cultivation to additional seasons (Rao, 1978). This allows farmers to benefit from surplus production which otherwise would not have been possible. As a result, groundwater became a chief source of irrigation primarily in dry sub-humid, semi-arid and arid areas and at the same time several problems like those mentioned above emerge due to heavy pumping.

Counter argument

Trees consume water. The more the aerial system of trees is developed, the more water they transpire. The desirability of tree planting in arid lands is debated because trees may consume more water than they provide to the water cycle. Some countries, such as South Africa, have imposed a tax on the water consumed by forests. In certain circumstances where trees consume all the rainwater, it may be judged better to harvest this water through a bare watershed, store it in a reservoir and use it to irrigate high-value agricultural crops. For example, in Yatir, Israel, where average precipitation is only 270 mm per year, more than 3000 ha of rainfed *Pinus halepensis* were planted in the early 1960s under a large-scale afforestation project. Although the forest provides carbon sequestration benefits and contributes to the livelihoods of nearby communities (particularly through fuel wood and non-wood forest products such as resins, fodder and medicinal and aromatic plants), it uses all the precipitation water. Furthermore, the forest has altered the biodiversity of the region, as new predation dynamics threaten endemic species. Rueff and Schwartz (2007) reported that the water that the watershed would have provided if it had not been afforested would have alleviated poverty better if it had been used for agriculture. They suggested that afforestation on a smaller scale, such as on farmers' plots, may yield similar benefits with fewer drawbacks, as combining tree planting and agriculture is less disturbing to the environment, improves agricultural yields, conserves water and soils and provides fuel wood for farmers.

Peak water and Future threat

Groundwater contributes 42%, 36% and 27% of water used for irrigation, households and manufacturing, respectively. In regions with extensive surface water irrigation, such as Indo-Gangetic plain, net abstractions from groundwater are negative, i.e. groundwater is recharged by irrigation. The opposite is true for areas dominated by ground water irrigation such as southern plateau regions covering Karnataka, Andhra Pradesh and Tamil Nadu where net abstraction of surface water is negative because return flow of withdrawn groundwater recharges the surface water compartments first and then excess flow downwards (Raghupathi and Ganeshamurthy, 2013).

The National Academy of Agricultural Science (NAAS) in its meeting on phosphorus in 2013 discussed about "Peak phosphorus" going to threaten future food security. But the real threat is the "Peak water". We may produce some food with low phosphorus supply, but we cannot produce food without water. Human beings on an average require four litres of water per day. But the water required for producing each day food per person is around 2,000 litters. This is 500 times as much compared to direct consumption of water by man. We must now understand that getting enough water to drink is relatively easy, but finding enough to produce the ever-growing quantities of food, fruits, vegetables, fodder and other requirements is a matter of serious concern. For example, it is a common scene seeing water tankers carrying water from tube wells from the farm land heading towards cities as a consequence of unplanned city expansions like those seen in Bangalore, Hyderabad, Chennai and Pune. There is concern that the state of peak water is being approached in many areas. Some areas are suffering from peak renewable water, where entire renewable flows are being consumed for human use, peak non-renewable water, where groundwater aquifers are being over pumped (or contaminated) faster than nature recharges them and peak ecological water, where ecological and environmental constraints are overwhelming the economic benefits provided by water use (Gleick and Palaniappan, 2010, 2011) if present trends continue.

In a short span of two to three decades the extraction of water began to exceed the recharge of aquifers from precipitation, and water tables began to fall. And then wells begin to go dry. For example, in the district of Chikkaballapur in Karnataka, Madanapalli in Andhra Pradesh, the farmers draw water worth 1800-2000 mm rainfall for rowing tomato after tomato, whereas the average precipitation is only 750-800 mm. In effect, over pumping creates a water-based food bubble, one that will burst when the aquifer is depleted and the rate of pumping is necessarily reduced to the rate of recharge. Definitely regions such as this have crossed the peak non-renewable water.

A World Bank study estimates that 15% of India's food supply is produced by mining groundwater. Stated otherwise, 175 million Indians are now fed with grains produced with the unsustainable use of water. As early as 2004, Fred Pearce reported in New Scientist that "half of India's traditional hand-dug wells and millions of shallower tube wells have already dried up, bringing a spate of suicides among those who rely on them. Electricity blackouts are reaching epidemic proportions in government where half of the electricity is used to pump water from depths of a kilo meter and above."

The excessive "mining" of our aquifers is causing environmental degradation on a potentially enormous scale (Raghupathi and Ganeshamurthy, 2013). As a country, we have dramatically increased our reliance on groundwater. This increase has dried up rivers and lakes, because there is a hydrologic connection between groundwater and surface water. Yet the legal rules governing water use usually ignore this link. This disconnection between law and science is a major cause of the problem. So too is our refusal to recognize the unsustainability of our water use. Significant reformsare necessary if we are to save our trees, prevent further degradation of our rivers, streams, lakes, wetlands, and estuaries. A final consequence of ground water pumping is its impact on surface water, including lakes, ponds, rivers, creeks, streams, springs, wetlands, and estuaries. These consequences range from minimal to catastrophic. An example of the latter is the Arkavati and Vrishabha vatirivers and a chain of 65 lakes in Bengaluru. Two lakes viz., Hessaraghatta lake and Tippagodanahalli lake provided sufficient good quality drinking water to metropolitan city "Bengaluru". It is more than two decades these very important water bodies have dried-up. Once a verdant riparian system with a lush canopy provided

by several tree species and big gardens, groundwater pumping has lowered the water table, drained the rivers and lakes of their flow, devastated vegetation and driven away the local birds and wildlife. The rivers and lakes have become an oxymoron–a dry river and lake-a pathetic desiccated sandbox. Other lake cities, Bhopal and Udaipur are in the verge of reaching the state of Bengaluru in near future if corrective measures are not taken.

How do water bodies go dry?

Groundwater and surface water are not separate categories of water. The designations groundwater and surface water merely describe the physical location of the water in the hydrologic cycle. Indeed, groundwater and surface water form a continuum. Virtually all groundwater was once stream flow that seeped into the ground. The converse is also true but not obvious. Groundwater pumping essentially interrupts the water cycle by removing water, directly or indirectly, that would otherwise discharge from aquifers to rivers, streams, and other surface water bodies.

As groundwater pumping lowers the water table, the direction of the flow of rivers streams and lakes changes. Once the water table is below the elevation of the rivers, streams and lakes, water flows from the water bodies toward the aquifer. This is what groundwater pumping did in areas where perennial fruit and nut trees are drying. Groundwater pumping literally sucked water from the rivers, streams and lakes and produced horrible environmental consequences. First, of course, the flow in the rivers and streams gets reduced and lakes dried and water-dependent species like areca nut, coconuts and mangos suffered heavily and areca nut and coconut trees withered and mango trees are in the queue.

In considering other examples of environmental problems caused by groundwater pumping, the first thing to note is that the impact of groundwater pumping on the environment is not confined to any given region. Like Karnataka and other southern states, the central Indian states also have similar problems. But the peak has reached in south and may take little more time for the other regions. In the North and Indo-Gangetic plain, the problem is similar but for the well supplied water from Himalayan river systems.

We use groundwater to grow all kinds of things, even when there is no need to do so. Until rather recently, many of our farms were "dryland" farmed. However, as the demand increased farmers shifted from dryland to irrigation farming. In places where only highly drought resistant crops like ragi and horse grams were grown, farmers shifted to highly water dependent crops like tomato, watermelons etc. with almost three-fold increase in cropping intensities, all through exploitation of ground water. We require 200 to 225 litres of water to produce one kilogram of tomato. We export this tomato to other countries at the rate of approximately Rs. 20/- per kg. Are we not foolish to do this and farmers suffering many a times from tomato glut? Such over pumping of water irrigates the surface layers of soil in annual crops like tomato and other vegetables. But the perennial crops in the region, particularly in areas like Srinivasapura in Kolar district in Karnataka and Nuzvid area in Andhra Pradesh under mango and Tumkur and Hassan districts in coconut and areca nut undergo severe stress due to continued profile drying.

Another pitiable example is our newfound fascination with bottled water. It is a scene even plaguing the rural areas. The domestic bottled water market (including organised and unorganised players) is estimated at Rs 8,000 crore. The bottled water market which has been growing at a CAGR of 19%, is expected to continue its growth momentum and grow over four-folds to Rs 36,000 crore by 2020 (Mukherjee, 2012). The industry is heavily dependent on ground water (onelitre bottled water = 1.8 litre of ground water) has become a competitor with the irrigation system.

The urgent need for reforms

The impact of groundwater pumping on agriculture in general and perennial horticulture in particular, is an example of what biologist Garrett Harden called "the tragedy of the commons." The legal rules governing groundwater use is not strong and the law makers are yet to understand the ground reality. We have failed to eliminate the gap between law and science. In lieu of legal reform, we have shown limitless ingenuity in devising technological fixes for water supply problems. These so-called solutions have altered the hydrologic cycle in order to sustain existing usage. As our water use spirals upward, we must begin to rethink the economic structure by which we value our water

resources. At the same time, we must act to protect our rivers, springs, lakes, estuaries and wetlands from groundwater pumping. There is considerable urgency. Because groundwater moves so slowly, it may take years or decades of groundwater pumping before the effect on the environment is apparent. The hidden tragedy is that groundwater pumping which has already occurred will cause irremediable environmental damage.

To control the impact of groundwater pumping on the environment, we must combine a command-and-control model of government rules and regulations with the market forces of transferable rights and price incentives. Any meaningful reform must do two things: protect the rights of existing users by creating quantified water rights that are transferable and therefore valuable; and break free of the relentless cycle of increasing use by placing restrictions on individual freedom to pump groundwater. The law makers must take cognizance of the following issues to save the environment, orchards, water bodies and our future generations.

- Government rules and regulations deserve a prominent place in our reform efforts as we attempt to protect the environment. The government should undertake a number of very specific reforms.
- Even though water is a scarce commodity, most of us have not yet faced the condition that economists call scarcity, which occurs when people alter their consumption patterns in response to price increases. Our habits of water use will not change until the cost of water rises sufficiently to force an alteration. Therefore, we must increase water rates so that all users pay the replacement value of the water, which includes environmental impact cost. Economists agree that significant price increases would create incentives for all users to conserve. All farmers, businesses, or industrial and other users could then decide which uses of water to continue and which to curtail. Rate increases would encourage the elimination of marginal economic activities and the movement of water toward more essential and productive uses.
- The government should carefully craft water conservation standards. However, the experience of some western government with conservation standards sends a mixed message. If the government attempt to impose elaborate and detailed conservation standards, the regulated groups will fight tooth and nail over every sentence in the proposed regulation. This process can consume enormous amounts of time, energy, and money. The lesson for government is that it is better to embrace simple conservation standards that are easy to administer and implement. They are likely to have the most practical effect in terms of actually saving water and will avoid prolonged political struggle.
- The government should establish minimum stream flows and protect those flows from pumping of hydrologically connected groundwater. The legislature should authorize the State Departments of Environment and forestry, agriculture and horticulture to establish minimum water levels for streams and lakes to protect water resources. The minimum levels become appropriations within the prior appropriation system and offer protection against subsequent groundwater pumping.
- The government should prohibit the drilling of new wells in areas that are hydrologically connected to surface flows. Generally speaking, the farther a well is from a watercourse, the less significant the impact of groundwater pumping from that well will be. Government has two options to solve this problem: They can make the ban on wells near watercourses turn on a hydrologic analysis of the particular region, or ban on drilling wells within, for example, a mile of the river.
- Both the state governments and Panchayats should commit resources to purchasing and retiring groundwater rights to protect critical catchments, watersheds and habitats. For example, the catchment area of critical water bodies like Badatalab of Bhopal or Hessaraghatta and Tippagodanahalli lakes in Bengaluru, Sukna lake in Chandigarh, Pichola, Fatehsagar, Jaisamand and Rajsamand lakes of Udaipur, Husain and Himayathsagar in Hyderabad.
- Government should foster a market in water rights by allowing the easy transferability of rights from existing users to newcomers. Enormous quantities of groundwater are used for extremely low-value economic activities. State law must facilitate the movement of water from these uses to higher-value ones by establishing a water rights market as the mechanism for accomplishing this shift.

- The government should impose an extraction tax on water pumped from any well within a certain distance of a river, spring, or lake. This tax would have two benefits: It would encourage existing pumpers to conserve water, and it would create an incentive for new pumpers to locate wells farther away from watercourses.
- The government should not allow land developers to drill wells in an aquifer already under stress and land developers should not be allowed to source water from agricultural areas.
- The government, especially through panchayats, should use financial incentives as a significant part of water policy. Quite simply, we are not paying the true cost of water. When homeowners or businesses receive a monthly water bill from the utility, that bill normally includes only the extraction costs of drilling the wells, the energy costs of pumping the water, the infrastructure costs of a distribution and storage system, and the administrative costs of the water department or company. Water rates, with rare exceptions, do not include a commodity charge for the water itself. The water is free.
- Unplanned urbanization has forced cities to depend on rural areas for sourcing water supplies. The flow of water from rural tube wells to urban areas for meeting domestic and industrial water requirements of cities must be stopped
- Several crops which need huge quantity of water are grown for export like sugarcane, gherkins, tomatoes, capsicum, scented rice etc. These crops are exporting water more than the produce. The actual cost of water is not calculated while working out the economics. Growing crops for export purpose using groundwater is not justified when local populations are going to suffer from severe shortage of water. Such activities must be restricted.
- The government certainly has powers to impose location specific regulations on groundwater pumpers, yet there are two good reasons why it should not do so. First, it would provoke a bruising political battle. The political capital expended to win that fight could be better spent elsewhere. Second, the impact of groundwater pumping on the environment is nuanced and site-specific, depending enormously on the particular hydrologic characteristics of an aquifer. Imposing a uniform template on the nation is likely to exclude some pumping that should be regulated and to include some pumping that poses no serious risk of harm.
- The impact of groundwater pumping on the environment is enormous. And it is getting worse. As the drought that frequently grips the country, farmers, cities and individual homeowners are scrambling in search of additional water supplies. They have often focused on groundwater; indeed, well-drilling businesses around the country are booming. The drought has prompted the media to pay remarkable attention to water issues. A massive campaign to save water is the need of the hour.

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Original Research Paper

Assessment of Genetic Divergence in Long Day Onion (*Allium cepa* L.) through Principal Component and Single Linkage Cluster Analysis

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ABSTRACT

To assess the nature and magnitude of genetic diversity in long day onion germplasm by using the principal component analysis and single linkage cluster analysis an experiment was carried out with 34 onion genotypes. High coefficient of variation with wide range in traits indicated an appreciable variability in germplasm. Genotypes were classified into seven principal components having Eigen value > 1, cumulatively accounted for 83.87% of total variability. Principal Component - I contributed for 24.73% of total variation for followed by principal component-II (15.27%). PC-I had high positive loading for bulb weight (0.401), marketable yield (0.338), total bulb yield (0.401) and PC-II had high positive loading for plant height (0.412), PC-III for high T.S.S. (0.276) PC -IV for A grade bulbs (0.436), PC-V for polar diameter of bulbs (0.514), PC-IV negatively loaded with purple blotch (-0.461) and PC-VII for narrow neck thickness (-0.515). Plotting PC-I aganist PC-II differenciated CITH-O-13, CITH-O-4, CITH-O-22, CITH-O-19, CITH-O-9, CITH-O-6 and CITH-O2 as most divergent genotype.On the basis of single linkage cluster means cluster-I was most importent for average bulb weight, minimum bolters, high marketble bulb percentage high marketable and total bulb yield whereas cluster -II was important for maximum nuber of leaves/plant and minimum neck thicknes. Highest inter-cluster distance was observed between cluter II and Cluster-I(873.5%).Most divergent genotypes with high inter cluter distance could be the most appropriate parents for crop impovement in onion.

Key Words: Genetic diversity, Onion, Principal component analysis, Single linkage cluster analysis

INTRODUCTION

Onion is an important vegetable crop used by all the sections of people,round the year throughout the world for its distinct flavour and health healing properties. It is a photosensitive crop and forms bulbs at certain day length. Long day onion requires 14 hours or more day length to initiate the bulbing. In India, majority of growing area is under short day onion except in hillyregion. Long day onion is grown in temperate region of India with productivity ranging from 10 to 23 t/ha.Though it covers large temperate area from Jammu and Kashmir to Arunachal Pradesh but efforts on varietal improvement programme on long day onion are very limited. There is no commercial long day variety available for cultivation except some old introductions like Yellow Globe and Brown Spanish. Farmers use their own seed without caring isolation distance to maintain the purity which leads to a great variability in shape and size of bulb with inherent low yield.

The magnitude of genetic diversity in onion germplasm is a critical component in breeding for new cultivar. Selection of genetically diverse parents in breeding programme on the basis of divergence would be more promising to get the heterotic F1, and to create a broad spectrum of variability in segregating generation (Meena and

Bahadur, 2015). Presence of genetic diversity play a vital role in plant breeding for getting higher yield, uniformly disired quality and resitance to biotic and abiotic stresses.A systematic understanding of gentic diversity in different traits is essential for targeted breeding programme. There are numerical taxonomic techniques available to classify the variation pattern at inter and intra specific level (Ario and Odulaza, 1999). Multivariate analysis is an effective tool for characterization and classification of plant genetic resources, when a large number of accessions are assessed for several traits (Peter and Matrinelli, 1989). Different type of multivariate analysis such as principal component analysis (PCA) and single linkage cluster analysis (SLCA) are used to identify groups of accessions that have desirable traits for breeding and assessing the pattern of variation in germplasm collection. PCA enables easier understanding of impact and relationship among the different traits. However PCA alone would not give an adequate character representation in term of relative importance when multiple characters are considered simultaneously (Shalini *et al.,* 2003). To complement the results of such multivariate analysis, Single Linkage Cluster Analysis(SLCA) is employed to classify the variation. It is an agglomerative technique which shows the patterns of exact genotype position in population. (Ariyo and Odulaza, 1999) by sorting them in distinct group.Thus this study is aimed to identify the major characters responsible for variation among the onion genotypes with a view to group accessions and for identifying the potential parental stocks within the group of local germplasm by employing the multivariate analysis.

MATERIALS AND METHODS

Thirty four long day onion accessions (*Allium cepa* L.) including two varieties collected from growing hot spot of Kashmir valley and conserved at active germplasm site of ICAR-Central Institute of Temperate Horticulture, Srinagar (J&K) were evaluated (Table 1). The seedlings of 45 day old were transplanted in main field during rabi season. Each accession was grown in ten rows of two metre length with a spacing of 10x15 cm. The experiment was conducted in randomized block design with three replications. Geographical position of the experimental site lies between latitude of $34^0$05 N and longitude of $74^0$50 E at an altitude of 1640 MSL. The average maximum and minimum temperature were 19.63^{0}C, 6.52^{0}C respectively with annual precipitation of 160.72 mm and relative humidity 58.35%, evaporation 2.45mm. The soil characteristics *viz.* pH= 6.81 and EC = 0.36 dSm^{-1} were recorded during the cropping season. Recommended uniform agronomic and cultural practices were adopted to obtain better expression of phenotypic characters. Data was recorded on nineteen quantitative traits. Disease severity rating was measured on 0-5 scale - (0 grade - No disease, 1 grade - 1-10%, 2 grade -11-20%, 3 grade -21-30%, 4 grade -31-50% and 5 grade -51-100%). Whereas, pest (thrips) damage (1-5 scale) - (1 -1-20% foliage damage, 2- 21-40 foliage damage, 3-41-60% foliage damage, 4-61-80% foliage damage and 5-81-100% foliage. The genotypes with <5% infestation was considered immune, 6-10% infestation resistant, 10-20 infestation moderately resistant, 21-40% infestation moderately suceptble, 41-60% infestation susceptible >60% infestation considered highly susceptible. Data collected on the quantitative characters were analyzed using SAS Microsoft Windows 9.2 (SAS Institute, 2011), employing the method outlined by Steel and Torrie (1980) using statistical XL STAT-2011. Principal Component Analysis and Single Linkage Cluster Analysis (SLCA) were used for the determination of genetic variation and percentage similarity within the genotypes. Eigen - Vectors and principal component score were used to assess the relative discriminatory power of its axis and their associated characters. The cluster procedure was used to produce distinct groups of 34 genotypes on the basis of genetic relationship while using the character variation. Average intra-cluster distance was calculated by the following formula as suggested by Singh and Choudhary (1985). SLCA summarized the position of accessions into a dendogramat an interval of 5% level of dissimilarity starting from 100 % level of dissimilarity (Kendall, 1980).

Table1. Accessions with their geographical information used in study

Genotype	Collection site	Latitude	Longitude	Altitude (Meter)	Genotype	Collection site	Latitude	Longitude	Altitude (Meter)
CITH-O-1	Budgam	34.63^0	74.04^0	3199.99	CITH-O-17	Srinagar	34.08^0	74.79^0	1585.01
CITH-O-2	Budgam	34.63^0	74.04^0	3199.99	CITH-O-18	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-3	Budgam	34.63^0	74.04^0	3199.99	CITH-O-19	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-4	Budgam	34.63^0	74.04^0	3199.99	CITH-O-20	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-5	Budgam	34.63^0	74.04^0	3199.99	CITH-O-21	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-6	Budgam	34.63^0	74.04^0	3199.99	CITH-O-22	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-7	Budgam	34.63^0	74.04^0	3199.99	CITH-O-23	Baramulla	34.198^0	74.36^0	1590.00
CITH-O-8	Gandharbal	34.23^0	74.78^0	1619.10	CITH-O-24	Shopian	34.81^0	75.01^0	2057.00
CITH-O-9	Gandharbal	34.23^0	74.78^0	1619.10	CITH-O-25	Shopian	34.81^0	75.01^0	2057.00
CITH-O-10	Gandharbal	34.23^0	74.78^0	1619.10	CITH-O-26	Badipora	34.50^0	74.68^0	1578.00
CITH-O-11	Gandharbal	34.23^0	74.78^0	1619.10	CITH-O-27	Bandipora	34.50^0	74.68^0	1578.00
CITH-O-12	Gandharbal	34.23^0	74.78^0	1619.10	CITH-O-28	Kulgam	33.65^0	75.02^0	1738.88
CITH-O-13	Srinagar	34.08^0	74.79^0	1585.01	CITH-O-29	Kulgam	33.65^0	75.02^0	1738.88
CITH-O-14	Srinagar	34.08^0	74.79^0	1585.01	CITH-O-30	Kupwara	33.65^0	75.02^0	1738.88
CITH-O-15	Srinagar	34.08^0	74.79^0	1585.01	CITH-O-31	Kupwara	33.65^0	75.02^0	1738.88
CITH-O-16	Srinagar	34.08^0	74.79^0	1585.01	CITH-O-32	Srinagar	34.08^0	74.79^0	1585.01
Coral Red (check)	ICAR-DOGR Pune		-	-	Brown Spanish	ICAR-DOGR Pune			

RESULTS AND DISCUSSION

The genotypes evaluated for all horticultural traits varied significantly (Table 2). The phenotypic variability expressed by range, standard deviation, and coefficient of variation. The plant height ranges from 63.33 to 91.66 cm. Genotype CITH-O-9 recorded highestplant height, whereas CITH-O-32 recorded lowest plant height (63.33 cm). Number of leaves ranged from 6.33 (CITH-O-9) to 14.0 cm (CITH-O-19. Polar diameter of bulb ranged from 5.18cm (CITH-O-7) to 11.97cm (CITH-O-13). Equatorial diameterof bulb ranged from 5.87cm (CITH-O-5) to 11.08 cm (CITH-O-8). Polar and equatorial diameter ratio reflects the bulb shape

Table 2. Variation in quantitative traits of onion accessions

Characters	Range				Mean	Standard deviation	CV (%)
	Minimum		Maximum				
	Value	Genotype	Value	Genotype			
Plant height (cm)	63.33	CITH-O-32	91.66	CITH-O-9	80.09	5.92	12.92
No. of Leaves/ plant	6.33	CITH-O-9	14.00	CITH-O-19	9.87	1.66	23.71
Polar diameter (cm)	5.18	CITH-O-7	11.97	CITH-O-13	7.44	1.23	20.58
Equatorial diameter (cm)	5.87	CITH-O-5	11.08	CITH-O-8	8.52	1.15	20.85
Polar Equatorial diameterratio	0.55	CITH—O-11	2.03	CITH—O-4	0.89	0.26	25.82
Neck thickness (cm)	0.42	CITH-O-7	2.46	CITH-O-19	0.96	0.38	30.56
A Grade bulb (%)	12.00	CITH-O	86.11	CITH-O-12	58.71	15.95	37.05
B Grade bulbs (5)	0.00	CITH-O	34.55	CITH-O	13.96	10.39	60.16
C Grade bulbs (%)	0.00	CITH-O	33.00	CITH-O	6.53	8.42	51.04
Doubles (%)	0.00	CITH-O	34.28	CITH-O	16.05	11.48	66.97
TSS %	0.36	CITH-O-5	16.00	CITH-O-29	10.92	4.36	54.51
Average Bulb weight (gm)	154.51	CITH-O-29	470.30	CITH-O-9	289.08	82.29	34.99
Purple Blotch (%)	7.00	Brown Spanish	30.71	CITH-O-29	19.52	50.88	33.13
Thrips/plant	7.66	CITH-O-17	31.00	CITH-O-6	24.26	5.44	35.12
Downy mildew (%)	13.48	CITH-O-9	30.50	CITH-O-2	20.80	4.92	32.25
Bolters (%)	0.00	CITH-O-9	3.66	CITH-O-11	0.91	1.10	60.31
Marketable bulbs (%)	48.26	CITH-O-9	100.00	CITH-O-28	82.35	12.60	25.20
Marketable yield (q/Ha)	331.28	CITH-O-9	1212.56	CITH-O-31	765.09	251.74	41.52
Total Yield (q/h)	494.45	CITH-O-9	1505.06	CITH-O-31	925.07	263.32	34.99

which is an important parameter indirectly related to yield storage life and market preference. The bulbs of genotype having < 1 Polar and equatorial diameter ratio (P: E) considered as flat and genotypes having P: E ratio 1 considered globe and those having P: E ratio> 1 were considered as torpedo. Genotype CITH-O-11, had P: E ratio 1, whereas CITH-O-4, CITH-O-32 and CITH-O-13 have < 1 P: Eratio and remaining genotypes were having >1 P: E ratio. Neck thickness of bulb affects the storage life. Neck thickness ranged from0.42 to 2.46 cm. The minimum neck thickness (0.42 cm) was observed with genotype CITH-O-7, whereas CITH-O-19 had maximum neck thickness (2.46 cm). Bulb grade determines the market price and quality. A grade bulb ranged from 12 to 86.11 per cent (Table 3). Genotype CITH-O-12 recorded highest A grade percentage of bulbs. B grade bulb ranged from 00 to 34.55 per cent. Double bulbs which are major drawback in onion production ranged from 00 to 34.28 per cent. Total soluble solids important quality trait in onion ranged from 0.36 to 16 per cent. Genotype CITH-O-29 scored highest TSS (16 %) whereas minimum TSS (0.36%) was recorded with genotype CITH-O-5. Average bulb weight which is directly correlated with yield, ranged from 154.51 to 470.30g. Genotype CISH-O-9 recorded the highest average bulb weight (470.30 g) whereas smallest bulb size was recorded with CITH-O-29 (154.51 g). Foliar disease of onion is major problem in long day conditions.The incidence of purple blotch ranged from 7.00 to 30.71%. Genotype CITH-0-29 has recorded highest infestation (30.31%) whereas,

Table 3. The Principal component latent vector for Eigen values and proportion of variance accounted for different components with respect of different traits

Characters	PC-I	PC-II	PC-III	PC-IV	PC-V	PC-VI	PC-VII
Plant height (cm)	-0.039	0.412	0.045	0.208	0.328	-0.215	0.018
No. of Leaves/ plant	-0.317	0.147	0.273	-0.001	0.192	-0.043	0.014
Polar diameter (cm)	-0.037	-0.315	-0.109	-0.262	0.514	-0.072	-0.162
Equatorial diameter(cm)	0.354	0.227	0.062	-0.018	-0.075	0.040	0.160
Polar Equatorial diameterratio	-0.204	-0.355	-0.168	-0.138	0.398	-0.108	-0.230
Neck thickness (cm)	0.040	0.288	0.283	-0.228	0.096	0.128	-0.515
A Grade bulb (%)	0.179	-0.111	0.278	0.436	0.234	-0.187	0.083
B Grade bulbs (5)	-0.208	0.034	0.346	-0.355	-0.111	0.250	0.081
C Grade bulbs (%)	-0.027	-0.336	-0.302	0.041	-0.403	-0.090	0.146
Doubles (%)	-0.243	0.198	-0.267	-0.325	-0.101	0.154	0.177
TSS %	-0.236	-0.142	0.276	-0.214	-0.135	-0.179	0.146
Average Bulb weight (gm)	0.401	0.034	-0.029	-0.309	0.104	0.014	0.022
Purple Blotch (%)	0.055	-0.082	0.198	-0.183	0.021	-0.461	0.376
Thrips/plant	-0.195	-0.170	0.305	-0.108	0.012	0.057	0.277
Downy mildew (%)	-0.006	-0.137	0.158	0.221	0.156	0.660	-0.002
Bolters (%)	-0.039	0.023	-0.331	0.029	0.322	0.280	0.564
Marketable bulbs (%)	0.148	-0.410	0.282	0.146	-0.098	0.137	0.030
Marketable yield (q/Ha)	0.388	-0.192	0.125	-0.199	0.025	0.083	0.063
Total Yield (q/h)	0.401	0.034	-0.029	-0.309	0.104	0.014	0.022
Eigen value	4.698	2.902	2.410	2.072	1.583	1.221	1.051
Variability (%)	24.726	15.271	12.686	10.904	8.333	6.424	5.530
Cumulative %	24.726	39.998	52.684	63.588	71.921	78.345	83.876

least infestation was observed with variety Brown Spanish (7%). Downy mildew infestation ranged from 13.48 to 30.50%. The lowest (13.48%) infestation of downy mildew was observed in genotype CITH-0-9 whereas highest infestation of was recorded with CITH-0-2 (30.50%). Thrips is major damaging insect in long day onion. Number of thrips/plant ranged from 7.66 to 31.00 / plant. The minimum infestation of thrips /plant was recorded with genotype CITH-0-6 (7.66/plant) whereas, maximum number of thrips / plant was observed with CITH -0-17 (31.00/plant). Premature bolting a burning problem in onion ranged from 0 to 3.66 % among the genotypes evaluated. The highest percent of bolting was observed in genotype CITH-0-11 (3.66%), whereas fourteen genotypes recorded 0% bolting. Marketable bulb percentageis an important trait from economic point of view. The percentage of marketable bulb ranged from 48.26 to 100 %. The lowest marketable bulb percentage (48.26%) was recorded with genotype CITH-09 whereas CITH-0-28 had recorded 100% marketable bulbs. Marketable bulbyield ranged from 331.28 to 1212.56 q/ha. The lowest marketable bulb yield was recorded with genotype CITH-0-31 (331.28 q/ha) where as highest marketable bulb was recorded with CITH-0-8 (1212.56 q/ha). Total yield ranged from 494.5 to 1505.06 q/ha. Among the genotype evaluated, CITH-09 recorded highest total yield, where as minimum total yield was observed with CITH-0-31. The genotype having the highest desirable traits may be utilised for crop improvement programme for a particular trait. Coefficient of variation (%) reflected the extent of variationfor evaluated phenotypic traits was highest for double bulb percentage and B grads of bulbs, T.S.S and marketable bulb yield (q/h). High coefficient of variation among studied traits indicated an appreciable variability which is prerequisite of a crop improvement program. Similar type of variability was also reported by Arya *et al*. (2017). The observed variability found among the onion genotypes might be related to genetic makeup of genotype as per Kandil *et al*. (2010).

Based on degree of divergence 34 genotypes were grouped into 7 principal component having Eigen value >1 and cumulatively accounted for 83.87% of total variability (Table 4a and 4b). The PC-I contributed for 24.73% of total variation was positively loaded with bulb weight, marketable bulb percentage, total and marketable bulb yield. It was negatively correlated with number of thrips per plant, downy mildew infestation, (%) bolters and doubles, B and C grades bulbs. The PC-II reflected 15.27% of total variability and was positively loaded with plant height, neck thickness and negatively with downy mildew infestation. The PC-III was positively loaded with T.S.S. (%), number of leaves/plant and contributed 12.69 % of total variation. The 4th principal component contributed 10.90% of total variability was, associated with A grade bulb (%) and negatively correlated with purple blotch disease.The PC-V accounted 8.33% of total variation was positively loaded with polar diameter, Polar: Equatorial. Diameter ratios, bolter percentage and negatively loaded with C grade bulb percentage. Principal Component-VI contributed 6.42% of total variation and was positively correlated with downy mildew (%) but it was negative associated with purple blotch. PC-VII accounted for 5.53% of total variation was positively loaded with bolter percentage, purple blotch percentage, and number of thrips/plant and was negativity correlated with neck thickness.

The positive and negative loading of quantitative traits reflects the positive and negative correlation trend between the components and variables suggesting that these principle components may be used to summarise the variables. The traits with largest absolute value closer to unit within first component influence the cluster more than those to lower absolute value closer to zero. Thus in present study the differentiation of genotypes in different principal component was because of high contribution of few characters rather than small contribution of each character. The desirable characters loaded positively and undesirable characters loaded negatively in first seven PC's could be in consideration while selecting the genotype for appropriate traits and yield potential. The principal component analysis has also been used for showing the genetic diversity in many species (Ravindra *et al.*, 2018; Singh *et al.*, 2017). The Bi-plot of PC-I & PC-II indicated that the some isolated genotype clearly define the diversity among the evaluated germplasm. The genotype CITH-0-13, CITH-0-4, CITH-0-22, CITH-0-19, CITH-0-9, CITH-06, CITH-O-2 and variety Coral Red were most

Table 4a. Cluster means for 19 characters in 34 onion genotypes based on agglomerative hierarchical clustering analysis

Characters	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
Plant height (cm)	63.33	83.00	79.66	79.33	80.00
No. of Leaves/ plant	7.33	12.00	10.33	8.33	11.00
Polar diameter (cm)	7.50	8.54	6.55	5.18	7.95
Equatorial diameter(cm)	7.16	5.87	9.32	9.48	8.99
Polar Equatorial diameter ratio	1.05	1.45	0.70	0.55	0.89
Neck thickness (cm)	0.98	0.66	1.21	0.42	0.88
A Grade bulb (%)	22.00	42.00	69.21	65.69	63.58
B Grade bulbs (5)	25.00	6.35	12.28	0.00	20.51
C Grade bulbs (%)	25.00	12.40	0.00	15.84	7.69
Doubles (%)	28.00	28.05	17.30	16.33	8.20
TSS %	15.06	9.50	14.00	2.23	14.10
Average Bulb weight (gm)	374.06	196.98	310.39	236.69	377.76
Purple Blotch (%)	8.24	10.26	13.09	8.39	9.10
Thrips/plant	24.66	21.33	24.00	9.33	29.00
Downy mildew (%)	16.08	19.96	20.16	18.06	17.01
Bolters (%)	0.00	1.18	1.21	2.13	0.00
Marketable bulbs (%)	92.66	71.94	82.70	83.66	91.79
Marketable yield (q/Ha)	1109.15	453.47	821.41	633.64	1109.59
Total Yield (q/h)	1197.01	630.34	993.24	757.40	1208.83

divergent (Fig 1) usually is customary to select one of the important variable from these identified groups for targeted improvement programme. Hence PC-I for higher total yield, PC-II for plant height, PC-III for high T.S.S, PC-IV for maximum A Grade bulb, PC-V for wider polar diameter of bulb, PC-VI for resistance to purple blotch, PC-VII for thin Neck thickness of bulb were ideal for selection . The results of present study are useful as it furnish the information about the group where certain traits are more important, allowing breeder to execute breeding for specific target. Biological implication of principal component analysis can be quantified by contribution of different variable in each PC as revealed by Eigen vector and cluster score at the component axis suggest that some relationship exist among the individuals with the cluster but not provided the exact position of genotypes in groups.

Based on single linkage cluster analysis genotypes were grouped into five clusters by quantifying their share and cluster means for all the traits (Table 4 a,b). The cluster-I and Cluster-III accommodated maximum number of genotype (9) followed by cluster II (8), Cluster-IV (7) and cluster -V(1) contributing 26.47, 23.53, 20.58 and 2.94%, of total population respectively. On the basis of cluster means, the cluster-I was important for high TSS (15.06%), marketable bulbs percentage (92.66%) powdery mildew (8.24%) and purple loch (16.08%) resistance. Cluster-II was important for plant height (83 cm) number of leaves/plant(12), polar diameter (8.54 cm) and P:E ratio (1.54) cluster -III was important A grade bulb percentage (69.21%)whereas cluster-IV was important equatorial diameter (9.48 cm),minimum neck thickness (0.42 cm) thrips resistance (9.33 thrips/ plant). Cluster -V was important for average bulb weight (377.76 g) marketable yield (1109.59 q/ha) and total yield (1208.83 q/ha). The genotype of cluster having high means value of particular traits would contribute more positively in their off springs if used as a parent. Arya *et al.* (2017) and Singh *et al.*

Table 4b. Grouping of 34 onion genotypes into five clusters based on agglomerative hierarchical clustering analysis

Characters	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
Number of genotypes	9	8	9	7	1
% of total genotypes	26.47	23.52	26.47	20.28	2.94
Position of genotype	CITH-O-1 CITH-O-2 CITH-O-3 CITH-O-8 CITH-O-9 CITH-O-18 CITH-O-27 CITH-O-32 Brown Spanish	CITH-O-4 CITH-O-14 CITH-O-16 CITH-O-21 CITH-O-22 CITH-O-24 CITH-O-30 CITH-O-31	CITH-O-5 CITH-O-6 CITH-O-10 CITH-O-11 CITH-O-12 CITH-O-20 CITH-O-23 CITH-O-26 Coral Red	CITH-O-7 CITH-O-13 CITH-O-15 CITH-O-17 CITH-O-19 CITH-O-25 CITH-O-28	CITH-O-29

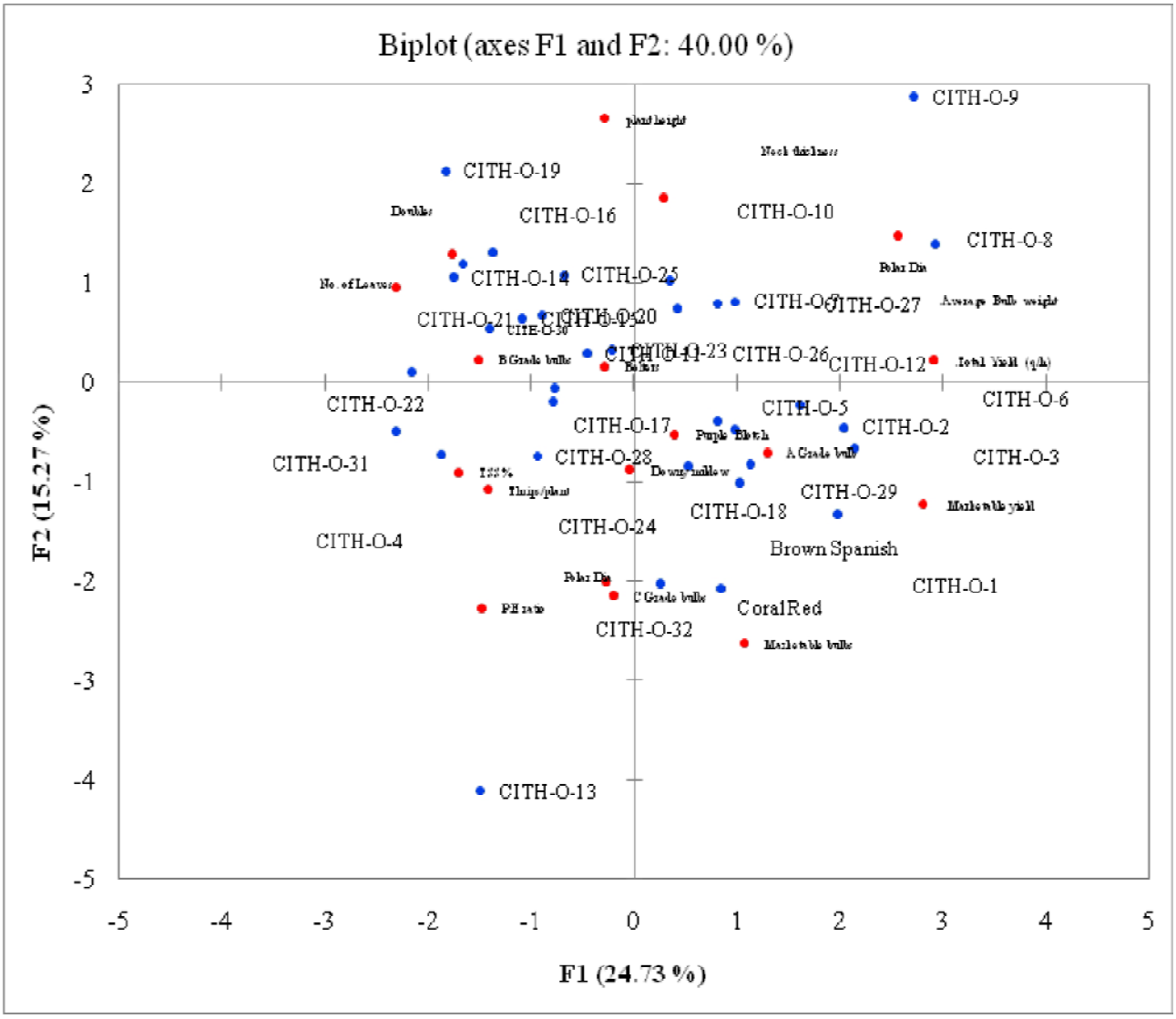

Fig. 1.: Bi-plot for 1st and 2nd PC for genotypes of onion in relation horticultural traits

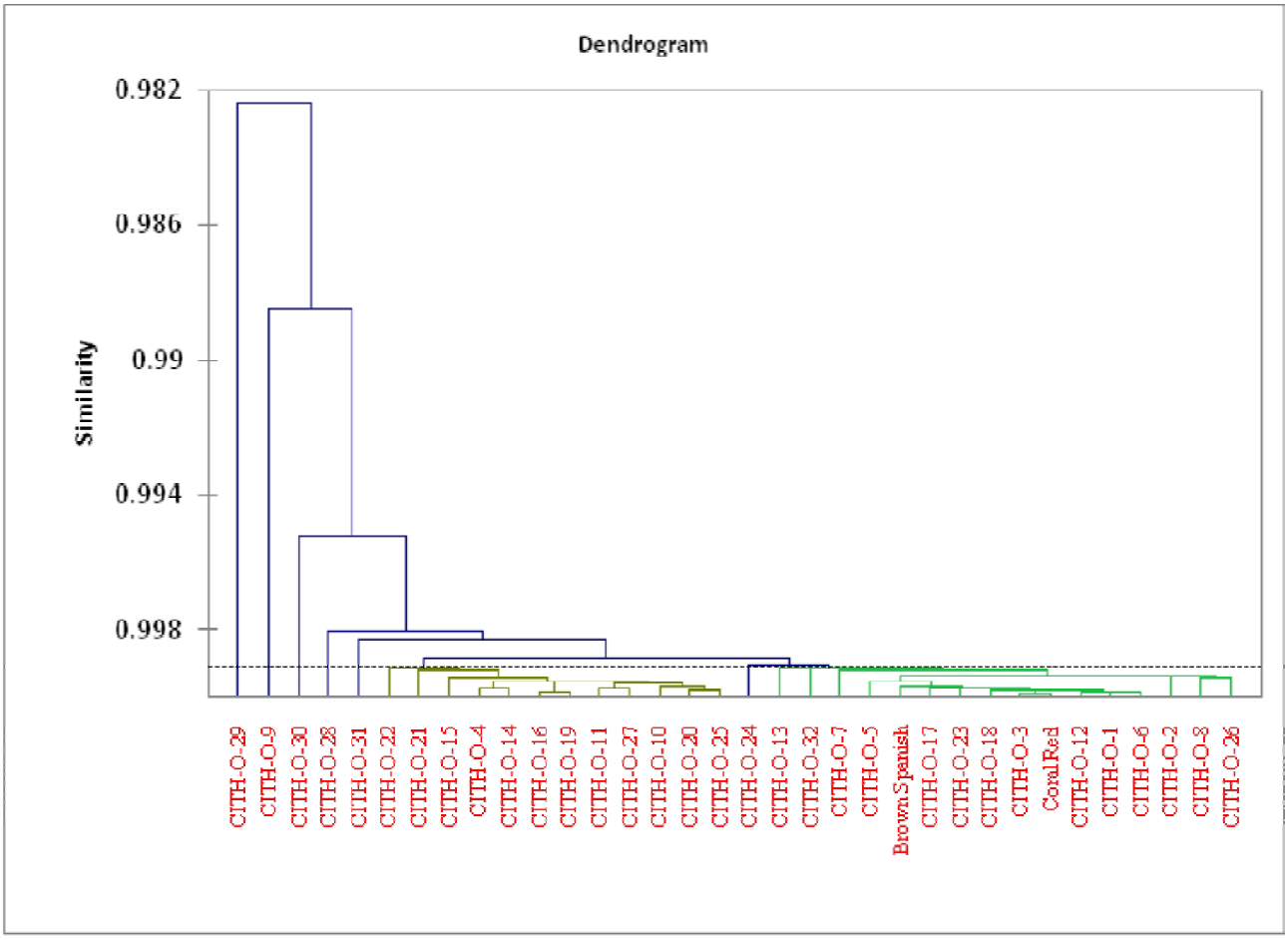

Fig. 2. Dendrogram depicting genetic relationship among 34 genotypes based on horticultural traits produced by complete linkage analysis (Scale- Euclidean distance at .05)

(2017) also suggest that clusters having high mean value of the traits may be used for hybridization program to get better segregates.

Proximity matrix obtained, suggest the resolution for 34 onion genotype distributed in five clusters with wide range of diversity for the traits (Table 5.) The highest inter cluster distance between cluster II and Cluster I (803.47%) followed by cluster-III and I (697.8) cluster V and cluster I (582.56). Based on convention that distantly related parents give better recombinants and hybrid. It could be expected that hybridization between genotype of these cluster will results high heterotic F_1, s and better recombinants in segregating generations. These genotypes of distant clustercould serve useful source of genes for different desirable traits in onion. The findings are in conformity with finding of Singh *et al.* (2017), Chattopadhay *et al.* (2015) and Ravindra *et al*. (2018) who reported that genotypes among the cluster having high distance

Table 5. Average Inter and intra cluster distance

	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V
Cluster-I	0.00	803.47	697.08	509.31	582.56
Cluster -II		0.00	488.05	334.36	435.95
Cluster-III			0.00	305.03	579.48
Cluster-IV				0.00	353.09
Cluster-V					0.00

when used in hybridization programme will obtain a wide spectrum of variation in sergeants.

Dendogram obtained from single linkage cluster analysis by using the Euclidian distance depicted the clear relationship and exact position of genotype in the clusters. All the genotype were distinct at 100 percent of dissimilarity and formed nine duster at 87% of dissimilarity, and formed five clusters at 57 of dissimilarity (Fig II). The dissimilarity ranged from 57 to 100% among the delineated genotypes enough to suggest the variability for crop improvement in onion. (Denton and Nwangburuka, 2011).

Genotypes CITH-0-29, CITH-0-26, CITH—8, CITH-0-24 and CITH-05 were divergent in cluster position on the basis of Euclidian distance which reflected higher distance among these genotypes and may be used for hybridization to get the better segregates. Singh *et al.*, 2013 and Santara *et al.,* 2017 also reported such variability by using the single linkage cluster analysis in Onion.This genetic diversity analysis would be useful to avoid the selecting parents from genetically homogenous cluster and maintain the broad genetic base for breeding programme in long day onion.

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Original Research Paper

Carotenoid Content in Cherry Tomatoes Correlated to the Color Space Values L*, a*, b*: A Non-destructive Method of Estimation

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ABSTRACT

Cherry tomatoes are rich sources of carotenoids. The carotenoids are known to be precursors of vitamin A and also act as an antioxidant. It is important to visually judge the tomato surface color for higher β carotene content since this is the major provitamin A carotenoid. Estimation of carotenoids by HPLC (High Performance Liquid Chromatography) and spectrophotometric methods in tomatoes are very expensive and time consuming. Therefore, colorimeters can be used to describe the color and determine the carotenoid content in a relatively easy and inexpensive manner. The objective of this study was to determine, if the carotenoid content within cherry tomatoes measured by conventional method could correlate with colorimetric CIE (Commission International del'Eclairage) L*, a*, b* color space values. Strong correlations were found between color surface value a* and total carotenoids (0.82) and lycopene content (0.87). We also observed positive correlation for the b* color value with β carotene (0.86). The L* value was negatively correlated (-0.78) with an increase in carotenoids. These close associations between color space values L*, a*, b* and carotenoids will help the breeders to quickly screen large germplasm/ breeding lines in their breeding program for improvement in carotenoid content through this time saving, inexpensive and nondestructive method at fully ripe stage.

Keywords: β carotene, Carotenoid, Lycopene, Tomato.

INTRODUCTION

Color is one of the important quality parameters of fruits and vegetables. The color of tomatoes is the most important quality character to determine the ripeness. The color of tomatoes is the initial external factor that makes them appealing to the consumer's decision for purchasing them. The complexity of tomato color is due to the presence of a diverse carotenoid pigment system with appearance conditioned by pigment types and concentrations, and subject to both genetic and environmental regulation (Radzevicius *et al.,* 2014). Color of tomatoes is an important desired character which can be achieved by genetic improvement of breeding lines with varying concentration of carotenoids. The tomatoes are harvested and consumed at the red ripe stage of ripening, which occurs due to the degradation of chlorophyll at green stage and rapid accumulation of carotenoids particularly lycopene and β carotene. In this study, we have assessed surface color differences among the cherry tomatoes and its relation to their total carotenoid, lycopene and β carotene content. Carotenoid content in fruits can be assessed in laboratory through spectrophotometer measurement of tomato fruit extracts, but this method is time consuming and tedious (Lichtenthaler 1987). Colorimeters can be used to determine the carotenoid content in fruits and vegetables in a quick, easy and in a non-destructive manner. In 1931, the Commission International del'Eclairage (CIE) made possible to express color in exact quantitative and numerical

terms. An improvement of this system was developed in 1976 by CIE, which defines color better related to human perception and where all conceivable colors can be located within the color sphere defined by three perpendicular axes, L* (from white to black),a* (green to red) and b* (blue to yellow). In the present study, an attempt was made to correlate tomato surface color values with actual carotenoids content so as to standardize a fast, inexpensive and non-destructive method.

MATERIALS AND METHODS

Plant material: Nine different cherry tomato lines such as IIHR 2754, IIHR 2857, IIHR 2858, IIHR 2861, IIHR 2862, IIHR 2863, IIHR 2864, IIHR 2865, IIHR 2866 were grown in the open field at ICAR–Indian Institute of Horticultural Research, Bengaluru, India. Fruits were harvested in ripe stages and brought to the laboratory for further examination of color and estimation of carotenoids.

Carotenoid profiling : Total carotenoids and lycopene content were analyzed by spectrophotometry method (Lichtenthaler 1987). Carotenoids were extracted using acetone and partitioned with hexane for the ripe stage.The carotenoids in the extract were estimated by reading absorbance at 470 and 503 nm for estimating total carotenoids and lycopene respectively. Thecarotenoid profiling was done using UPLC, as per themethod reported by Serino *et al*. (2009) with modifications.

Color measurement : The surface color (values of L*,a*, b*, C* and hue angle) was measured on fresh tomatoes using a color Reader, CR-10 (Minolta Co. Ltd, Osaka, Japan; measuring area of 8mm with 8/d viewing geometry using CIE Standard Illuminant D65). Three different measurements were taken at three equidistant points on the equatorial region of individual fruit. The value L*(lightness) indicates the ratio of white and black color, value a* is the ratio of red and green colors, value b* is the ratio of yellow and blue colors. Chroma/Chromaticity (C*) is the saturation or vividness of color. As chromaticity increases, acolor becomes more intense; as it decreases, a color becomes more dull. Hue angle isthe basic unit of color. Both chroma and hue are derived from a* andb* using the following equations: chroma: $C^* = \sqrt{(a^*)^2 + (b^*)^2}$and hue angle: $h^0 = \tan^{-1}(b^*/a^*)^0$ (Itle *et al.*, 2009). It should be noted that all color space values L*, C, a* and b* are measured in NBS units, hue angle h° in degrees from 0 to 360°. NBS unit is a unit of USA National Standard Bureau and corresponds to one threshold of color distinction power, *viz*. the least distinction in color, which the trained human eye can notice (Juskeviciene *et al.*, 2014).

***Statistical analysis*:** Correlation analysis and regression analysis was conducted for total carotenoids, lycopene and β carotene with color space values using statistical package SPSS ver. 19 (SPSS Inc., Chicago, IL, USA) software (Wellman 1998). Microsoft Excel program was used to plot the scatter plot and calculate regression equation. Mean cd and standard error was also calculated.

RESULTS

Colorimetric measurements: Significant differences were observed among the cherry tomato lines for color values L*, a*, b*, C* and hue angle. Beginning with the L* value a range from lightness (48.9) to darkness (37.40) was observed in tomato lines evaluated. Highest L* value of 48.9 NBS units was observed for IIHR 2866 and IIHR 2754 showed the least L* value of 37.40. Mean color space value a* ranged from 35.43 in IIHR 2754 to 18.03 in IIHR 2866. The mean color space value b* ranged from 42.99 in IIHR 2866 to 21.03 in IIHR 2754. C*, Chroma/chromaticity ranged from 46.61 in IIHR 2866 to 34.46 in IIHR 2857. Hue angle ranged from 67.25^0 in IIHR 2866 to 30.69^0 in IIHR 2754 (Table. 1 & Fig. 1). There was a significant difference in the total carotenoid content among the tomato lines. The dark red fruit line IIHR 2754 contained highest carotenoid content with 23.80 mg/100g FW. The lycopene content was also more in IIHR 2754 with 15.10 mg/100g FW and β-carotene content was 3.02 mg/100g FW. IIHR 2865 contained the least amount of carotenoids with 8.20mg/100g FW. IIHR 2866 contained the lowest lycopene content 0.85 mg/100g FW and highest β-carotene content 8.56mg/100g FW (Fig. 2).

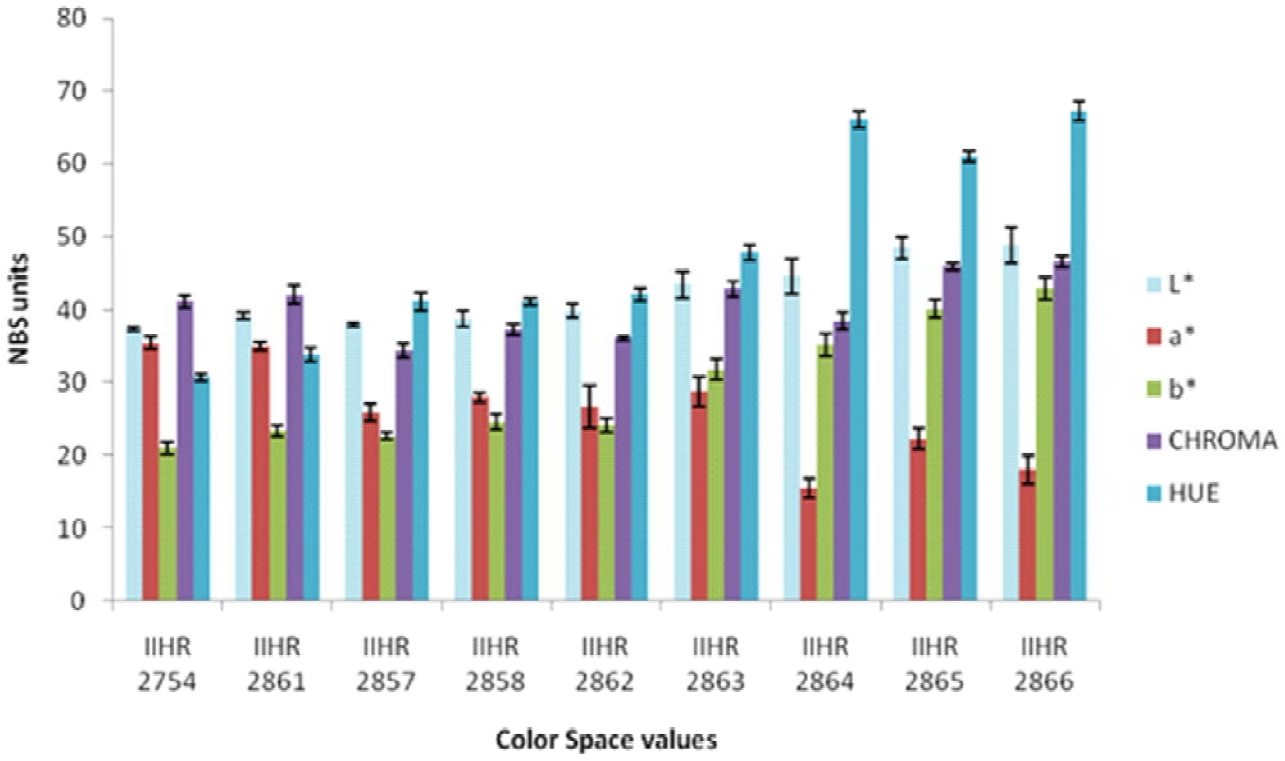


Fig. 1. Color indexes L*, a*, b*, C* and hue angle in cherry tomato lines. Error bars indicate the extent of variation among genotypes.

Table 1. Each observation is a mean ±SD of three replicate experiments of color indexes L*, a*, b*, C*, hue angle (h°) and total carotenoids, lycopene and β-carotene content in cherry tomato lines.

GENOTYPES	L	a*	b*	Chroma angle (h°)	Hue	Tot car	Lycopene	β-carotene
IIHR 2754	37.40 ± 0.49	35.43 ± 0.62	21.03 ± 0.70	41.21 ± 1.25	30.69 ± 0.98	23.80 ± 1.26	15.10 ± 0.69	3.02 ± 1.25
IIHR 2861	39.20 ± 0.29	34.93 ± 1.16	23.37 ± 0.54	42.03 ± 0.96	33.78 ± 1.25	17.90 ± 1.54	11.60 ± 0.87	1.23 ± 0.96
IIHR 2857	37.93 ± 1.08	25.93 ± 0.70	22.70 ± 1.04	34.46 ± 0.69	41.20 ± 0.54	13.70 ± 2.36	8.30 ± 1.06	1.79 ± 0.69
IIHR 2858	38.77 ± 0.94	28.07 ± 2.98	24.53 ± 0.97	37.28 ± 0.25	41.16 ± 0.85	12.10 ± 0.98	5.20 ± 1.89	0.80 ± 0.25
IIHR 2862	39.83 ± 1.89	26.73 ± 1.98	24.20 ± 1.37	36.06 ± 1.02	42.15 ± 1.06	11.00 ± 1.06	6.20 ± 1.65	1.63 ± 1.02
IIHR 2863	43.43 ± 2.36	28.83 ± 1.41	31.80 ± 1.54	42.93 ± 1.26	47.80 ± 1.15	10.70 ± 1.54	6.10 ± 0.84	1.87 ± 1.26
IIHR 2864	44.60 ± 1.47	15.57 ± 1.47	35.13 ± 1.21	38.43 ± 0.48	66.10 ± 0.75	9.74 ± 1.97	3.30 ± 1.78	6.44 ± 0.48
IIHR 2865	48.50 ± 2.50	22.30 ± 1.96	40.13 ± 1.58	45.91 ± 0.78	60.94 ± 1.35	8.20 ± 2.01	2.00 ± 1.30	5.65 ± 0.78
IIHR 2866	48.90 ± 0.69	18.03 ± 1.02	42.99 ± 0.66	46.61 ± 1.36	67.25 ± 1.06	9.50 ± 1.23	0.85 ± 0.56	8.56 ± 1.36

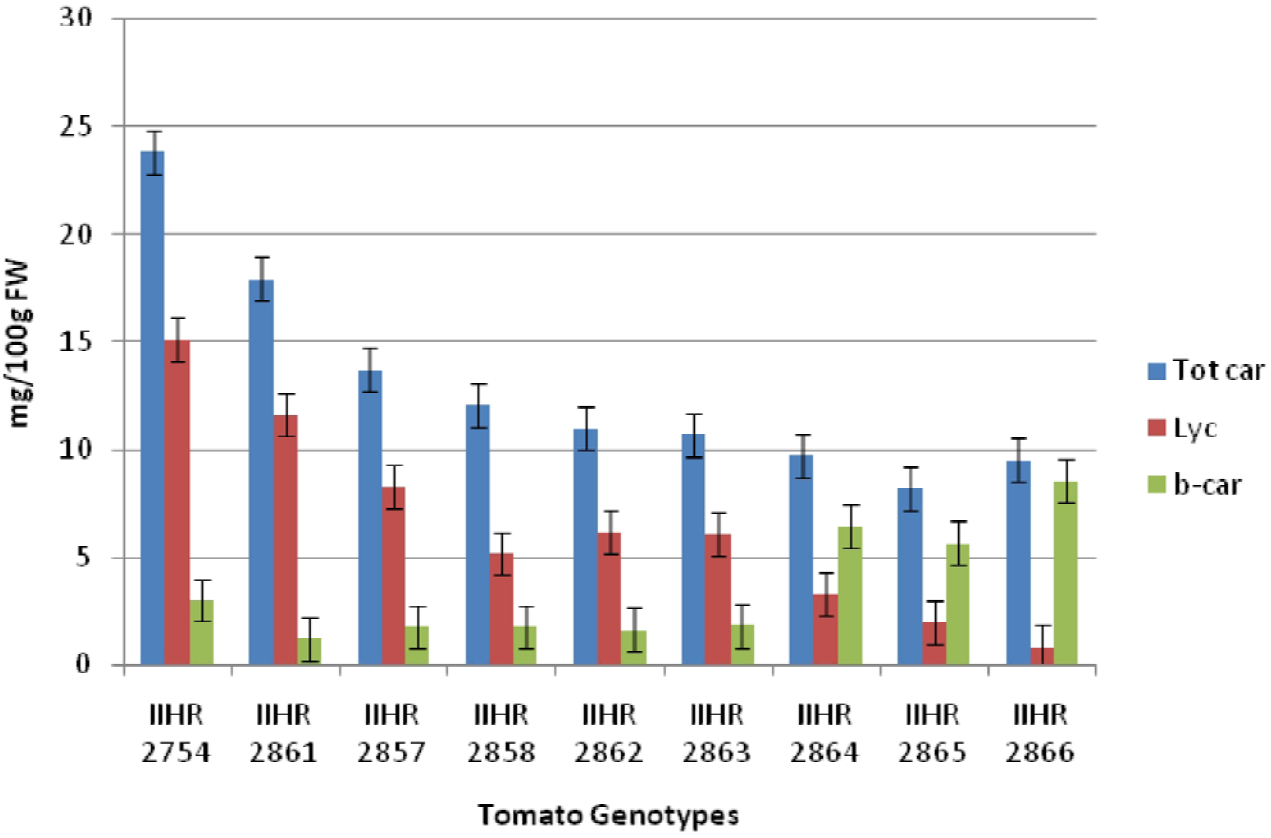


Fig. 2. Total carotenoids, lycopene and β-carotene content in cherry tomato lines. Error bars indicate the extent of variation among genotypes.

DISCUSSION

The color change in tomato is primarily observed from the immature green stage to the red ripe stage. During the process of ripening chlorophyll gets disappeared and carotenoids start accumulating giving the red or the orange color in tomatoes. Color is an important quality attributes in the food and bioprocess industries, and it influences the consumer's choice and preferences (Pathare *et al.*, 2013). Most of the tomato literature defines color in terms of the achromatic descriptors *viz*. L*, a*, b*. The color indexes a* and b* are combined and used by various researchers in different mathematical models to express color changes (Lopez Camelo *et al.*, 2004) in tomato.

In this study, cherry tomato lines were studied for surface color changes associated with carotenoid content in them. The cherry tomato lines used in this study included both red and the orange colored tomatoes. Lightness (L*) values ranged from 48.9 to 37.40. We observed that the L* value which indicates lightness was more in orange fruited tomatoes compared to the red tomatoes, this is because red colored tomatoes synthesize more lycopene and appear darker than the orange colored tomatoes. The L* value of IIHR 2866 was highest (48.9 NBS units) and these tomatoes were lighter than the red colored tomatoes with lower L* values in genotypes such as IIHR 2754 (37.40), IIHR 2861 (39.2).

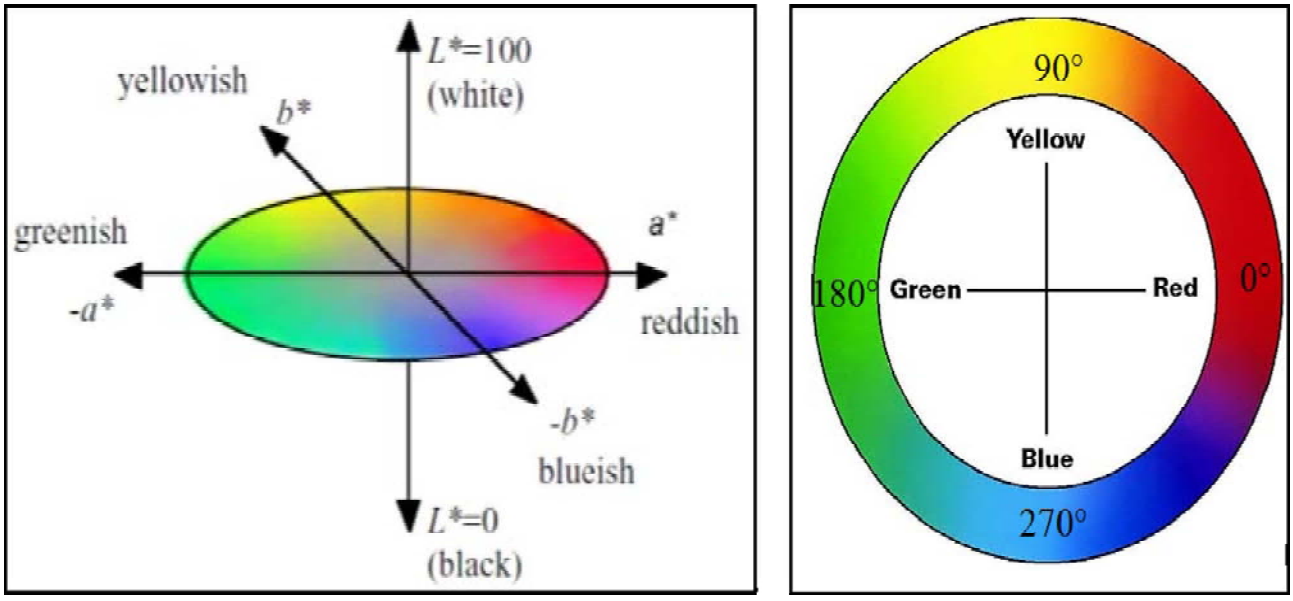


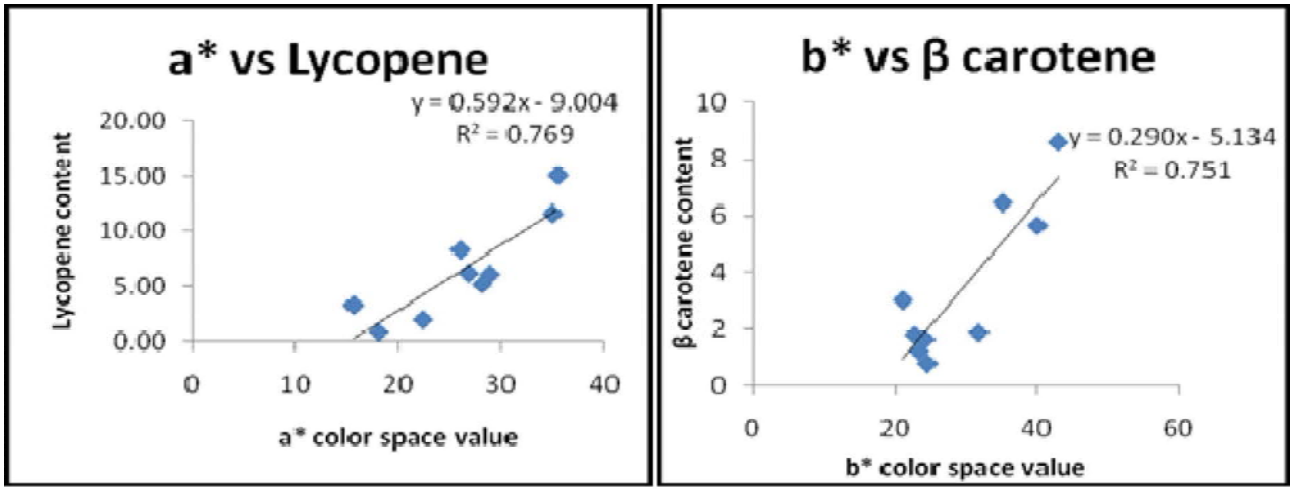
Fig. 3. A three-dimensional representation of CIE (L*, a*, b*) color space. The figure shows horizontal oval disk, with four orthogonal axes radiating out from the center of the disk in the horizontal plane. One set of horizontal axis ranges from -a* (greenish) to +a*(reddish).The other set ranges from -b*(blueish) to +b*(yellowish). Inside the horizontal disk, the range of perceived colors is shown. An orthogonal vertical axis runs through the center of the disk, this vertical axis portrays the lightness dimension, ranging from L*= 100 for white at the top and L*=0 for black at bottom (CIE Publication15.2-1986).

We observed that the correlation between L* and total carotenoids was -0.78($P<0.05$) (Table 2) *viz.* as the total carotenoids in tomato lines increase, the fruit surface L* color space value decreases. A similar study by Itle *et al.*, in 2009 on pumpkins and squashes reported that there was negative correlation between L* and carotenoid content. The color space value a* was found to be higher in IIHR 2754 (35.43) that had high total carotenoids and lycopene content. We observed that, as the a* value decreased in different tomato lines there was concomitant decrease in carotenoids (Table 1). There was a positive correlation between a* value to total carotenoids (0.82) ($P<0.01$) and lycopene content (0.87) ($P<0.01$) where as a negative correlation was observed between a* and β-carotene content (-0.77)($P<0.05$). As indicated in the Table 1, in red colored tomato lines lycopene constitutes major part of total carotenoids which are red color pigments. As higher a* values indicate more redness, the tomato lines with higher surface a* values had more lycopene pigments indicating positive correlation as reported in (Table 2). The orange colored tomatoes showed a* value lower than red tomatoes, as shown in Fig 3 that a* value in horizontal axis is negative for green color and gradually increases with a* value becoming positive as there is change in color from orange to orange red and then to red. The b* value was highest in IIHR 2866 (42.99) which had highest β-carotene of 8.56 mg/100g FW.

Table 2. Pearson correlation coefficients (r) (2 tailed) (n = 10) between color space values (L*, a*, b*, chroma, and hue angle) and total carotenoids, lycopene and β-carotene content. Significant correlations of two-tailed tests are indicated: *, $P < 0.05$; **, $P < 0.01$

	L*	a*	b*	Total carotenoids	Lycopene	β carotene	Chroma	Hue angle(h°)
L*	1	-0.738*	0.993**	-0.788**	-0.817**	0.840**	0.737	0.913**
a*	-0.738**	1	-0.780**	0.822**	0.877**	-0.772*	-0.128	-0.943**
b*	0.993**	-0.780**	1	-0.789**	-0.835**	0.867**	0.709	0.939**
Total carotenoids	-0.788**	0.822**	-0.789**	1	0.976**	-0.507	-0.245	-0.842**
Lycopene	-0.817**	0.877**	-0.835**	0.976**	1	-0.613	-0.286	-0.895**
β carotene	0.840**	-0.772*	0.867**	-0.507	-0.613	1	0.596	0.865**
Chroma	0.737	-0.128	0.709	-0.245	-0.286	0.596	1	0.438
Hue angle	0.913**	-0.943**	0.939**	-0.842**	-0.895**	0.865**	0.438	1

We observed a positive correlation between b* and β-carotene content (0.86) (P<0.01) and there was a negative correlation between b* and total carotenoids (-0.78) (P<0.01) & lycopene content (-0.83) (P<0.01). The surface b* values indicate yellowness and the tomato lines with higher b* values had higher β-carotene content giving positive correlation between b* and β-carotene. Chroma value C showed no significant differences among the genotypes (Table 1). It is reported that although chroma sub model has been proposed (Thai *et al.*, 1990), it is not a good indicator of tomato ripening because it essentially is an expression of the purity or saturation of a single color (differentcolors may have the same chroma values) (Lopez Camelo and Gomez *et al.*, 2004).In the case of tomato ripening, different colors are present simultaneously since chlorophyll is degraded from green to colorless compounds at the same time that carotenoids are synthesized from colorless precursor (phytoene) to β-carotene (pale yellow), lycopene (red), β-carotene (orange) and xanthophylls and hydroxylated carotenoids (yellow) (Giuliano *et al.*, 1993), in a kind of parallel biosynthetic pathway (Horton & Stark,1969). Hue angle, h° was more in IIHR 2866 (67.25) and was less in red colored tomato IIHR 2754 (30.69). Lower hue angle means redness and higher hue angle indicates yellowness. The negative correlation (-0.84) (P<0.01) observed between hue and total carotenoids is perfectly reflected by lower carotenoid readings in tomato lines with higher hue angles. A similar negative correlation was observed by Itle et al., (2009) in pumpkins and squashes; they suggest that as hue angle decrease the carotenoid content increase. But we could observe positive correlation (0.86) (P<0.01) between β-carotene and hue angle. Also, hue angle and b* value strongly correlated (0.93) (P<0.01), as we discussed earlier with increase in β-carotene the b*values also increased and showed positive correlation. So, it can be assumed that b* value and hue angle are clearly associated with β-carotene content in tomatoes.



4(a) 4(b)

Fig. 4(a). Correlation between a* and lycopene content, **4(b).** Correlation between b* and β carotene content (n=10).

CONCLUSION

From this study, it is clear that there was a change in a* value due to accumulation of lycopene. The a* value increased as lycopene content increased and b* value increased with increase in β-carotene content. In the tomato lines selected in our study we observed the total carotenoid content was more in lines where there was more lycopene content, hence there was positive correlation between a* to total carotenoids and lycopene content. Hue angle also showed a strong positive correlation to β-carotene content. Based on these results from this study, we could identify strong correlations between colorimetric values and the carotenoid content. These results confirmed the feasibility of obtaining precise indirect estimation of lycopene and β-carotene content from chromaticity readings. The methodology described here could be useful for large scale selection of tomato lines with improved levels of lycopene without high prices and likewise prevents the residue disposal problems associated with the employment of organic solvents in the standard spectrophotometric methods. The utilization of portable hand held colorimeters for estimation of carotenoids in tomatoes is less clumsy and convenient when compared to other methods.Therefore, the close association between color and carotenoids established through colorimeter readings can be utilized or applied for breeding purposes to improve the nutritional value of tomatoes in very easy, inexpensive, less time consuming and a non-destructive method.

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Original Research Paper

Standardization of Spacing and Soil Volume Wetting for Drip Irrigation in Papaya (*Carica papaya* L.)

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ABSTRACT

Field experiments in two crops of papaya were conducted at ICAR-Indian Institute of Horticultural Research for four years during 2016-19 to standardise spacing with optimum soil volume wetting for drip irrigation. Narrowing the plant rows drastically reduced the plant height while leaf production affected significantly due to reduction in intra row spacing. The height at first fruiting was significantly lower with a spacing of 1.8 m x 1.5 m (56.4 cm) significantly differing from both 1.5 m x 1.5 m (60.9 cm) or 1.8 m x 1.8 m (66.8 cm). Significantly higher mean fruit yield (42.2 t/ha) was recorded with the spacing of 1.5 m x 1.5m as compared to either 1.8m x 1.5m (23.4 t/ha) or 1.8m x 1.8m (22.1 t/ha). Significantly higher water use efficiency (71.3 kg/ha.mm) was recorded in papaya by following closer spacing of 1.5 m x 1.5 m. Among the interactions, higher papaya yield (48.0 t/ha) was recorded with normal drip irrigation (80% soil volume wetting) under closer spacing (1.5 m. x 1.5 m). Further, higher water use efficiency (129 kg/ha. mm) could be obtained by scheduling the irrigation at 30% soil volume wetting especially by planting at 1.5 m. x 1.5 m. spacing suggesting its suitability for water scarcity areas.

Key words: Papaya yield, Scheduling irrigation, Soil volume wetting, Spacing, Water use efficiency.

INTRODUCTION

Papaya is an important fruit crop cultivated in tropical and subtropical regions. Being a shallow rooted crop, papaya needs regular irrigation for its rapid growth and development. Further, the orchard should have a good drainage system and any amount of water logging will affect the growth. In papaya, stomata are found only on the abaxial leaf surface. They are sensitive to changes in the saturation deficit of the air. Stomata also respond quickly to changing light conditions. On clear days, midday suppression of photosynthesis occurs as a result of partial closure of the stomata (Carr, 2014).

A properly designed and operated drip irrigation system has to supply the water amount required by the crop and should also wet enough soil volume. Unlike surface and sprinkler irrigation, drip irrigation only wets part of the soil root zone. This may be as low as 30 per cent of the volume of soil wetted by the other methods. The wetting patterns which develop from dripping water onto the soil depend on discharge and soil type. Although only part of the root zone is wetted it is still important to meet the full water needs of the crop. Two of the key factors in the design of micro-irrigation systems to obtain the maximum benefits from these practices are the amount of water used and the volume of soil to be wetted.

The restricted volume of the wetted soil under drip irrigation and depth-width dimensions of this volume are of considerable practical importance. The volume of the wetted soil represents the amount of soil water stored in the root zone, its depth dimension should coincide with the depth of the root system while its width dimension should be related to the spacing between the emitters and lines.The parameters which influence the wetted soil volume are the available water holding capacity of the soil and the peak daily crop water use representing specific field conditions. The irrigation interval and the management-allowed deficit are additional parameters which affect the wetted volume and could be changed depending on

crop sensitivity as well as water and irrigation equipment accessibility. A truncated ellipsoid is assumed to best represent the geometry of the wetted soil volume under an emitter.The partial soil wetting pattern by micro irrigation requires assessment of the percentage of soil volume that is wetted (Moshe. 2006). The distance between emitter on lateral pipe and distance of lateral pipes from each other should be determined based on the degree of wetted soil diameter by emitters. The duration of irrigation also depends on the fact that at what time after commencement of irrigation, the wetting front reaches depth of plant's root or a multiple of it. The distance of outlets, discharge rate and time of irrigation in drip irrigation have to be determined so that volume of wetted soil is close to volume of plant's root as much as possible. The volume of wetted soil surface and moisture in onion shape depends on different factors including soil texture and layering, soil homogeneity, dripper flow rate, primary moisture of soil, consumption water and land slope. Analyses on the effects of application rate on the water distribution pattern demonstrated that increasing the water application rate allows more water to distribute in horizontal direction, while decreasing the rate allows more water to distribute in vertical direction for a given volume applied (Li *et al*., 2004).

Although papaya is generally considered to be drought sensitive and responsive to irrigation, there is a shortage of good experimental evidence to support this view. There is a need to establish practical irrigation schedules for this remarkable crop. Further, to enhance the productivity of the crop, optimum plant population is very crucial. When spacing is varied, it further warrants for understanding the requirement of wetted volume for standardizing the drip irrigation practice in a given agro-climatic condition. Keeping this in view, field experiments were conducted to adjudge an optimumsoil volume wetting in papaya along with different plant spacing.

MATERIALS AND METHODS

Field experiments were conducted during 2015 to 2019 at ICAR- Indian Institute of Horticultural Research, Hessaraghatta, Bengaluru located at a latitude of 13°8'12"N and a longitude of 77°29'45"E. The experimental soil was sandy loam in texture with a pH of 6.14 and an EC of 0.067 dSm^{-1}. The maximum temperature during the experimental period ranged from 24^0C to 36^0C and the minimum temperature ranged between 10^0C to 22^0C. The period between March to May are the warm months with higher temperatures and evaporation while the period between November to January were the cooler months with low temperature and evaporation. The average relative humidity was higher during September and October months. The average rainfall of the region is around 850 mm with two peak periods of rainfall during June- July and September- October months. Pre-experimental soil had a pH of 6.32 with almost no salts (0.14 dSm^{-1}). The organic carbon content of the soil was quite low (0.15 %). Although the available nitrogen content of the soil was low (56 kg/ha), the available phosphorus was higher (51.5 kg/ ha). Further, the available potassium content of the soil was also higher (315 kg/ha).

Field experiments were conducted in papaya (Cv. Red Lady) in split plot design with three plant spacing (1.5 x 1.5 m., 1.8 x 1.5 m. and 1.8 x 1.8 m.) as main plot treatments and three levels of soil volume wetting (30%. 50% and 70%) as sub plot treatments in four replications. The crop was raised with recommended package of practices except for irrigation. Irrigation was scheduled based on pan evaporation and the per cent soil volume was restricted by inserting a barrier in the root zone. The calculated amount of water for each irrigation was either partially wetted or fully wetted in the root zone depending on the treatment. All the growth and yield parameters were recorded in both the crops. The root length and depth were recorded based on longest horizontal and vertical growth, respectively and the root volume was measured based on the quantity of water displaced by immersing in water. The root dry weight was recorded by carefully collecting the roots and drying in hot air oven till constant weight was obtained. All the mean data was analyzed as per standard statistical procedures (Panse and Sukhatme, 1985).

RESULTS AND DISCUSSION

Plant Growth

Although majority of the growth parameters showed non-significant differences among different per cent soil volume wetting irrigation levels, plant spacing was found to affect the plant stature, leaf production and height at first flowering significantly (Table 1). Giving wider spacing of 1.8 m x 1.8 m for papaya was found to favour the crop growth through higher plant height

(1.57 m) significantly differing from both 1.8 m x 1.5 m and 1.5 m x 1.5 m plant spacing. The leaf production followed a similar trend with significantly higher leaf production at wider spacing of 1.8 m x 1.8 m (24.5 leaves/plant). It is worth to note that narrowing the plant rows drastically reduced the plant height while leaf production affected much due to reduction in intra row spacing. This assumes significance in papaya as source sink balance is critical in papaya fruit set, development and sugar accumulation and in general each mature leaf can provide photo assimilate for about three fruits (Zhou *et al*., 2000). Further, the height at first fruiting was significantly lower with a spacing of 1.8 m x 1.5 m (56.4 cm) significantly differing from both 1.5 m x 1.5 m (60.9 cm) or 1.8 m x 1.8 m (66.8 cm) plant spacing. Similar results were reported in papaya by Singh *et al*. (2010) wherein vegetative growth characters in papaya like plant height, numbers of leaves and inter-nodal length showed significant difference among all the treatments.

Root growth parameters in papaya

Root growth in general was significantly influenced both by the plant spacing as well asirrigation levels. Root length was significantly higher with 1.5 m. x 1.5 m spacing (109.8 cm) ascompared to either 1.8 m. x 1.8 m. (100.3 cm) or 1.8m. x 1.5 m. (91.9 cm). Among the irrigation levels, meeting 50% ER and irrigating in part of the root zone (50%) was found to have higher root length (114.7 cm) significantly differing from others. Further, the interaction between spacing and irrigation levels was significant with highest root length (126.7cm) recording in1.5m x 1.5m spacing with 50% ER irrigating in 50% of the root zone (Table 2).

The rooting depth in papaya was influenced significantly both by plant spacing and interaction of spacing with irrigation levels. Planting at a distance of 1.8m x 1.5m was found to produce significantly deeper roots (54.2 cm) over other spacings and among the interactions, planting at 1.8m x 1.5m and irrigation meeting 30% of ER wetting 100% of the root volume resulted in significantly higher rooting depth (61.7cm).

The root volume in general differed significantly both with the plant spacing and the irrigation levels. Closer planting at 1.5m x 1.5m distance had shown significantly higher root volume (2148 cm^3) as compared to either 1.8m x 1.5m (1983 cm^3) or 1.5m x 1.5m (1671 cm^3). Among the irrigation levels, meeting 70% ER and irrigating in part of the root zone (70%) was found to have higher root volume (2572 cm^3) significantly differing from others. Further, the interaction between spacing and irrigation levels was significant with highest root volume (2833.3 cm^3) observed under 1.8m x 1.5m spacing with irrigation meeting 70% ER and wetting 70% of the root zone.

The oven dry weight of roots was significantly influenced by plant spacing. The significantly higher oven dry weight of root in papaya was observed under plant spacing of 1.5 m x 1.5m (516.7 g/plant) as compared to either 1.8m x 1.5m (237.2 g/plant) or 1.8m x 1.5m (279.6 g/plant) which may be attributed to the higher growth of roots in competing environments in search of resources at closer spacing with higher intra-plant competitions.Wang *et al*. (2006) also reported that root development and distribution are affected by spatial and temporal soil water distribution.

Photosynthetic rate

The photosynthetic response of papaya is strongly linked to environmental conditions through stomatal behavior (Zhou *et al*., 2004). The photosynthetic rate recorded in papaya in different treatment combinations is depicted in Fig.1. It was observed that the spacing of papaya plant to a distance of 1.8m x 1.8 m influenced the photosynthetic rate (10.85 µ $mol/m^2/s$) significantly as compared to 1.5 m. x 1.5 m. (9.09 µ $mol/m^2/s$) although it was at par with the spacing of 1.8 m. x 1.5 m. (9.48 µ $mol/m^2/s$). Among the irrigation levels, meeting 70% of the ER and wetting 70% soil volume recorded the highest photosynthetic rate (10.52 µ $mol/m^2/s$) which was found to differ significantly with 50% of the ER wetting 50% of the soil volume (8.41 µ $mol/m^2/s$). Although the interaction effects were not significant, the highest photosynthetic rate of 11.73 µ $mol/m^2/s$ was recorded with 1.8 m x 1.8 m spacing at 70%ER irrigation wetting 70% soil volume. Santas *et al*., (2015) also affirmed that the fruit production and physiological characteristics of papaya depend on planting spacing.

Table 1. Growth attributes in papaya as influenced by spacing and soil volume wetting irrigation treatments

Spacing	Irrigation level	Plant height (cm)	Collar girth (cm)	No. of leaves/ plant	Canopy spread (m)		Height at first fruiting (cm)
					E-W	N-S	
1.5m x 1.5m	Irrigation at 30% ER wetting 30% soil volume	133.0	24.3	17.4	142.0	147.0	62.8
	Irrigation at 50% ER wetting 50% soil volume	140.0	26.4	20.0	154.0	168.0	58.6
	Irrigation at 70% ER wetting 70% soil volume	143.0	28.0	21.4	154.0	156.5	58.0
	Irrigation at 70% ER wetting 100% soil volume	137.7	26.1	17.5	167.0	138.7	64.0
	Mean	138.4	26.2	19.1	154.3	152.6	60.9
1.8m x 1.5m	Irrigation at 30% ER wetting 30% soil volume	132.0	26.6	24.4	146.0	144.0	61.8
	Irrigation at 50% ER wetting 50% soil volume	146.0	28.2	23.8	165.0	156.0	55.0
	Irrigation at 70% ER wetting 70% soil volume	138.0	26.8	22.4	154.0	152.0	54.0
	Irrigation at 70% ER wetting 100% soil volume	143.0	24.7	22.5	150.3	152.7	54.8
	Mean	139.8	26.6	23.3	153.8	151.2	56.4
1.8m x 1.8m	Irrigation at 30% ER wetting 30% soil volume	167.0	28.0	25.8	162.0	161.0	66.8
	Irrigation at 50% ER wetting 50% soil volume	140.0	25.0	21.6	154.0	158.0	66.4
	Irrigation at 70% ER wetting 70% soil volume	162.0	28.2	21.8	163.0	161.0	69.4
	Irrigation at 70% ER wetting 100% soil volume	159.0	29.2	28.7	169.7	174.8	64.7
	Mean	157.0	27.4	24.5	162.2	163.7	66.8
	S.Em ±						
	Main	2.47	0.60	0.84	3.10	5.874	1.19
	Sub	2.65	0.73	1.17	4.18	6.559	1.52
	Main x Sub	4.93	1.19	1.68	6.20	11.748	2.39
	C.D.(P=0.05)						
	Main	8.17	NS	2.79	NS	NS	3.95
	Sub	NS	NS	NS	NS	NS	NS
	Main x Sub	13.75	NS	NS	NS	NS	NS

Table 2. Root growthin papaya as influenced by plant spacing and irrigation levels

Spacing	Irrigation level	Root length (cm)	Root depth (cm)	Root volume (cm^3)	Dry weight of roots (g/plant)
1.5 m x1.5m	Irrigation at 30% ER wetting 30% soil volume	110.0	56.7	2866.7	688.3
	Irrigation at 50% ER wetting 50% soil volume	126.7	48.3	2000.0	356.2
	Irrigation at 70% ER wetting 70% soil volume	102.5	52.5	2800.0	540.7
	Irrigation at 70% ER wetting 100% soil volume	100.0	42.5	925.0	481.5
	Mean	**109.8**	**50.0**	**2147.9**	**516.7**
1.8 m x 1.5m	Irrigation at 30% ER wetting 30% soil volume	91.7	61.7	2350.0	317.4
	Irrigation at 50% ER wetting 50% soil volume	100.0	46.7	1850.0	232.7
	Irrigation at 70% ER wetting 70% soil volume	103.3	50.0	2833.3	299.5
	Irrigation at 70% ER wetting 100% soil volume	72.5	58.5	900.0	99.2
	Mean	**91.9**	**54.2**	**1983.3**	**237.2**
1.8 m x1.8m	Irrigation at 30% ER wetting 30% soil volume	75.0	40.0	800.0	171.3
	Irrigation at 50% ER wetting 50% soil volume	117.5	45.0	2000.0	368.5
	Irrigation at 70% ER wetting 70% soil volume	111.3	43.3	2083.3	337.6
	Irrigation at 70% ER wetting 100% soil volume	97.5	45.0	1800.0	241.1
	Mean	**100.3**	**43.3**	**1670.8**	**279.6**
	S.Em ±				
	Main	1.87	2.18	98.49	48.00
	Sub	3.06	1.85	119.55	61.09
	Main x Sub	4.96	3.53	204.60	103.45
	C.D. (P=0.05)				
	Main	6.59	7.68	347.46	169.33
	Sub	8.93	NS	348.76	NS
	Main x Sub	14.89	11.11	625.80	NS

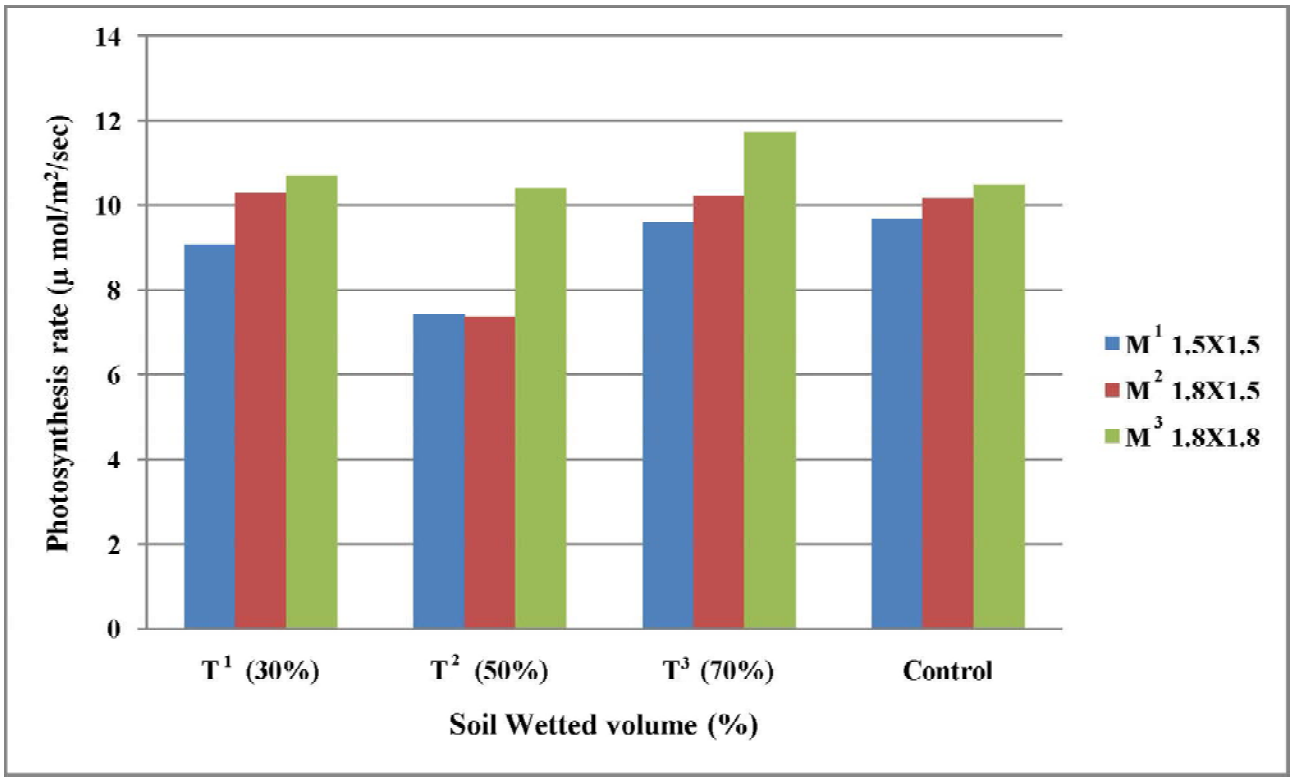

Fig 1. Effect of different spacing and soil volume wetting irrigation on photosynthetic rate in papaya

Fruit yield

Although the mean fruit number per plant not affected significantly by plant spacing, different levels of soil volume wetting irrigation had a significant influence (Table 3). Irrigating the plant to wet 70% of the soil volume resulted in significantly higher number of fruits (17.2/plant) as compared to 30% (10.6/plant) and normal drip irrigation (13.3/plant) although it was on par with 50% of the soil volume (14.8/plant). Although the fruit yield in general was slightly lower in the crop owing to the incidence of PRSV disease (even with sufficient care to combat the disease), the treatment effects were very clear both for the spacing and the irrigation levels.

Significantly higher mean fruit yield (42.2 t/ha) was recorded with the spacing of 1.5 m x 1.5m as compared to either 1.8m x 1.5m (23.4 t/ha) or 1.8m x 1.8m (22.1 t/ha).The mean increase in yield on the basis of two crops ranged from 80.3 per cent (over 1.8 x 1.5m spacing) to 91.0 per cent (over 1.8m x 1.8 m spacing). This increased fruit yield at closer spacing of 1.5m x 1.5m was not only due to more number of plants per unit area but also was due to higher number of fruits (16.4/plant) over other spacings. Although different soil volume wetting irrigation failed to affect the mean fruit yield significantly, irrigation at 70 per cent ER and wetting either 70% of soil volume (31.7 t/ha) or 100% of soil volume (31.2 t/ha) showed higher fruit yield. The response in total yield to the irrigation applied was quadratic and increasing in the range to the amounts of water applied from 30, through 50 to 70 per cent of the ER. Similar results of higher yield with increasing trend in irrigation levels each year was also reported earlier in orange by Petillo (2004).

Significantly higher water use efficiency (71.3 kg/ha.mm) was recorded in papaya by following closer spacing of 1.5 m x 1.5 m which decreased drastically with increase in spacing either to 1.8 m x 1.8 m (34.6kg/ha.mm) or 1.8 m x 1.5 m (37.3 kg/ha.mm).This suggests that under limited water situations following an ideal agronomic package is also essential to get more output per unit amount of water used.

Table 3. Fruit yield and water use efficiency in papaya as influenced by spacing and soil volume wetting in two crops of papaya

Spacing	Soil volume wetting (%)	No. of fruits /plant			Fruit yield / plant (kg)			Papaya yield (t/ha)			Water use efficiency (kg/ha.mm)		
		2016-17	2018-19	Mean	2016-17	2018-19	Mean	2016-17	2018-19	Mean	2016-17	2018-19	Mean
1.5m x 1.5m	Irrigation at 30% ER wetting 30% root zone volume	13.00	19.90	16.50	10.30	10.40	10.30	45.6	46.03	45.79	110.08	148.01	129.04
	Irrigation at 50% ER wetting 50% root zone volume	11.30	20.40	15.90	8.00	8.77	8.38	35.6	39.70	37.63	51.55	74.88	63.21
	Irrigation at 70% ER wetting 70% root zone volume	16.90	19.90	18.40	8.95	10.40	9.66	39.8	34.90	37.30	40.92	52.57	46.75
	Normal drip irrigation	18.10	11.30	14.70	11.2	5.84	8.53	49.9	46.10	47.98	62.69	29.62	46.15
	Mean	14.80	17.90	16.40	9.61	8.83	9.22	42.7	41.7	42.20	66.31	76.27	71.29
1.8m x 1.5m	Irrigation at 30% ER wetting 30% root zone volume	9.70	8.63	9.15	5.95	4.46	5.21	22.05	16.50	19.30	53.29	53.14	53.21
	Irrigation at 50% ER wetting 50% root zone volume	13.70	12.60	13.10	6.38	6.75	6.56	23.60	25.00	24.30	34.25	48.04	41.15
	Irrigation at 70% ER wetting 70% root zone volume	9.70	20.50	15.10	3.90	11.2	7.54	14.50	41.40	27.90	14.96	47.25	31.10
	Normal drip irrigation	11.60	11.70	11.60	6.40	5.48	5.94	23.70	20.30	21.99	24.53	23.15	23.84
	Mean	11.20	13.30	12.20	5.66	6.97	6.31	20.96	25.80	23.40	31.75	42.89	37.32
1.8m x 1.8m	Irrigation meeting 30% ER wetting 30% soil volume	3.43	8.68	6.05	1.60	4.57	3.09	4.93	14.10	9.51	11.90	45.35	28.63
	Irrigation meeting 50% ER wetting 50% soil volume	10.18	20.40	15.28	5.30	11.20	8.25	16.38	34.50	25.50	35.60	66.38	50.99
	Irrigation meeting 70% ER wetting 70% soil volume	12.40	23.50	17.96	8.58	10.70	9.66	26.45	33.20	29.80	27.38	37.85	32.61
	Irrigation meeting 70% ER and wetting 100% soil volume	10.90	16.40	13.70	6.35	9.02	7.69	19.60	27.80	23.70	20.28	31.78	26.03
	Mean	9.23	17.24	13.20	5.46	8.88	7.17	16.80	27.40	22.10	23.79	45.34	34.57
	S.Em ±												
	Main	1.54	1.75	1.26	0.86	1.09	0.85	3.79	4.06	3.46	4.44	6.86	4.82
	Sub	0.97	1.81	1.02	0.77	1.00	0.63	3.09	3.84	2.54	6.19	8.47	5.40
	Main x Sub^{-1}	2.12	3.23	1.98	1.45	1.86	1.23	5.99	7.05	5.15	10.30	14.43	9.43
	C.D (P=0.05)												
	Main	NS	NS	NS	3.05	NS	NS	13.38	NS	12.20	15.6	24.2	17.00
	Sub	2.82	5.29	2.97	NS	2.93	1.85	NS	NS	NS	18.0	24.7	15.80
	Main x Sub $^{-1}$	6.86	NS	NS	4.53	NS	4.05	NS	NS	NS	31.2	44.1	29.00

Among the interactions, higher papaya yield (48.0 t/ ha) was recorded with normal drip irrigation (80% soil volume wetting) under closer spacing (1.5m x 1.5 m). However, higher water use efficiency (129 kg/ ha.mm) could be obtained by scheduling the irrigation at 30% soil volume wetting especially by planting at 1.5 x 1.5m spacing.

The economics of papaya cultivation

The economics of growing papaya under different spacings with irrigation levels is presented in Table 4. It was observed that the cost of production of papaya was although 33 per cent higher under closer spacing of 1.5 m x 1.5 m (Rs.3,69,400/ha), the gross returns were also significantly higher (Rs.6,32,740/ha). The higher cost of production with closer spacings may be attributed to the additional investment cost on planting material, pit making and other inputs like manures and fertilizers. Sagar *et al.,* (2012) also found that papaya was highly capital intensive crop and average cost of cultivation per hectare was Rs.176660. The higher gross returns with closer spacing may be attributed not only to the more number of yielding plants but also higher yield per plant. Higher net returns were recorded with closer spacing of 1.5 m x 1.5 m (Rs.2,63,290/ha). Further, benefit cost ratio was although higher (1.72) with closer spacing, the results were found to be non-significant.

Table 4. The mean economics of papaya cultivation under different spacing and irrigation levels

Spacing	Irrigation level	Cost of production (Rs/ha)	Gross returns (Rs/ha)	Net returns (Rs/ha)	B:C ratio
1.5m x1.5m	Irrigation at 30% ER wetting 30% root zone volume	3,47,700	6,86,900	3,39,020	1.98
	Irrigation at 50% ER wetting 50% root zone volume	3,65,060	5,64,400	1,99,340	1.55
	Irrigation at 70% ER wetting 70% root zone volume	3,82,430	5,59,980	1,77,550	1.46
	Normal drip irrigation	3,82,430	7,19,670	3,37,240	1.88
	Mean	3,69,400	6,32,740	2,63,290	1.72
1.8 m x1.5m	Irrigation at 30% ER wetting 30% root zone volume	2,97,810	3,91,740	93,930	1.31
	Irrigation at 50% ER wetting 50% root zone volume	3,15,180	3,64,690	49,510	1.16
	Irrigation at 70% ER wetting 70% root zone volume	3,32,550	4,18,790	86,240	1.26
	Normal drip irrigation	3,32,550	3,91,740	93,930	1.31
	Mean	3,19,520	3,91,740	80,900	1.26
1.8 m x1.8m	Irrigation at 30% ER wetting 30% root zone volume	2,55,970	3,94,910	1,38,940	1.54
	Irrigation at 50% ER wetting 50% root zone volume	2,73,340	3,81,880	1,08,540	1.4
	Irrigation at 70% ER wetting 70% root zone volume	2,90,710	4,47,040	1,56,330	1.54
	Normal drip irrigation	2,90,710	3,55,820	65,100	1.22
	Mean	2,77,680	3,94,920	1,17,230	1.43

Among the irrigation levels, the gross returns were relatively higher (Rs. 4,91,180/ha) with irrigation at 30% ER and 30 per cent soil volume wetting, which may be attributed to the better soil moisture availability under the treatment in turn improving the productivity. Similarly, the net returns were also relatively higher with the treatment. Closer spacing of 1.5 m x1.5m also recorded higher benefit cost ratio which may be attributed to both higher plant population (44% higher) and the yield per plant (28% higher). Higher benefit cost ratio with spacing of 1.5m x 1.5m clearly indicated that it is worth to spend more for the inputs with closer spacing. Further, benefit cost ratio was relatively higher with 70 per cent soil volume wetting as compared to other irrigation levels.

CONCLUSION

The results of four years field trial in papaya on spacing and different soil volume based irrigation levels clearly indicated that under water scarcity conditions, it is worth irrigating papaya to meet only 30 per cent of the soil volume through a package of 1.5 m x 1.5m spacing so as to enhance the water use efficiency to the highest level (129.04 kg/ha.mm).

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Original Research Paper

Variability and Genetic Divergence in Vegetable Cowpea Germplasm of Goa

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ABSTRACT

Vegetable cowpea or Yard long bean [*Vigna unguiculata* var. *sesquipedalis* L. (Walp)] is a warm season leguminous crops grown especially for vegetable purpose along the west coast of India. In Goa, pole type varieties are preferred over bushy types as they offer multiple harvests with comparatively longer pods. There is wide variability found for different morphological and other traits in the local types cultivated in the state of Goa. Exploration of genetic variability in the available germplasm is a prerequisite for initiation of any successful breeding programme. Twenty nine genotypes of vegetable cowpea including three improved varieties collected from different parts of Goa state were evaluated for twelve quantitative characters including yield. High variability was observed for pod yield/plant, number of pods/plant and pod length. The high variability for pod yield per plant is apparent as the pod yield ranged from 315.25 to 2070.45 g/plant with an average of 827.48 g per plant. Pod yield depends on number of pods per plant, pod length and pod weight. Number of pods per plant ranged from 36.65 to 147.80. Pod weight depends on pod length, number of seeds per pod and hundred seeds weight. Wide variation was observed for all these characters in the present study. The GCV value was maximum for pod yield per plant (g) followed by pod weight (g) and number of pods per plant. Low values of GCV were observed for days to first flowering, days to first harvest and number of seeds per pod. In the present study, the twenty nine genotypes could be grouped into fourteen clusters based on genetic distance. High coefficient of variation was observed for pod yield per plant, pod weight, number of pods per plant and pod length indicating their significant contribution in determining the inter cluster distances.

Key words: Correlation coefficient, Genetic divergence, Quantitative character, Vegetable cowpea

INTRODUCTION

Vegetable cowpea popularly known as Yard long bean (*Vigna unguiculata* var. *sesquipedalis*) is an important leguminous vegetable crop of South India. Vegetable cowpea is an important vegetable grown as intercrop in different cropping systems. (Khanpara *et al.,* 2016). In Vegetable cowpea, among the different parts analyzed shells were rich in dietary fiber. Seeds were nutrient dense as compared to pods and shells, but more in anti- nutrients (Tiwari *et al.,* 2019). In Goa, pole type cowpea with indeterminate growth habit producing long green fleshy pods are preferred and fetch premium price in the market through out the year. There are many varieties released in case of bush type of cowpea but the availability of improved varieties in pole type vegetable cowpea is rather scanty. Not much work has been carried out on the genetic improvement of pole type vegetable cowpea. There is wide variability found for different morphological and other traits in the local types cultivated in the state of Goa. Exploration of genetic variability in the available germplasm is a prerequisite for initiation of any successful breeding programme. In spite of its popularity and importance very little effort has been

made to upgrade the genetic makeup of this crop. Hence, the present investigation was carried out systematically to evaluate the local accessions to estimate the extent of genetic variability, heritability, genetic advance and genetic divergence in the locally collected germplasm of vegetable cowpea.

MATERIALS AND METHODS

Twenty nine genotypes of vegetable cowpea collected from different parts of Goa including three released varieties were evaluated in a randomized block design with two replications during *rabi* seasons starting from 2012-2016 in paddy fallow land. The soil is sandy loam with a pH of 5.1 with medium phosphorous and potassium availability. Recommended package of practices were followed to raise a good crop (Anon. 2004). Observations were recorded on twelve important quantitative characters, *viz.,* plant height (cm), number of primary branches, leaf length (cm), leaf width (cm), days to first flowering, days to first harvest, pod length (cm), pod weight (g), pods per plant, number of seeds per pod, 100 seeds weight (g) and pod yield per plant on five randomly selected plants/genotype/replication. The analysis of variance was carried out as suggested by Panse and Sukhatme (1985). The genotypic and phenotypic coefficient of variation was calculated as per the formula suggested by Comstock and Robinson (1952). Heritability (broad sense) and genetic advance were worked out as per the procedure given by Burton and De Vane (1953) and Allard (1960).

RESULTS AND DISCUSSION

Among the twelve quantitative characters studied, the twenty nine vegetable cowpea genotypes exhibited highly significant differences for all the characters indicating high variability in the cowpea accessions (Table 1). Wide range of variation was observed for all the characters studied. Highest variation was observed for pod yield/plant, number of pods/plant and pod length (Table 2). Such a high variability for the above characters was also reported earlier by De Mooy (1985), Resmi (1998) and Narayanankutty *et al.* (2003).

In the present study, all the characters exhibited narrow differences between the value of PCV and GCV. This indicated low impact of environment on the expression of all the quantitative characters. The same was reported earlier by Narayanankutty *et al.* (2005).

The GCV value was maximum for pod yield per plant (g) followed by pod weight (g) and number of pods per plant. Low values of GCV were observed for days to first flowering, days to first harvest and number of seeds per pod (Table 2). Shobha and Vahab (1998) and Narayanankutty *et al.,* (2003) reported high GCV for yield per plant and pod weight in vegetable cowpea. Low GCV values for days to first flowering and number of seeds per pod has been reported by Sreekumar *et al.,* (1996). The results of analysis of variance for different traits are given in Table 3.

The high variability for pod yield per plant is apparent as the pod yield ranged from 315.25 to 2070.45 g / plant with an average of 827.48 g per plant. Pod yield depends on number of pods per plant, pod length and pod weight. Number of pods per plant ranged from 36.65 to 147.80. Pod weight depends on pod length, number of seeds per pod and hundred seeds weight. Wide variation was observed for all these characters in the present study. Similar findings have been reported by other workers (De Mooy(1985), Resmi (1998), Shobha and Vahab(1998) and Narayanankutty *et al.*,(2005)

With the help of variability and subsequent GCV alone, it is not possible to determine the amount of genetic variation that is heritable to the further generations. Burton and De Vane (1953) suggested that GCV combined with estimates of heritability would give the best results of genetic advance to be expected from selection. In the present study, heritability values were high (>90%) for all the characters studied except number of seeds per pod. High values of heritability for quantitative characters have also been reported by earlier workers, Sobha and Vahab (1998) and Narayanankutty *et al.* (2003). The accurate value for heritable variation can be estimated when heritability is combined with genetic advance. In the present study, high heritability coupled with high genetic advance was observed for pod yield per plant (g) and pod weight (g). This may be due to the preponderance of additive gene action for these characters there by indicating the advantages of selection for their

Table 1. Mean performance of vegetable cowpea germplasm of Goa

S.No.	Accession number	Plant height	No of 1° branches	Leaf length	Leaf width	Days to 1st flowering	Days to 1st harvest	Pod length	Pod weight	Pods per plant	No of seeds per pod	100 seeds weight	Pod yield per plant
1	VCG1	4.49	6.60	13.48	9.68	43.00	57.60	35.17	10.58	77.00	14.61	17.32	820.50
2	VCG2	5.29	8.25	11.39	8.25	50.40	71.15	40.77	12.36	77.65	14.70	27.23	950.50
3	VCG3	4.85	5.25	10.63	7.77	51.30	71.00	47.35	13.90	74.40	13.76	25.65	1030.45
4	VCG4	6.29	4.10	12.54	9.56	46.40	60.00	64.70	35.52	58.35	13.19	19.22	2070.45
5	VCG5	3.46	5.60	9.98	6.47	52.20	69.70	40.85	9.26	54.50	17.34	25.25	505.50
6	VCG6	4.37	5.85	11.29	8.67	56.15	69.90	39.42	9.00	50.45	16.97	19.82	455.40
7	VCG7	3.90	5.25	11.13	9.33	48.50	62.90	37.53	8.47	67.15	15.15	14.90	565.65
8	VCG8	4.26	4.60	12.21	9.14	43.50	60.00	37.90	11.58	147.80	15.63	18.47	1710.65
9	VCG9	4.35	4.85	11.94	9.34	45.20	60.90	36.69	9.80	92.45	14.52	22.50	910.40
10	VCG10	4.41	6.65	10.84	7.97	50.60	66.90	40.32	11.13	72.50	14.77	13.45	810.00
11	VCG11	4.99	4.40	11.88	7.40	46.40	64.20	41.24	10.89	78.50	15.88	20.11	850.45
12	VCG12	4.64	4.80	11.08	7.27	51.90	77.10	43.01	9.60	58.40	16.55	15.39	555.40
13	VCG13	3.72	5.05	11.78	8.29	54.50	74.30	40.55	12.84	51.50	17.91	20.31	660.25
14	VCG14	4.31	4.15	13.73	9.93	44.20	61.20	41.19	9.74	65.05	15.51	20.51	635.25
15	VCG15	4.38	5.00	12.78	10.66	43.60	60.00	39.02	10.68	85.35	15.97	19.29	910.45
16	VCG16	4.75	4.85	12.24	9.58	45.30	60.70	27.97	6.22	83.60	15.26	20.93	520.65
17	VCG17	4.08	4.70	13.00	9.80	51.60	62.80	31.54	10.61	59.10	15.99	22.33	625.20
18	VCG18	4.46	4.25	13.60	9.93	55.20	67.00	33.36	8.66	36.65	16.11	20.11	315.25
19	VCG19	3.39	5.05	10.54	10.39	42.10	66.00	46.13	12.08	44.15	15.75	15.04	527.30
20	VCG20	4.64	4.85	11.42	9.05	44.40	71.90	51.95	12.03	52.20	14.97	17.55	606.20
21	VCG21	4.43	5.55	11.89	9.24	44.00	71.60	48.14	11.13	86.45	13.03	20.05	908.45
22	VCG22	4.95	6.25	12.07	10.21	46.10	70.20	59.50	19.13	72.50	16.86	22.29	1374.15
23	VCG23	4.10	6.15	11.74	9.87	44.00	65.60	55.64	10.57	105.50	16.04	20.39	1183.55
24	VCG24	4.72	5.30	11.14	9.61	51.80	78.40	46.87	13.31	91.60	13.06	23.56	1262.45
25	VCG25	4.86	4.95	12.33	9.10	51.00	77.60	48.77	14.09	48.80	13.61	20.61	697.50
26	VCG26	3.95	4.65	13.13	11.20	48.70	62.50	51.01	13.94	64.10	13.61	19.84	783.10
27	Vijayanthi	2.90	6.15	11.13	10.95	50.70	72.00	47.23	10.75	50.70	12.94	23.21	408.60
28	A Garima	1.00	5.20	10.00	10.89	42.90	64.60	51.40	10.95	71.30	12.20	21.96	707.80
29	A Suman	1.05	5.10	9.39	10.09	45.90	65.00	54.28	12.07	65.20	13.05	19.94	635.45
	CD(0.05)	0.61	6.60	0.82	0.71	3.06	3.98	3.36	1.63	7.36	1.92	1.62	123.72

Table 2. Range, mean, PCV, GCV, heritability and genetic advance for twelve quantitative characters in vegetable cowpea

Characters	Mean ± SEm	Range	Phenotypic variance	Genotypic variance	PCV	GCV	Heritability (%)	Genetic advance (%)
Plant height	4.170±0.210	1.00 - 6.29	2.31	2.22	36.45	35.74	96.19	72.21
No of 1° branches	5.29±0.24	4.10 - 8.25	1.59	1.47	23.81	22.95	92.92	45.58
Leaf length	11.73±0.29	9.39 - 13.73	2.41	2.25	13.24	12.79	93.28	25.44
Leaf width	9.29±0.25	6.46 - 11.19	2.69	2.57	17.64	17.25	95.55	34.73
Days to 1st flowering	47.98±1.06	42.10 - 56.15	33.88	31.64	12.13	11.72	93.40	23.34
Days to 1st harvest	66.99±1.38	57.60 - 78.40	67.72	63.95	12.28	11.94	94.43	23.89
Pod length	44.12±1.16	27.97 - 64.69	142.48	139.79	27.06	26.80	98.12	54.69
Pod weight	12.09±0.56	6.22 - 35.52	51.48	50.85	59.31	58.94	98.77	120.67
No of pods/plant	70.45±2.54	36.65 - 147.80	967.57	954.66	44.17	43.86	98.67	89.75
No of seeds/pod	14.99±0.66	12.20 - 17.91	4.48	3.60	14.11	12.66	80.43	23.38
100 seed weight	20.25±0.56	13.45 - 27.23	20.50	19.88	22.36	22.02	96.96	44.67
Yield per plant	827.48±42.72	315.25 - 2070.45	303498.45	299849.38	66.58	66.18	98.79	135.49

Table 3. Analysis of Variance for Different traits in Vegetable Cowpea germplasm of Goa

Sources of Variation	d.f.	Mean Sun of Squares											
		Plant height	No of branches	Leaf length	Leaf width	Days to 1st flowering	Days to 1st harvest	Pod length	Pod weight	Pods per plant	No of seeds per pod	100 seeds weight	Pod yield per plant
Treatments	28.00	64.67*	44.42	67.53	75.31	948.59*	1896.24	3989.55*	1441.39*	27091.81	125.41	97956.69	574.08*
Replications	1.00	0.47	0.08	0.07	0.05	0.03	0.38	1.32	0.65	0.02	1.07	1383.77	0.21
Residual	28.00	2.47	3.15	4.54	3.35	62.58	105.70	75.19	17.72	361.41	24.55	2174.08	17.48

*Significant at 1% level

Table 4. Clustering pattern of vegetable cowpea genotypes

Cluster No.	Number of genotypes	Constituent genotypes
I	5	VCG1, VCG2, VCG3, VCG9, VCG15
II	2	A Suman , A Garima
III	2	VCG6, VCG13
IV	2	VCG18, VCG17
V	2	VCG11, VCG14
VI	2	VCG25, VCG20
VII	2	VCG10, VCG12
VIII	2	VCG7, VCG19
IX	2	VCG24, VCG21
X	2	Vijayanthi, VCG26
XI	2	VCG23, VCG22
XII	2	VCG5, VCG16
XIII	1	VCG4
XIV	1	VCG8

Table 5. Phenotypic correlation coefficient among different quantitative characters in vegetable cowpea

Traits	Plant height	No of 1° branches	Leaf length	Leaf width	Days to 1st flowering	Days to 1st harvest	Pod length	Pod weight	No of pods/ plant	No of seeds/ pod	100 seed weight	Yield per plant
Plant height	**1.000**	-0.176*	0.688**	0.345*	0.150**	0.060	-0.076	0.360*	0.104	0.239	0.008	0.423*
No of 1° branches		**1.000**	-0.373	-0.286	0.061	0.201	0.084	-0.130	0.083*	-0.093	0.219	-0.046
Leaf length			**1.000**	0.829	0.033	-0.141	-0.297	0.047	0.064	0.258**	-0.022	0.135
Leaf width				**1.000**	-0.164	-0.157	-0.073	0.063	0.050	-0.022	-0.024	0.109
Days to 1st flowering					**1.000**	0.567**	-0.229	-0.080	-0.469	0.280*	0.242*	-0.318*
Days to 1st harvest						**1.000**	0.237*	-0.039	-0.326	0.013	0.220*	-0.195
Pod length							**1.000**	0.700**	-0.077	-0.390**	0.055	0.489*
Pod weight								**1.000**	-0.083*	-0.267	0.044	0.737**
No of pods/plant									**1.000**	-0.099	0.072*	0.596**
No of seeds/pod										**1.000**	-0.139**	-0.203*
100 seed weight											**1.000**	0.096

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

improvement. High heritability coupled with high genetic advance for above characters in vegetable cowpea has been reported by Resmi (1998) and Narayanankutty *et al.* (2003). Other characters *viz.,* days to first flowering, days to first harvest, number of seeds per pod and leaf length has recorded high heritability of more than 90 per cent but their genetic advance is very low (<30%) indicating the non additive gene action for these traits. This implies improvement of above traits by pyramiding desirable genes through suitable hybridization programmes.

The success of any hybridization programme depends on the genetic diversity present in the parents. In the present study, the twenty nine genotypes could be grouped in to fourteen clusters based on genetic distance (Table 4). The cluster I was the largest comprising of five genotypes and remaining clusters had two genotypes each except thirteen and fourteen that had one genotype each.

The clustering pattern in the present study did not follow any uniform pattern. The clustering pattern was irregular and the same type of distribution was earlier reported by Patil and Bhapkar (1987) and Narayanankutty *et al.* (2005).

The correlation studies provide reliable information on the nature and extent of relationship for bringing about improvement in yield and other traits. The estimates of correlation coefficients is presented in Table 5. Characters showing positive and highly significant correlation with yield per plant were pod weight (0.737) and number of pods per plant (0.596). On the other hand, yield had negative and significant correlation with days to first flowering (-0.318) and number of seeds per pod (-0.203). This is in accordance with the results of Narayanankutty *et al.* (2005).

In the present study, high coefficient of variation was observed for pod yield per plant, pod weight, number of pods per plant and pod length indicating their significant contribution in determining the inter cluster distances. Hence, selection of parents differing in traits such as pod weight, pod yield per plant , number of pods/plant and pod length will be more useful in future breeding programmes.

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Original Research Paper

Validation of Molecular Markers Genetically Linked to *S*-Cytoplasm and Restoration-of-fertility (*Rf*) Loci in Hot Pepper (*Capsicum annuum L.*)

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ABSTRACT

Existence of CGMS system in hot pepper is due to the rearrangements in the mitochondrial genome and is largely used in economized and pure F_1 hybrid seed production around the world. The *orf456*, a new ORF present at flanking region of the *coxII* gene at the 3' end, was distinguished male sterile cytoplasm in hot peppers along with *atp6-2*gene. In the current study, eighteen pepper genotypes (nine each of A and corresponding B lines) of varied origin were used to validate with two male sterile cytoplasm (*S*-cytoplasm) specific sequence characterised amplified region (SCAR) markers *viz*., *atp6-2*$_{(875\ bp)}$ and *orf456*$_{(456\ bp)}$ and one restoration-of-fertility (*Rf*) locus specific marker, CRF$_{(550\ bp)}$. The results clearly showed that the presence of CMS-*S*-cytoplasm and absence of restoration-of-fertility (*Rf*) gene in the pepper genotypes studied and is comparable with the phenotypic data. In view of the outcomes it has been reasoned that the accessible *S* and *Rf* markers available in the public domain are reproducible and can be promptly utilized for marker assisted selection (MAS) in hot pepper crop improvement program.

Keywords: CGMS, Hot pepper, Marker Assisted Selection, Mitochondria, ORF.

INTRODUCTION

Peppers are commercially grown as a spice and vegetable crop. Hot pepper is a Solanaceous crop, originated in Central and South America, and is introduced to India over 500 years ago.Among the domesticated species, *Capsicum annuum* L is one of the most extensively cultivated pepper species in India. In India, 75% of chilli production is from the southern states *viz*., Andhra Pradesh, Telangana, Karnataka, Tamil Nadu and Maharashtra. Concerted efforts in thecrop improvement program in pepper resulted in release of many improved varieties and F_1 hybrids for commercial cultivation. Utilization of male sterile systems in F_1 hybrid seed production of peppers is exceptionally economical.

Male sterility in crops is due to a failure to produce functional pollen or anthers (Grelon*et al.,* 1994, Pruitt and Hanson, 1991; Budar and Pelletier, 1994). CMS/ CGMS is exploited for the development of F1 hybrids in many crops around the world (Hanson, 1991; Hanson and Bentolia, 2004; Miller and Bruns, 2016). Generally,CMS resulted due to the rearrangements in the mitochondrial genome sequences,which in turn results in the arrangement ofnew open reading frames (ORF) which alter the expression of normal genes of the mitochondrial ATP synthesis complex (Pruitt and Hanson,1991; Budar and Pelletier, 1994). The rearrangements within the sub unit genes of ATP synthesis, such as atp 4, 6, 8 and 9 (Pruitt and Hanson, 1991; Hanson and Bentolia, 2004; Schanable and Wise, 1998 Pruitt and Hanson, 1989) are responsible for the CMS in crops and other gene rearrangements observed in pepper lines will be contributed by *coxII* and *nad9*.

Hot pepper genotype PI164835, a collection from India was the first CMS line reported (Peterson, 1958), and is being used in production of F_1 hybrid seeds all over the world (Reddy *et al.,* 2002). In this CMS line, a new ORF viz., *orf456* was found as flanking region of the *coxII* gene at the 3' end. The *atp6-2* gene is believed to be regulated through restoration-of-fertility (*Rf*) loci at the transcriptional level and the *orf456* is regulated at post transcriptional or translational level

Table 1. Molecular markers used for the validation of male sterile lines in the present study

Marker name (Nature)	Primer Sequence (52 to 32)	Annealing temperature (^{O}C)	Expected amplicon size of primer (bp)	Reference
atp6-2 (SCAR)	F AGTCCACTTGAACAATTTGAAATAATC R - GTTCCGTACTTTACTTACGAGC	58	875 bp	Ji *et al.* (2013)
orf456 (SCAR)	F - ATGCCCAAAAGTCCCATGTA R - TTACTCGGTTGCTCCATTGTTT	60	456 bp	Kim *et al.* (2007)
CRF (SCAR)	F - GTACACACCACTCG-TCGCTCCT R - TTCTTGGGTCCCTTT-CTTCCAA	55	870 bp	Gulyas *et al.* (2006)

(Kim *et al.,* 2006; Kim *et al.,* 2007), are responsible for CMS trait.In the present study, the four stable CGMS lines developed and being use dinpepper improvement program at ICAR-IIHR, Bangalore are validated with the two male sterile cytoplasm (*S*-cytoplasm) trait linked markers, *atp6-2* and *orf456* and one restoration-of-fertility (*Rf*) loci linked to *CRF* marker.

MATERIALS AND METHOD

Plant material

An aggregate of nine male sterile and their comparing nine maintainer lines were utilized in the current study are referenced in the Table 2.

Phenotypic evaluation of male sterility

The phenotypic evaluation of male sterility and fertility in lines were carried out by the visual observation at the flowering stage.The male sterile plants showed no pollen grains with shriveled anther lobes, whereas the male fertile plants have bulged anther lobes with abundant pollen grains (Plate 1).

Pollen morphologyandsize

The freshly unopened flower samples of male sterile and male fertile plants were gathered from the field in the early dawn,and put away in impenetrable zip lock polythene covers over the ice package to keep up the freshness. The pollen grains were collected from the dehisced anther lobes independently from individual flowers, frozen on the liquid nitrogen and stored them at -195^{0} for further studies. For morphological examinations, the individual pollen grains were directly dusted on to the slides and length and breadth of the individual grains were measured using scanning electronmicroscope (TM3000, Hitachi, Japan). The reproductive parts of both male sterile and male fertile flowers and the cross section of the anther lobes were additionally seen under the scanning electronmicroscope (TM3000, Hitachi, Japan), in order to study the morphological difference between the male sterile and male fertile flowers.The stereo microscopy images of the dehisced flowers were additionally examined (ZEISS Stereo zoom microscope Stemi 508 doc, Germany).

DNA extraction:

The total genomic DNA was isolated from the leaves of one month old seedling using 4% CTAB plant extraction protocol (Doyle and Doyle, 1990). The genomic DNA samples were qualitatively checked in 0.8% agarose gel and quantitatively by using UV-spectrophotometer. The concentrated DNA was diluted to 20ng/µL according to the spectrophotometer reading and thus diluted DNA is used as the template in PCR for genotyping with specific molecular markers.

PCR conditions and validation of molecular markers:

The polymerase chain reaction master mixture contained 2µL of 10X buffer, 2µL 25 mM $MgCl_2$, 2.5µL 1mM dNTP, (3b Blackbio, Spain) 1.5µL of 10µM of forward and reverse primer, 0.5µL 1U *Taq* DNAPolymerase (3b Blackbio, Spain) and 2µL of 20ng template DNA. The PCR conditions for the validation of the three SCAR markers were carried out as mentioned here. Initial denaturation at 95^{0} for 5 minutes accompanied with 30 repeated cycles of denaturation at 94^{0} for 60 seconds, annealing as given in the Table 1 for 60 seconds, extension at 72^{0} for 60 seconds and final extension at 72^{0} for 5 minutes. The reactions were carried out in the thermocycler (Eppendorf, Germany). PCR amplified fragments were separated on 1.5% agarose gel/1X TBE (w/vol), stained with ethidium bromide dye and

Table 2. Results of the markers screened for CGMS lines

Sl.No.	Sample Name	PCR Amplification of SCAR markers			Observed Phenotype	Expected Genotype
		atp6-2	*orf 456*	*CRF*		
Male sterile lines						
1	IIHR 3285 A	+	+	-	Sterile	S
2	IIHR 3226 A	+	+	-	Sterile	S
3	IIHR 3287 A	+	+	-	Sterile	S
4	IIHR 3228 A	+	+	-	Sterile	S
5	IIHR 4560 A	+	+	-	Sterile	S
6	IIHR 4561 A	+	+	-	Sterile	S
7	IIHR 4558 A	+	+	-	Sterile	S
8	IIHR 4553 A	+	+	-	Sterile	S
9	IIHR 4555 A	+	+	-	Sterile	S
Male fertile lines						
10	IIHR 3285 B	-	-	-	Fertile	N
11	IIHR 3226 B	-	-	-	Fertile	N
12	IIHR 3287 B	-	-	-	Fertile	N
13	IIHR 3228 B	-	-	-	Fertile	N
14	IIHR 4560 B	-	-	-	Fertile	N
15	IIHR 4561 B	-	-	-	Fertile	N
16	IIHR 4552 B	-	-	-	Fertile	N
17	IIHR 4554 B	-	-	-	Fertile	N
18	IIHR 4556 B	-	-	-	Fertile	N
19	Control R- line	-	-	+	Fertile	N

(+) amplification; (-) non-amplification

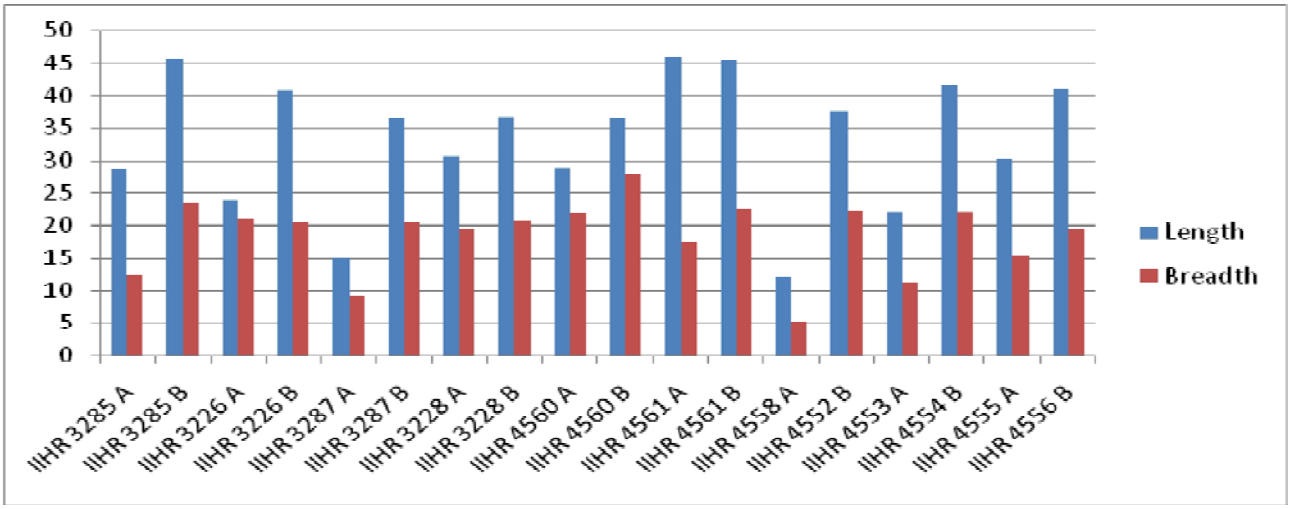


Fig 1. Bar diagram showing the measurement of individual pollen grains size in male sterile vs male fertile flowers using scanning electron microscope

documented under neath the ultra violet light (UVI Pro Platinum, Cambridge, U.K). The experiments were repeated for three consecutive times with each marker for confirmation of results.

Cloning and sequencing:

The PCR amplified fragments of atp6-*2* gene in male sterile lines were separated on 1% agarose stained with EtBr gel, excised and purified the fragments using Nucleospin® Gel and PCR Clean-Up Kit (Macherey-Nagel, Germany). Five µL of the eluted product was ligated into pTZ57RT cloning vector system. The pTZ57RT vector containing the ligated DNA was successfully transformed into DH5α strain of *E.coli.* Transformed colonies were spread on Luria Bertani agar/Ampicillin/X-gal/IPTG plates and were identified through blue white screening after incubation at 37^0 overnight. Recombinant colonies were confirmed using colony PCR, further plasmid was isolated using alkaline lysis method. The isolated plasmids were confirmed for the presence of insert (*atp6-2 gene)* by digestion with the restriction enzyme, EcoRI and the restriction digested products were separated on 1% agarose/ EtBr gel to differentiate two distinct bands of vector and the 850bp insert respectively. Before sequencing, PCR product clean-up was performed using Nucleospin® Gel and PCR Clean-Up Kit (Macherey-Nagel, Germany). The sequencing was carried out in ABI-3710 Prison automated DNA analyzer (Europhins, India).

RESULTS AND DISCUSSION

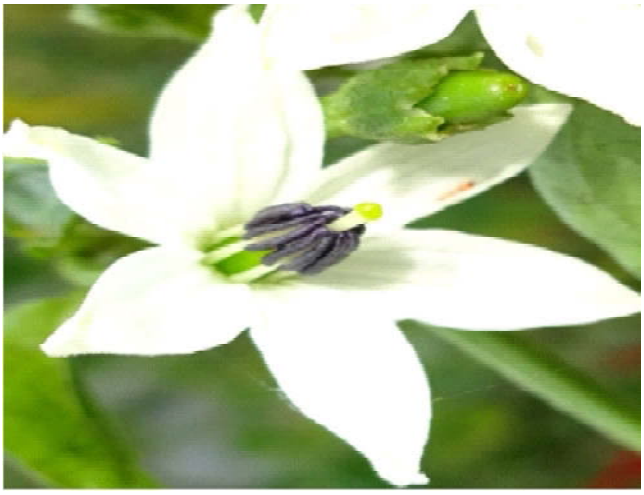
We used two male sterile cytoplasm (*S*-cytoplasm) trait linked markers,*atp6-2*$_{875}$and *orf456(*Ji *et al.,*2013and Kim *et al.,* 2005, 2007) and one restoration-of-fertility (*Rf*) loci linked marker *CRF* (Gulyas *et al.,*2006) to validate nine male sterile and their corresponding nine maintainer lines. CMS linked SCAR marker*orf456* amplified in allthe male sterile genotypes (*S*-cytoplasm), at an expected base pairs of 456 as shown in the Fig. 2 and this 456bp amplicon size was absent in all corresponding maintainer lines (*N*-cytoplasm) (Fig.2c, Table 2).Instead of amplifying at expected amplicon size of 875bp, *atp6-2* marker amplified at 850 bp in all the nine male sterile genotypes (*S*-cytoplasm) (Fig.2b, Table 2).In order to confirm the 25bp difference in the amplicon size, further cloning and sequencing was undertaken. Five clones each of the male sterile lines were selected, plasmid isolated, purified and further sequenced (ABI-3710 Prisom automated DNA analyzer). Sequence obtained from ABI-3710 Prisom automated DNA analyzer was analysed from NCBI site (www.ncbi.nlm.nih.gov) and checked for nucleotide sequence identity of the observed sequences and found that there is almost 99% identity for *Capsicum annuum atp6-2* subunit.The presence of the expected amplicon pattern in all nine male sterile genotypes (*S*-cytoplasm) proved that the mitochondrial gene associated *atp6-2* subunit is responsible for the transcription of the *orf456* novel gene which indeed responsible for the cause of CMS in the cultivar varieties of hot peppers. Meanwhile, the nine corresponding male fertile/ maintainer lines (*N*-cytoplasm) failed to amplify at the expected amplicon size. The CMS lines which are phenotypically male sterile are genotypically carrying a sterile cytoplasm, *S* with *rfrf* loci and all the maintainer or fertile B lines are genotypically carrying a normal cytoplasm, N with *rfrf* loci.The one *CRF-SCAR* marker specific to restoration-of-fertility (*Rf*) locus as expected failed to amplify the 550bp fragment in any of the nine cytoplasmic male sterile (A) lines or cytoplasmic male fertile/maintainer (B) lines (Fig 2,Table 2).The complete absence of the *CRF-SCAR* marker in all genotypes used for the current study proves that these samples didn't carry a restoration-of-fertility*, Rf* loci, indicating that the cytoplasm looks genotypically normal, N or sterile, S. Even though there are markers for identification of restoration-of-fertility (*Rf*) in hot pepper (Kumar *et al.,* 2009, Kim *et al.*, 2005, Zhang *et al.,* 2000),*CRF-SCAR* marker (Gulyas *et al.,*2006) is the most commonly and widely used molecular marker for the detection of presence or the absence of restoration-of-fertility in CMS lines of hot pepper.

Further, using the scanning electron microscope (SEM) the morphological variation in pollen grain size among the nine male sterile and their corresponding maintainer lines (Fig.1& Plate 2) was studied measuring the length and breadth of pollen grains (Plate 2). Maximum variation in pollen grain length was observed among A lines compared to B lines, and it ranged from 12.2 to 46.2µM and 36.4 to 45.7µM, respectively. Similarly, maximum variation in pollen grain breadth was observed among A lines and ranged from 5.24 to 21.9 µM, whereas it ranged from 19.4

Plate 1. Images of male sterile *vs* male fertile flowers

IIHR3285 A

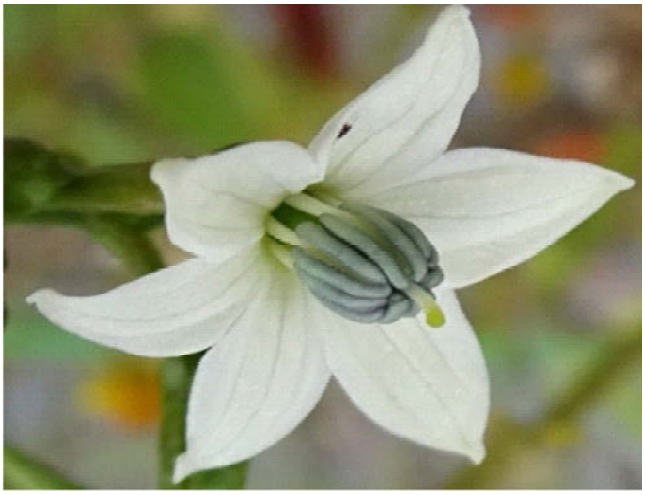
IIHR3285 B



IIHR3287 A

IIHR3287 B

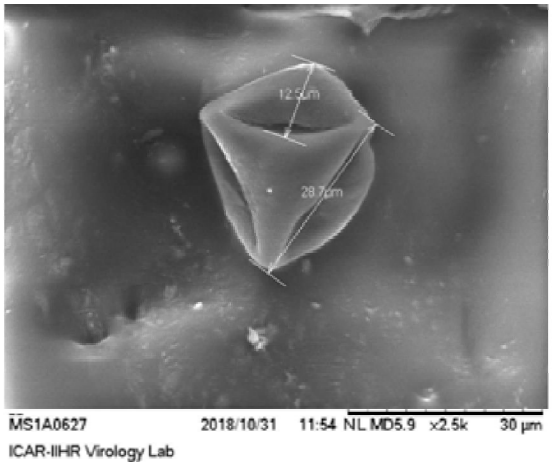
IIHR3228 A



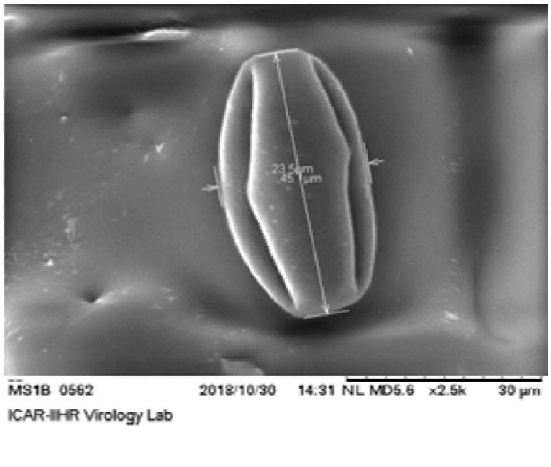
IIHR3228 B

Male sterile (A) lines showing shrinked anther **Maintainer (B) lines showing bulged anther**

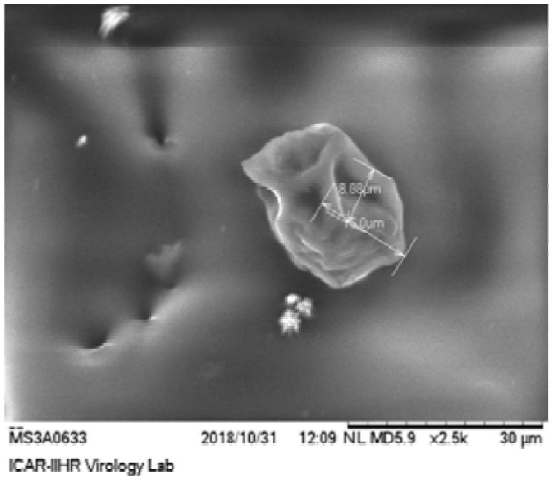
Plate 2. Images of male sterile *vs* male fertile anthers (Single pollen SEM images at 2.5k magnification)



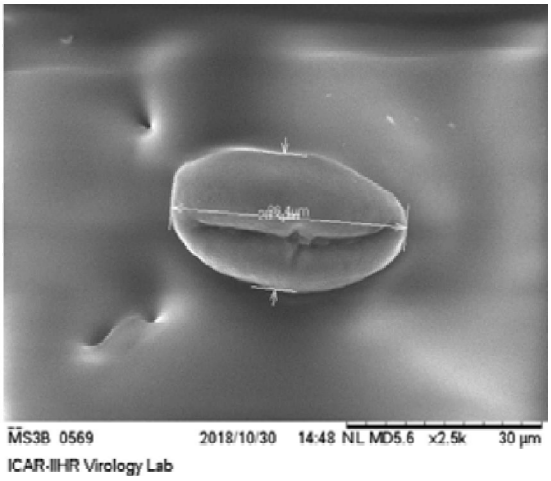
IIHR3285 A



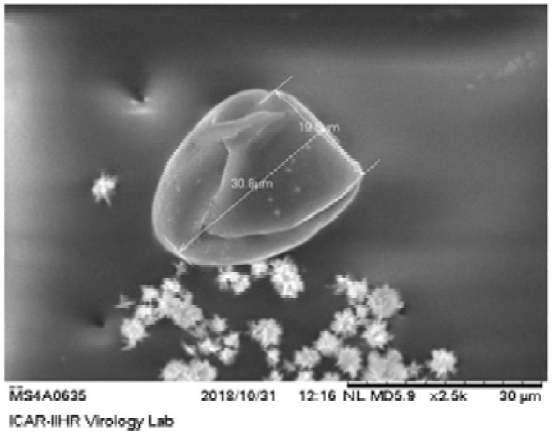
IIHR3285 B



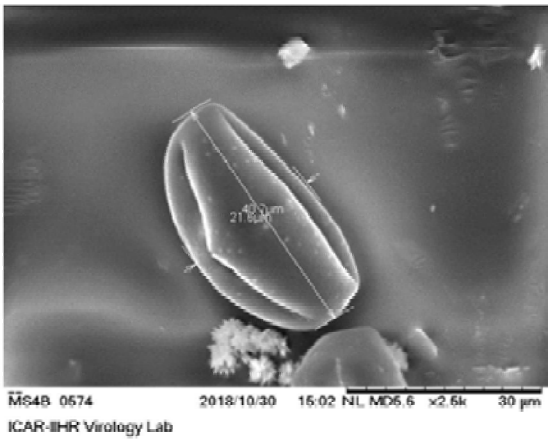
IIHR3287 A



IIHR3287 B



IIHR3228 A



IIHR3228 B

Male sterile (A) lines showing shrinked pollen grains

Maintainer (B) lines showing normal pollen grains

to 27.8µM among B lines (Fig. 1 & Plate 2), respectively.

The SEM images of the reproductive parts of the male sterile flowers morphologically found to be very shorter in size compared to the male fertile plants. The anther lobes of the male sterile flowers appeared to be shrivelled with less or shrunken pollen grains, whereas the male fertile plants have bulged anther lobes with abundant pollen grains (Plate 3b). So as to see the distribution of the pollen grains inside the anther lobe, the cross section of the anther lobe was studied. The SEM images clearly distinguished the male sterile plants had no visible pollens inside the tetrad pollen chambers, rather the male fertile plants produced numerous functional pollens (Plate 3c) attached to the tetrad anther chambers. The stereo

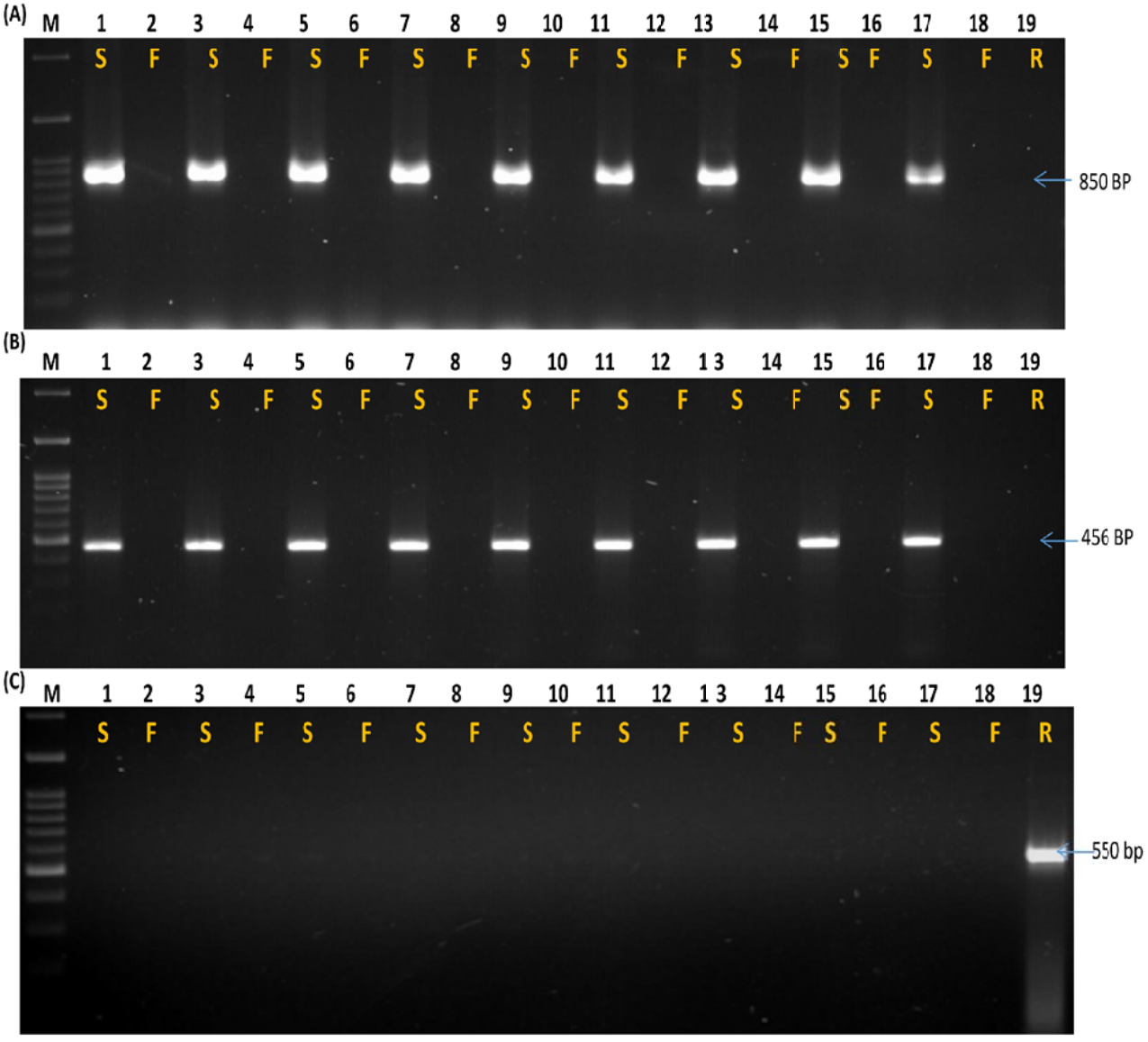
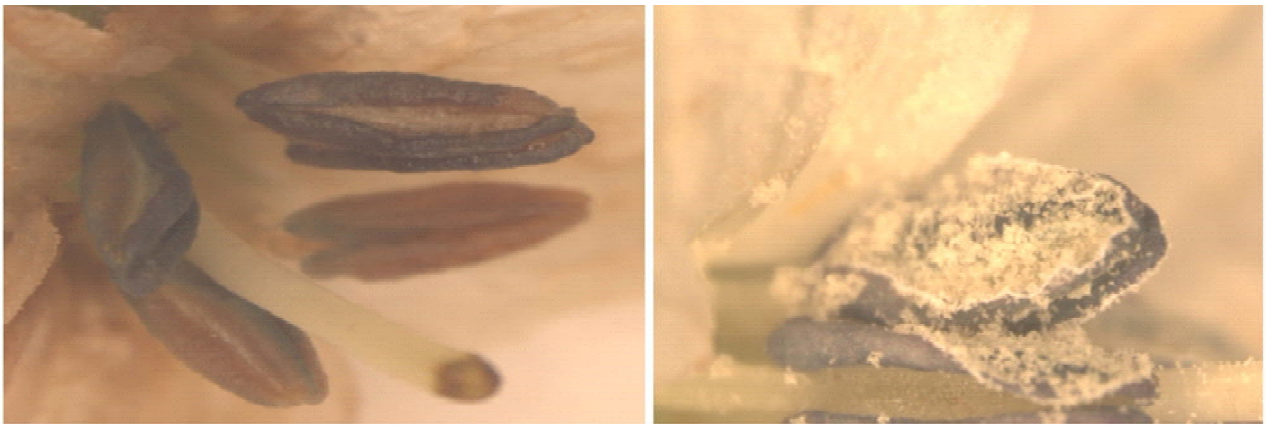


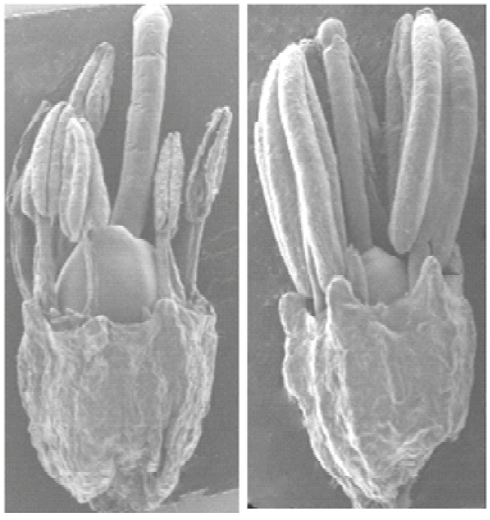
Fig 2: Gel picture showing the amplification results with the three molecular markers across eight pairs of sterile and fertile lines used (**A**) *atp6-2* marker (**B**) *orf 456* marker and (**C**) restorer of fertility gene specific *crf*marker. All PCR products were separated on 1.5% 1X TAE- agarose gel, stained with ethidium bromide dye. M= 100 bp ladder, serial number 1-18 indicates the sample order as given in the table no.2. S= male sterile line, F= male fertile line and R= restoral line. Arrow head indicates the band size obtained.

Plate 3. Male sterile *vs* male fertile (a) flower, (b) reproductive part and (c) cross section of anther lobe

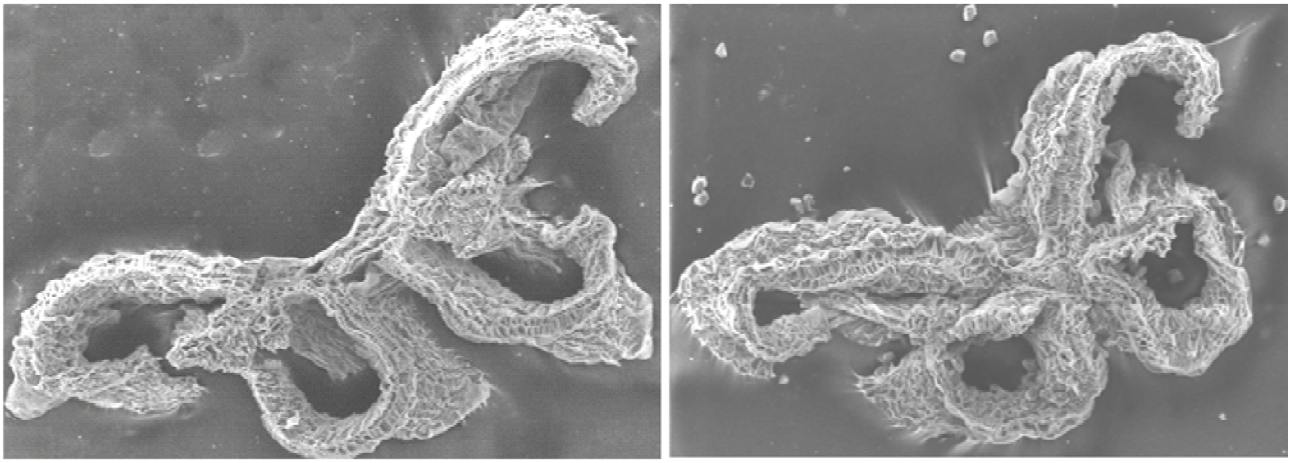
Male sterile **(a)** **Male fertile**



(b)



(c)



Male sterile (A) lines with no or very less pollen grains

Maintainer (B) lines with abundant pollen grains

microscopy images of the dehisced flowers clearly showed the absence of pollens at different magnification in male sterile plants where as presence of abundant pollen grains were visible in and out of the anther lobes of male fertile plants as shown on Plate 3a.

CONCLUSION

CMS in crops is caused due to a failure to produce functional pollen or anthers (Gómez 1999, Pruitt and Hanson, 1991). Previously, the two male sterile cytoplasm (*S*-cytoplasm) trait linked molecular markers viz., *atp6-2* and *orf456 (*Ji *et al.,*2013 and Kim *et al.,*2005,2007)were identified and characterised in CMS lines of hot pepper, were further used for the hybrid seed production in a commercial scale. The CMS pepper lines, were validated with the existing SCAR markers linked to the male sterility in pepper.The eight hot pepper lines namely IIHR 3285, IIHR 3226 , IIHR 3287, and IIHR 3228 (four CMS and 4 maintainer lines) developed at ICAR-IIHR, Bangalore and the other ten hot pepper lines (5 CMS and 5 maintainer lines) received from AVRDC, Taiwan, are having common sterile cytoplasm and restoration-of-fertility genes as were successfully validated using the three already known SCAR markers i.e., two male sterile cytoplasm (S-cytoplasm) trait linked to *atp6-2* and *orf456 (*Ji *et al.,*2013 and Kim *et al.,* 2005, 2007) and one restoration-of-fertility (*Rf*) loci linked marker *CRF* (Gulyas*et al.,*2006) and these molecular markers are highly reproducible at the genotypic level. Thus, these molecular markerscan be effectively used to recognize CMS from maintainer lines and fertility restorer lines and helps to fasten the breeding work to incorporate the CGMS system with varied fruit types and to incorporate disease resistant genes into A, B and R lines.

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Original Research Paper

Breeding for Improvement of High Temperature Tolerance in Garden Pea (*Pisum sativum* L.) for off Season Cultivation

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ABSTRACT

The present investigation is aimed towards breeding varieties of garden pea for early summer cultivation (March-May) that can tolerate temperatures up to 35^{0}C. High temperature tolerant accessions KTP-4, Arka Sampoorna, Oregon Sugar, Magadi local were crossed with Arka Ajit, Arka Pramodh, Arka Priya having superior pod quality, yield and followed by pedigree method of breeding, superior transgressive segregants from these crosses were advanced up to F_7 generation. In F_7, six selected advanced breeding lines were assessed for their performance in the field with checks during early summer for four years in succession. Results revealed significant differences between selected lines and checks wherein all the lines surpassed checks with yield ranging from 5.9-7.6 t/ ha and in checks it was only 2.6-3.1 t/ha. Among these six breeding lines, three lines were selected based on high yield (6.7-7.6 t/ha), pod quality characters and identified to be highly suitable for early summer cultivation.

Key words: Breeding, Early summer, Garden pea, High temperature, Stress tolerance

INTRODUCTION

Globally, vegetable legumes are conventionally identified as indispensable sources of nutrition and health to humankind besides radically influencing agricultural sustainability. Garden pea, one among the commercially cultivated leguminous vegetables is a dense source of nutrients and vital source of health promoting antioxidants, minerals, vitamins and phytonutrients (Dahl *et al.* 2012). In India, garden pea is grown in an area of 0.55 million hectares (m.ha) with an annual production of 5.52 million tonnes (m.t) and productivity of 10.03 t/ha (NHB, 2018-19). Various factors are known to influence yield of which, abiotic stresses especially temperature, drought and salt stress take away major share in causing severe yield losses by impairing growth and development of plants in majority of the crop species(Suzuki *et al.,* 2014).Within these factors, temperature stress imposes most protracted effects on plant development and reproduction accompanied with severe reduction in yield potential of many subtle crop species (Bita and Gerats, 2013). Garden pea being extremely sensitive to temperature stress, if subjected to higher temperatures responds in an exacerbated manner resulting in drastic reduction of yield. This strictly hampers summer cultivation of the crop where there exists a great demand for peas during off season. Hence, in India it is traditionally cultivated during rabi season when the temperatures fall between 15 to 27^{0}C that highly favor crop growth and yield (Mohan *et al.,* 2013). Summer cultivation of the crop is restricted to high altitude areas where congenial conditions for crop growth exist and in plains cultivation during summer often influences principal morphological, physiological, biochemical and molecular plant processes in a sequential manner affecting the overall plant growth and productivity remarkably (Petkova *et al.* 2009; Todorova *et al.,* 2016). Among various growth phases of the crop, reproductive stage is highly vulnerable to elevated temperatures (>30^{0}C) affecting pollination, flower shedding, flower abortion, seed loss, pod filling and ultimately lowers the yield (Guilioni *et al.,* 2003; Bueckert *et al.,* 2015). In the recent years, demand for off season peas has enormously increased and is

still anticipated to increase in the coming future owing to its nutritional and health benefits.To meet the ever growing demand for garden pea during off season, peas are often frozen and preserved for months together. Since the main growing season of the crop is confined to rabi, at present there are no commercial varieties of garden pea suitable for cultivation at least during early summer. Although varieties like Magadi local are cultivated during early summer on a commercial scale in certain parts of Southern India, being a pulse type (*arvense* group) with small pods its yields are exceedingly low with 2.5 t/ha. The realistic approach to surmount this barrier unequivocally includes initiation of breeding programmes to develop resilient varieties tolerant to high temperature (up to 35^0C) that could suit off season cultivation. With this objective, breeding work was started at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru, India during 2007 and aimed towards development of high temperature tolerant varieties (33-35^0C) suitable for early summer cultivation.

MATERIALS AND METHODS

A field experiment was started in 2007 at Indian Institute of Horticultural Research, Bengaluru, India (13.13° N, 77.49° E) located at an altitude of 890 m above mean sea level. Initially, 200 pea germplasm lines of garden pea were screened for three consecutive years during summer 2007 to 2009 for identification of lines tolerant to high temperature. Average maximum and minimum temperatures recorded during the crop growth period were 35^0C and 26^0C respectively. Screening and selection of tolerant lines was based mainly on yield related traits such as pods per plant, pod filling, seeds per pod, shelling percent and yield *per se*. Among 200 lines screened, accessions KTP-4, Magadi local, Arka Sampoorna and Oregon sugar were identified to be tolerant to high temperature and performed superior in terms of yield and other related traits for three consecutive years. During 2009, selected high temperature tolerant lines were used as parents and crossed with high yielding varieties Arka Ajit, Arka Priya and Arka Pramodh having average pod yield of 10-12 t/ha to generate F_1 population. Initially, crosses were attempted between (Arka Ajit × Arka Sampoorna) and (Arka Pramodh × Oregon Sugar) separately and in the resultant segregating generation F_2, superior lines derived from both the crosses were further crossed and advanced upto F_7 followed by pedigree method of breeding. Simultaneously in another cross, superior F_2 transgressive segregants developed from the cross (Arka Pramodh × Oregon Sugar) were selected and crossed to Arka Priya to improve pod filling and advanced up to F_7 generation followed by pedigree breeding. From both the crosses [(Arka Ajit × Arka Sampoorna) × (Arka Pramodh × Oregon Sugar)] and [(Arka Pramodh × Oregon Sugar) × Arka Priya], six advanced breeding lines were selected in F_7 generation during 2014 and were evaluated in randomized block design with three replications using three checks *viz.,* Magadi local (tolerant to high temperature), Arka Ajit and Arka Pramodh (high yielding) during summer for four consecutive years from 2014-2017. Standard package of practices was followed and no rainfall was received during the entire crop growth period. The maximum temperature recorded during reproductive and pod setting stages did not exceed 35^0C. Data on plant height, days to 50% flowering, pod length, pod width, 10 pod weight, pods per plant, seeds per pod, pod yield per plant, pod and seed color were recorded from 10 plants in each of the three replications. Data obtained was subjected to Analysis of variance (ANOVA) using the GENSTAT 9.1 package to assess significant differences among the breeding lines and checks based on mean performance.

RESULTS AND DISCUSSION

Results of ANOVA revealed significant differences among different advanced breeding lines for various morphological, yield and yield related traits in the present study (Table 1). The average plant height in the advanced breeding lines ranged from 64.0 to 127.0 cm. Highest plant height of 127.0 cm was recorded in the line IIHR 12-3 followed byIIHR 15-21 with 126.7 cm and least of 57.3 cm was reported in check variety Arka Ajit. Days taken for 50% flowering in the lines ranged from 44.0 to 48.3 as compared to checks with 42.3 to 48.7 days. With respect to this trait, lowest of 42.3 days was reported in check Magadi local followed by IIHR 15-6 with 44 days and highest of 48.7 days was recorded in Arka Pramodh. In connection to days to pod maturity, variability in the lines ranged from 60.0 to 65.7 and in checks it was 58.3 to 65.0 days. This clearly illustrates that no significant difference exist between lines and checks for the two traits *viz*., days to 50% flowering and days to pod maturityand all the selected

breeding lines fit into the category of mid-season varieties that can arrive to pod maturity within 60-65 days of flowering. With respect to pod length and width, selected breeding lines had higher pod length ranging from 6.5 to 7.9 cm and width of 1.4 to 1.6 cm in comparison to checks with pod length and width of 4.2 to 6.7 cm and 1.2 to 1.6 cm respectively. In terms of pod length, highest of 7.9 cm was observed in IIHR 15-6 followed by IIHR 15-21 with 7.0 cm and least of 4.2 cm was found in check Magadi local. Similar trend was reported in case of pod width wherein IIHR 15-6 followed by IIHR 1-1 recorded highest pod width of 1.6 cm and 1.5 cm respectively and lowest of 1.2 cm was recorded by check Magadi local.

Table 1. Mean performance of selected lines and checks for plant and pod characters during summer (2014-2017)

S.No.	Advanced breeding lines & checks	Plant height (cm)	Days to 50 % flowering	Days to pod maturity	Pod length (cm)	Pod width (cm)	10 pod wt.(g)	Shelling %	Seeds per pod	Pods per plant	Pod & seed colour
1.	IIHR 15-6	67.7	44.0	60.3	7.9	1.6	63.7	56.0	7.7	16.3	DG
2.	IIHR 1-2	64.0	48.0	65.0	6.8	1.5	55.7	56.3	6.3	15.0	DG
3.	IIHR 1-1	65.7	48.3	65.7	6.9	1.5	55.0	57.0	6.3	14.0	DG
4.	IIHR 15-21	126.7	45.3	64.0	7.0	1.4	57.3	60.0	7.3	17.0	G
5.	IIHR 12-3	127.0	45.0	60.0	6.5	1.4	37.7	54.0	5.7	17.7	LG
6.	IIHR 15-15	124.3	45.7	62.3	7.0	1.4	55.7	58.3	6.7	22.3	DG
7.	Magadi local (C)	122.3	42.3	58.3	4.2	1.2	23.3	57.0	4.3	24.0	LG
8.	Arka Ajit (C)	57.3	46.3	64.0	6.7	1.4	43.0	34.3	3.0	10.0	LG
9.	Arka Pramodh (C)	58.0	48.7	65.0	6.7	1.6	42.3	25.0	2.0	8.0	DG
	S.E.(m) ±	2.59	1.03	0.82	0.16	0.04	2.14	1.30	0.49	1.05	
	CD@5%	7.19	2.85	2.27	0.44	0.11	5.92	3.59	1.36	2.90	
	CV%	4.10	3.17	1.84	3.33	3.45	6.27	3.60	12.59	6.55	

DG-Dark Green, LG-Light Green, G-Green, C-Check variety

Among traits governing yield such as pod weight, shelling percent, number of pods per plant and number of seeds per pod significant differences in mean values were observed between checks and selected advanced breeding lines.With respect to 10 pod weight, all the selected lines recorded significantly higher pod weight ranging from 37.7 to 63.7 g as compared to checks with 23.3 to 43.0 g. Highest pod weight of 63.7 g was recorded in IIHR 15-6 and lowest of 23.3 g in check Magadi local. All the breeding lines except IIHR 12-3 (37.7 g) performed significantly superior than all the checks for this specific trait. Concurrent to this, shelling percent was also found to be high in the selected lines ranging from54 to 60% as compared to checks with 25 to 57%. Results from mean of shelling percent revealed highest performance in the line IIHR 15-21 (60%) followed by IIHR 15-15 (58.3%) and least of 25% was recorded in check variety Arka Pramodh. Further, number of seeds per pod in the selected lines were markedly higher ranging from 5.7 to 7.7 as compared to checks with only 2.0 to 4.3 seeds per pod. For this respective trait, IIHR 15-6 followed by IIHR 15-15 were found to have more seeds per pod with 7.7 and 6.7 respectively than checks. These results emphasize that pollination in check varieties was critically impaired due to exposure to high temperature eventually leading to seed abortion and lesser number of seeds with smaller size. The observed results were in accordance with the findings of few authors who reported lesser seed number and size in case of field pea after exposure to higher temperatures (Lambert and Linck, 1958; Jeuffroy *et al.*, 1990; Poggio *et al.,*2005). In contrast to this, for the trait pods per plant resistant check Magadi local reported highest of 24 pods whereas temperature sensitive high yielding check Arka Pramodh had lowest of 8 pods per plant. Elsewhere, in the selected

lines it ranged from 14.0 to 22.3 wherein IIHR 15-15 and IIHR 1-1 recorded highest of 22.3 pods per plant and lowest of 14.0 pods per plant respectively. The reason behind abruptly low number of pods per plant in case of high yielding checks Arka Ajit (10.0) and Arka Pramodh (8.0) could be directly attributed to lack of high temperature stress tolerance. Similar trend of decline in yield of heat susceptible cultivars has been reported in case of bean (*Phaseolus vulgaris*) by exposing plants to higher day temperatures of more than 28^{0}C (Prasad *et al.,* 2002). Although tolerant check is performing superior with respect to this trait, yield was on the higher side in selected advanced breeding lines owing to increased pod weight and more seeds per pod as compared to tolerant check. Further, comparison of yield *per se* between checks and selected breeding lines over average of four years were convincing and disclosed significant differences between checks and breeding lines with selected lines dominating and out yielding all the three check varieties. Average pod yield based on mean of four years in selected lines ranged from 5.9-7.6 t/ha in selected lines and in checks it was significantly lower with 2.6 to 3.1 t/ha. Among the six advance breeding lines, highest yield of 7.6 t/ha was reported in IIHR 15-15 followed by IIHR 15-21 with 7.1 t/ha and IIHR 15-6 with 6.7 t/ha(Table 2). All the three checks recorded significant reduction in yield levels that could be ascribed to effects of heat stress which potentially evoked flower drop, reduction in reproductive phase, reduced pod filling, abortion of seeds within pods finally lowering yield. In agreement to this, decline in yields of field pea cultivars due to high temperature stress was previously reported by Guilioni *et al.* (1997), Vijaylaxmi (2013) and Bueckert *et al.* (2015). Eventhough all the six selected breeding lines were reported to be superior over checks, three lines *viz.*, IIHR 15-15, IIHR 15-21 and IIHR 15-6 proved to be outstanding owing to minimal reduction in yield when exposed to higher temperatures in comparison to others. Further, percent increase in yield over lowest yielding check was reported to be 192.3% (IIHR 15-15), 173.1% (IIHR 15-21) and 157.7% (IIHR 15-6) in these three lines. Additionally, the three selected breeding lines were also superior in terms of pod qualitycharacteristics such as colour along with other yield attributing traits and released as Arka Uttam, Arka Chaitra and Arka Tapas respectively.

Table 2. Mean performance of selected breeding lines for pod yield (t/ha) during summer 2014 to 2017

Sl. No.	Advanced breeding lines and checks	2014 Summer mean	2015 Summer mean	2016 Summer mean	2017 Summer mean	Average	Per cent increase over check
1.	IIHR 15-6	6.6	6.7	6.6	6.9	6.7	157.7
2.	IIHR 1-2	6.2	6.0	5.9	6.3	6.1	134.6
3.	IIHR 1-1	5.9	5.9	5.6	6.0	5.9	126.9
4.	IIHR 15-21	7.1	7.1	6.9	7.3	7.1	173.1
5.	IIHR 12-3	7.0	6.9	6.6	6.9	6.9	165.4
6.	IIHR 15-15	7.6	7.5	7.3	7.8	7.6	192.3
7.	Magadi local (C)	2.6	2.6	2.4	2.8	2.6	-
8.	Arka Ajit (C)	3.1	3.0	2.7	2.8	2.9	-
9.	Arka Pramodh (C)	2.7	3.3	3.0	3.2	3.1	-
	S.E (m)±	0.18	0.15	0.17	0.18		
	CD@5%	0.51	0.42	0.47	0.49		
	CV%	4.80	3.90	4.60	4.49		

IIHR 15-6, IIHR 15-15 and IIHR 15-21 are selected advanced breeding lines identified for release as high temperature tolerant varieties.

These findings clearly illustrate that current breeding programme aimed towards incorporation of high temperature tolerance (33-35^{0}C) followed by rigorous selection procedures could successfully integrate heat stress tolerant genes into the selected lines as obvious from the results obtained from the study. Further, the average yield obtained from the lines during off season cultivation *i.e.,* summer was more than double in comparison to stress tolerant and high yielding checks. Even though yield (6.7-7.1 t/ha) obtained is not on par with the actual yields that could be realized from high yielding cultivars (10-12 t/ha) during normal season of cultivation *i.e.,* rabi, existing gap in yield levels can be compensated by the higher prices fetched for summer peas. Hence, the three varieties generated from this breeding programme invariably suit for cultivating garden pea in off season preferably during early summer in regions where temperatures donot exceed 35^{0}C. Further, the breeding material generated from the study tend to serve as base material for accomplishing in depth investigations on physiological and molecular mechanisms involved in regulating tolerance to high temperature in garden pea in the coming future.

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Original Research Paper

Evaluation of Hybrids and Cultivars of Single type Tuberose (*Polianthes tuberosa*)

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ABSTRACT

Hybrids and cultivars of single type tuberose was evaluated to fulfill the need to develop new hybrids as demanded by commercial growers. Evaluation of fifteen genotypes showed significant variation in growth, floral and bulb characters. Cultivar Arka Prajwal was significantly superior over all genotypes, which recorded least number of days for opening of 1st floret (78.55 days) with maximum diameter of spike (1.18 cm), length of floret (6.05 cm), weight of individual floret (3.12 g) and weight of spike (121.43 g).The hybrid genotype L1P4 (Variegated X Phule Rajani) was observed to be superior in terms of rachis length (39.78 cm), inter-nodal length (7.25 cm), length of bulb (8.09 cm), diameter of bulb (3.76 cm) and diameter of bulb-lets (1.85 cm). Among the hybrid genotypes L1P4 also recorded maximum plant height (116.39 cm), spike length (109.58 cm), weight of cut spike (105.08 g) and vase life (11.00 days). However, it was foundto be at par for number of florets per spike (57.25), length of floret (5.92 cm) and number of spikes per clump (10.14) with all other cultivars and hybrids tested. From the overall performance, it was found that the cultivar Arka Prajwal was the best. Genotype L1P4 found promising for loose as well as cut flower production because of its number of florets, inter-nodal length and spikes per clump which are important characters considering loose flower for taking maximum number of pickings. However, characters such as rachis length, spike length, vase life and weight of spike which are imperative for cut flowers are also noted superior in genotype L1P4.

Key words: Bulb, Flower, Growth, Hybrid and Single type tuberose

INTRODUCTION

Tuberose (*Polianthe stuberosa*) is one of the most importantcut and loose flowers in India. It is anornamental bulbous plant, native to Mexico from where it has spread to different parts of the world during the 16th century. It belongs to family Asperagaceae and is popularly known as '*Rajnigandha*'(Yadav and Maity,1989). The nomenclature of different types of tuberose is based on the number of rows of petals each flower possesses. The cultivar with single row of petals is designated as 'Single' while the one which bears more than three rows of petals is called 'Double'. The cultivar 'Semi-Double' bears flowers with two-three rows of petals. Valuable natural aromatic oil is extracted from the flowers for the high cost perfume industry. The flower of single petalled cultivars reported to contain 0.08-0.135 % concrete and yield 0.08-0.11 % essential oil in India (Singh, 2006). The serene beauty of flower spikes, bright white flowers, sweetness of blooms and delicacy of fragrance of this ornamental crop transform the entire area into a nectarine and joyous. Varieties which perform well in one region may not do well in other locations due to varying climatic conditions. Hence, it is important to study morphological variation and performance of genotypes for important yield contributing characters. Hence, the present investigation was undertaken to evaluate the single type tuberose for growth, flower and bulb yield for Western Maharashtra.

MATERIALS AND METHODS

The present study was conducted at the National Agricultural Research Project Ganeshkhind, Pune-7, MPKV; Rahuri, during 2014-2015. Geographically, Pune is situated at 18°32' North latitude and 73°51' East longitude on Deccan plateau at the confluence of Mula and Mutha rivers. The controlled hybridization programme

using available cultivar of single type of tuberose is already in progress at All India Co-ordinated Research Project on Floriculture at NARP, Ganeshkhind. Experimental materials consisted of 15 genotypes of single type tuberose obtained from AICRP on Floriculture.Seedling selection from hybrid progenies of different crosses identifiedeight new promising single type tuberose genotypes. The experiment was laid out in randomized block design with three replications.The land was ploughed to a medium depth. FYM was spread evenly @ 25 tonnes per hectare and recommended fertilizer dose 200:150:200 Kg NPK per hectare was incorporated. 100Kg Nitrogen was given as basal dose and two split doses 50 kg each at 60 days and 90 days respectively was spread after planting of bulbs. Flat beds of 1.8 x 1.5 m plot size were laid and medium sizedbulbsof 2-2.5 cm diameter were planted at a spacing of 30x30 cm which accommodated 30 bulbs per plot. Standard cultural practices were followed throughout experimentation. The data were recorded on three selected plants from each treatment and replication for vegetative, floral, bulb and bulb-lets characters.

RESULTS AND DISCUSSION

Vegetative characters

The mean performance of cultivars for vegetative growth characters (Table 1) reflected the variations among the cultivars. The earliest spike emergence was observed in cv. Arka Nirantara (60.89 days) but the genotype GK-T-C-4 required maximum days for spike emergence (67.89 days). Significantly highest plant height was recorded in cv. Variegated (138.64 cm) and less in hybrid Phule Rajani X Arka Suvasini (61.76 cm) compared to other genotypes. This is in accordance with the results of Ranchana *et al.* (2013).More number of leaves per plant were observed in genotype GK-T-C-2 (24.11) and less in cv. Local Single (17.00). Flower bud was initiated at 5.78th node in hybrid Phule Rajani x Arka Suvasini while cv. ArkaPrajwal flower bud started at 12.84th node which was noted to be the highest. The significantly longest spike was observed in cv. Variegated (124.37cm) and shortest in hybrid Phule Rajani x Arka Suvasini (56.52 cm). Hybrid L1P4 recorded significantly maximum rachis length (39.78 cm) amongst 15 genotypes under study. The variation

Table 1. Performance of different tuberose genotype on vegetative growth characters

Treatment	Days to spike emergence	Plant height (cm)	No. of leaves	Node at which floret started	Spike length (cm)	Rachis length (cm)	No. of florets per spike
Local Single	63.33	94.81	17.00	10.56	89.73	20.49	39.56
Arka Shringar	65.78	78.86	18.78	9.89	72.81	30.28	52.01
Phule Rajani	65.44	74.49	20.45	8.84	69.74	33.61	52.84
Hyderabad Single	66.67	77.47	20.00	9.73	72.46	28.41	47.59
Arka Nirantara	60.89	95.27	21.89	12.17	86.93	26.77	54.25
Arka Prajwal	62.78	94.83	23.55	12.84	91.14	32.53	57.56
Variegated	62.99	138.64	21.45	11.33	124.37	24.55	45.36
Variegated x Phule Rajani L9P7	65.11	71.33	19.34	10.22	64.93	25.14	57.28
Variegated x Phule Rajani L1P4	62.22	116.39	20.56	11.78	109.58	39.78	57.25
Variegated x Phule Rajani L9P2	66.22	84.51	17.45	9.45	80.56	24.75	34.53
Local Single x Arka Shringar GK-T-C-1	63.89	76.93	19.56	9.23	70.71	30.62	55.14
Local Single x Arka Shringar GK-T-C-2	65.89	84.63	24.11	10.89	79.22	25.67	49.45
Local Single x Arka Shringar GK-T-C-7	67.00	76.56	18.78	8.33	70.83	30.66	58.58
Phule Rajani x Arka Suvasini	64.22	61.76	18.89	5.78	56.52	36.72	49.95
Local Single x Arka Shringar GK-T-C-4	67.89	74.57	17.67	9.45	68.29	27.82	50.92
SE(m) ±	1.25	2.03	1.14	0.44	1.65	0.94	1.63
C.D. at 5%	3.63	5.92	3.33	1.29	4.81	2.72	4.76

among different vegetative characters are attributed due to the difference in their genetic makeup. The number of florets recorded to be highest in genotype GK-T-C-7 (58.58). However, less number of florets was recorded in genotype L9P2 (34.53). Number of florets is an important character, as single type flowers are mostly used as loose flower. The variation in florets per spike may be due to genetic variability, disparity in storage of food among different cultivars and prevailing environmental condition.

Flowering characters

Among the fifteen genotypes evaluated for their floral characters (Table 2), the minimum days required for opening of first floret was noted in cv. Arka Prajwal (78.55days) whereas, significantly maximum days was required in genotype L9P7 (85.76 days). Significantly maximum inter-nodal length was noted in genotype L1P4 (7.25cm).Cultivar Arka Prajwal recorded significantly thick diameter of spike (1.18 cm) while it was thin in genotype L9P2 (0.75 cm). The diameter of spike influences the spike strength and reserved food material in it. The existing environmental condition and genetic factors influence the variation in spike thickness among different genotypes under study. Patil *et al*. (2009) and Arya *et al.*(2006) also reported similar results in tuberose.

Cultivar Arka Prajwal recorded maximum floret length (6.05 cm) and significantly minimum length of floret was recorded in cv. Hyderabad Single (4.89 cm). The diameter of floret was noted significantly maximum in hybrid L9P7 (6.34 cm) and minimum was recorded

Table 2. Performance of different tuberose genotype on flowering characters

Treatment	Days for opening of 1st floret	Inter-nodal length (cm)	Diameter of cut spike (cm)	Length of floret (cm)	Diameter of floret (cm)	Weight of cut spike (g)	Weight of individual floret (g)	Vase life (days)	Spike per clump	Spike per hector
Local Single	79.22	4.60	0.88	5.29	4.45	64.90	1.47	9.50	7.88	390799
Arka Shringar	80.77	4.18	0.86	4.90	5.05	88.24	1.79	10.17	9.87	489284
Phule Rajani	81.33	4.73	0.91	5.12	5.64	96.16	1.97	11.50	9.71	481187
Hyderabad Single	83.44	3.25	0.95	4.89	5.37	81.53	1.74	10.00	8.13	403027
Arka Nirantara	79.66	4.50	1.11	5.76	5.79	91.69	2.59	10.00	8.11	402036
Arka Prajwal	78.55	4.97	1.18	6.05	5.65	121.43	3.12	10.17	9.04	448304
Variegated	82.99	4.68	0.87	5.34	5.18	94.41	1.49	9.00	6.85	339409
Variegated X Phule Rajani L9P7	85.76	4.21	0.87	6.04	6.34	88.89	2.49	9.00	7.77	385346
Variegated X Phule Rajani L1P4	83.33	7.25	0.85	5.92	5.67	105.08	1.80	11.00	10.14	502669
Variegated X Phule Rajani L9P2	81.22	3.60	0.75	5.28	5.08	57.52	1.94	9.17	10.57	523985
Local Single X Arka Shringar GK-T-C-1	82.88	4.66	0.81	5.95	5.11	67.10	2.32	8.50	8.93	442520
Local Single X Arka Shringar GK-T-C-2	81.55	3.67	0.91	5.66	5.59	81.10	2.07	9.67	10.06	498538
Local Single X Arka Shringar GK-T-C-7	83.33	4.17	0.83	5.52	5.45	78.56	2.35	8.83	8.35	413933
Phule Rajani X Arka Suvasini	80.22	5.79	0.85	5.22	4.52	48.57	1.94	9.17	8.22	407324
Local Single X Shringar GK-T-C-4	81.33	4.15	0.86	5.31	4.99	67.15	1.94	9.83	8.90	441033
SE(m) ±	1.24	0.27	0.02	0.24	0.26	2.99	0.17	0.52	0.42	
C.D. at 5%	3.61	0.77	0.05	0.69	0.76	8.70	0.49	1.52	1.22	

in cv. Local Single (4.45 cm). This variation among length and diameter of floret may be due to difference in the genetic makeup of cultivars. Significantly heavier cut spike (121.43g) and maximum individual floret weight (3.12g) were noted in cv. Arka Prajwal. The variation in weight of individual and cut spike among different genotype is due to genetic factors, length and thickness of floret and spike respectively. These results are in consonance with findings of Mahawer *et al*. (2013) in tuberose.The longest vase life duration was observed in cv. Phule Rajani (11.50 days) which was at par with genotype L1P4 (11.00 days) whereas, shortest vase life was observed in genotype GK-T-C-1 (8.50 days). The variation in vase life of cut spike may be due to different genetic makeup of each tuberose genotype with prevailing environmental condition, which finally affects physiological processes like cell turgidity, water uptake through xylem tissues, water loss through transpiration, respiration and breakdown of reserved food material. Maximum number of spikes per clump and hectare was recorded in genotype L9P2 i.e. 10.57 and 523985.55 respectively. However, minimum number of spikes per clump and hectare were observed in cv. Variegated i.e. 6.85 and 339409.12 respectively.

Bulb and bulb-lets characters

Total number of bulbs per clump (11.00) and per plot (329.90) was produced more in genotype L9P2. While, minimum bulbs per clump (7.01) and per plot (210.20) was recordedin cv. Variegated. Maximum number of bulb-lets per clump was recorded in hybrid

Table 3. Performance of different tuberose genotype on bulb and bulb-lets characters

Treatment	No. of bulb per clump	No. of bulb-lets per clump	Length of bulbs (cm)	Diameter of bulb (cm)	Weight of individual bulb (g)	Weight of bulb-lets (g)	Diameter of bulb-lets (cm)	Total bulbs per plot
Local Single	8.33	17.11	6.54	2.64	23.51	7.11	1.47	249.90
Arka Shringar	10.28	23.44	6.08	3.39	39.13	7.67	1.58	308.30
Phule Rajani	9.78	25.89	6.21	2.95	35.06	7.77	1.76	293.30
Hyderabad Single	9.10	22.33	5.97	2.98	31.46	7.63	1.62	273.10
Arka Nirantara	8.55	13.45	7.08	3.72	59.91	9.16	1.68	256.40
Arka Prajwal	9.30	12.50	7.55	3.56	56.90	9.43	1.75	279.00
Variegated	7.01	21.21	6.18	3.47	40.24	5.40	1.41	210.20
Variegated X Phule Rajani L9P7	8.60	15.89	5.87	3.15	38.73	7.83	1.63	258.10
Variegated X Phule Rajani L1P4	10.78	18.67	8.09	3.76	52.21	9.28	1.85	323.50
Variegated X Phule Rajani L9P2	11.00	25.33	5.87	3.26	43.23	8.39	1.58	329.90
Local Single X Arka Shringar GK-T-C-1	9.87	17.10	6.04	3.26	34.29	9.86	1.54	296.00
Local Single X Arka Shringar GK-T-C-2	10.52	16.33	5.46	2.98	43.47	8.43	1.56	315.50
Local Single X Arka Shringar GK-T-C-7	8.48	24.09	5.65	2.91	28.30	7.05	1.48	254.50
Phule Rajani X Arka Suvasini	8.22	28.66	6.02	3.04	35.07	7.67	1.40	247.93
Local Single X Arka Shringar GK-T-C-4	9.26	23.78	5.73	3.13	43.31	8.41	1.64	277.80
SE(m) ±	0.49	1.63	0.22	0.12	1.89	0.36	0.06	14.45
C.D. at 5%	1.41	4.75	0.66	0.35	5.50	1.05	0.19	42.07

Phule Rajani x Arka Suvasini (28.66) whereas, minimum were observed in cv. Arka Prajwal (12.50). Genotype L1P4 exhibited maximum length of bulb (8.09 cm) while genotype GK-T-C-2 recorded minimum (5.46 cm). The variation in number of bulbs produced per clump might be due to genetic factor which is further modified by prevailing environmental condition and the results are in consonance with finding of Chaturvedi *et al*. (2014) and Mahawer *et al*. (2013) in tuberose.Hybrid L1P4 exhibited maximum diameter of bulb (3.76 cm) and bulb-lets (1.85 cm) while minimum diameter of bulb was recorded by cv. Local Single (2.64 cm). However, heavier bulb was produced by cv. Arka Nirantara (59.91 g) while it was lighter in cv. Local Single (23.51 g). Genotype GK-T-C-1 recorded maximum weight of bulb-lets (9.86 g). The variation in bulb weight per plant among different genotype at bulb harvesting stage might be due to the distinguished varietal genetic makeup with more leaves to improve photosynthetic activity, source sink relationship to accumulate more carbohydrate and prevailing condition.

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Original Research Paper

Soil and Plant Analysis - A Strategic Tool to Diagnose Micronutrient Imbalance in Lime and Sapota Orchard in Tablelands of Chambal Ravine Region of India

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ABSTRACT

Micronutrient imbalance in lime and sapota fruit crops result in unstable fruit yield, fruit shedding and degrade quality of the produce. A study was therefore conducted to evaluate micronutrient statusoflime and sapota orchard by analysing soil and plant samples. Soil samples were collected from surface (0-15cm) and sub-surface (15-30cm)depth representing whole orchard. At the same time, plant samples including 35-40 each for leaves and petiole samples each from lime and sapota field was also collected.Available micronutrients from soil samples were extracted using diethylenetriaminepenta acetic acid (DTPA) and it was in the order of manganese (Mn)> iron (Fe)> zinc (Zn)> copper (Cu) in both lime and sapota plantations. DTPA- extractable Zn and Cu showed low status, marginal status of Fe and sufficient level of Mn in soils of sapota plantations. In plant analysis, high concentration of Cu (869 mg kg^{-1}) and Zn (411mg kg^{-1}) was observed in lime leaves; however, in sapota crop Cu and Zn content was 8.25mg kg^{-1} and 16.7mg kg^{-1} respectively. Similarly, Fe and Mn content of lime leaves was 197 and 43 mg kg^{-1} which was slightly higher than sapota leaves that recorded 128 and 49mg kg^{-1} of Fe and Zn respectively. In sapota plants, higher Mn and Cu concentration in leaf resulted in Zn deficiency symptoms such as shortened internodes or rosette disorders of sapota plants. Thus, correcting micronutrient deficiency is pre-requisite for qualitative and quantitative fruit production in tablelands of India.

Keywords: Copper, Iron, Leaf analysis, Manganese, Micronutrient deficiency, Sapota, Zinc

INTRODUCTION

Ravines are typical examples of land degradation covering approximately 2.06 Mha and gully formation occurs in 8.31 Mhaarea in India (ICAR-NAAS, 2010). Generally, these ravine lands also known as badlands are situated near rivers and typically know for deep ravines cutting with extension overnearby arable lands (Pani and Carling 2013). Cultivation of crops is practised on the top slope called tablelands and adjacent undulating topography of gully eroded areas of Chambal ravines. Fruit crops like lime is of great significance in semi-arid regions of Rajasthan due to its hardy nature and low water requirement. However, sapota crop is recently introduced in the region and therefore information on area and production of sapota in Rajasthan is not readily available. Lime per unit production in Rajasthan is 4.0 t ha^{0-1} which is low against India's national average of 8.33 t $ha^{"1}$ (Srivastava and Shyam, 2008). The area under sapota in India is estimated to be 1.77 lakh hectares, with an annual production of 1.74 million metric tonnes and productivity of 9.91 Mg ha^{-1} (Sharma, 2015). Major sapota growing state includes Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Tamil Nadu, Kerala, Punjab, West Bengal, and Haryana.

In citrus crops necrosis, die back, chlorosis symptoms are commonly observed in the region due to nutrient deficiency resulting in decline of lime yield (Somasundaram *et al*., 2011). Nutrient imbalance in sapota crop is visualised by poorfruit setting,quality, shedding of fruitsand low productivity. Guvvali (2016)

reported thatonly 10-12% of the total fruits set, and retains until maturity in sapota crop. Lower fruit production in north western India is mainly due to nutrient imbalance or disorders which cause considerable yield reduction with huge economic loss (Somasundaram et al., 2011; Guvvali and Shirol, 2017). Subsequently, orchards provide sub optimal fruit yield with increasing gap between the amount of nutrient added and demand of crop (Srivastava and Singh, 2006). In Rajasthan, about 57, 34, 28 and 9% soils are deficient in zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu) respectively (Shukla, 2018). Micronutrients are required by plants to perform specific biochemical reactions, metabolism required for its growth and productivity. Thus in order to avoid yield and quality loss, nutrient requirements of lime and sapota crop need to be carefully monitored through soil and plant analysis for evolving nutrient management strategies. Besides soil analysis, leaf sample analysis is considered a more direct method of plant nutritional status evaluation, especially, for fruit crops as these differ from seasonal crops in nutrient requirement due to their size, population density, rate of growth and rooting pattern (Motsara and Roy, 2008). In ravine landforms, very scanty information is available on micronutrientdeficiency in fruit crops (Somasundaram *et al.,* 2011; Meena *et al.,* 2019). Therefore, the present study was conducted with the hypothesis that diagnosing micronutrient disorders of sapota crop is vital to achieve optimum fruit yield so as to improve orchard efficiency with advancing age of crop. The objective of this study was to analyse micronutrient deficiency or sufficiency level through soil and plant analysis in lime and sapota crop and its management for sustainable productivity in semi-arid regions of ravine ecosystem.

MATERIAL AND METHODS

Brief description of experimental site

The study area comprises two distinct landscapes, the agricultural tablelands and the ravenous lands adjoining Chambal river. The physiography is constituted of gently sloping (<2% slope), moderately well-drained tablelands in the immediate vicinity of ravines. The experimental orchard area is a tableland located at Research Farm, ICAR- Indian Institute of Soil and Water Conservation, Research Centre, Kota, situated at 25º 11' N latitude and 75º 51' E longitudes at an elevation of 256.9 meters above mean sea levelwith fairly levelled topography. According to Koppen's climate classification subtype, the climate of Kota is semi-arid type (Mid latitude steppe). More than 90 per cent of rainfallis received during mid-June to September with scanty showers during winter months (Nov-Dec). This region is characterized by mild and dry winters and hot summers with average rainfall of 740mm (mean of last 5 years) of which most of the rainfall is received during July month (300 mm).

Lime (Kagzi lime variety) crop planted at 4.5 x 4.5 m (row x plant) during 2001; sapota cv. 'Kalipatti' trees planted at 8 x 8 m spacing (row x plant) during 2008. The study was carried out during 2018-2019at the research farm of Indian Institute of Soil and Water Conservation, Research Centre, Kota, Rajasthan. The soils are brown to dark grey brown in colour, generally non calcareous occurring on flat gently sloping land with less than 2% slope. The soils of the region are moderately well drained fine textured soils classified as Typic Chromoustert belonging to Kota soil series. The region comprises of two diverse geology namely sandstone quartzite, silicaceous limestone and dolomite where a vast area is formed from the alluvium brought down by Chambal and its tributaries passing through the residual hillocks and gently sloping rocky plateau (Shyampura and Sehgal, 1995). The physico-chemical properties of the orchard soil are given in Table 1. The irrigation water used in sapota orchard contained bicarbonate, calcium and magnesium of 600, 66.8 and 33.5 mg l^{-1} respectively, with pH of 7.6 and electrical conductivity (EC) of 2.76 dSm^{-1}.

Orchard Management

The experimental trees were managed with uniform cultural practices as per the standard recommendations with respect to manures and fertilizers, irrigation and plant protection measures,etc. Nutrients were regularly supplied to lime crop duringcritical period of crop growth for better production and explained in Table 2. Recommended dose of nitrogen (N), phosphorus (P_2O_5), and potash (K_2O) was applied beyond a 30-cm radius from the tree trunk of lime. After ten years, fertilizer were mixed @ 750g N, 450g P and 750g K was applied to each lime plant every year. Fertilizers were applied to each tree in two or

Table 1. Soil properties under lime and sapota orchard

Soil parameters	Lime		Sapota	
Depth (cm)	**0-15**	**15-30**	**0-15**	**15-30**
pH(1:2.5)	7.74	7.51	7.81	7.62
EC (dS m^{-1})	0.57	0.62	0.55	0.59
OC (g kg^{-1})	4.7	3.6	4.5	3.4
Available nutrients (kg ha^{-1})				
Nitrogen (N)	365.7	304.4	342.3	288.7
Phosphorus (P)	17.4	13.9	15.41	11.4
Potassium (K)	436.5	412.4	386	344.5
Exchangeable cations (cmol p^+ kg^{-1})				
Na	4.9	5.7	3.2	3.5
Ca	18.2	17.9	17.7	17.8
Mg	7.6	6.1	7.5	6.4
Cation Exchange Capacity (CEC) (cmol p^+ kg^{-1})	27.6	22.5	33.4	32.8
Soil texture (%)				
Sand	27.8	29.3	27.2	29.7
Silt	42.2	41.5	44.3	42.3
Clay	30	29.2	28.5	28

three split doses when soil is moist. In sapota orchard, recommended doses of fertilizer were mixed @ 1000 g N, 500 g P and 500 g K per plant for ten-year-old sapota plants. For the application of full recommended dose of NPK,2174 g urea (1000 g N), 3125 g single super phosphate (500 g P) and 833 g murate of potash (500 g K) per plant were applied from 6th year onwards. Full amount of phosphorus, potash and half dose of nitrogen in various treatments were applied as basal dose before vegetative sprouting in the month of June. Remaining half dose of nitrogen was applied after fruit set in the month of December.

Collection of plant and soil samples

A systematic survey of lime and sapota orchard was conducted to assess the micronutrient status in 15 and 10 year old plantation covering an area of 2 and 0.4 ha respectively. For leaf sample collection, uniform area was selected in the orchard and 30 trees were selected as shown in the Fig 1. In both lime and sapota orchard, recently matured leaf were collected from north, south, east, and west quarters of thetrees (Reuter *et al.,* 1997) during September and October.About 35-40 fully developed leaf samples were collected, from which petioles samples were separated.The sampling pattern is shown in figure 1 omitting the border plants. From the 35-40 leaf samples collected, petiole samples (40) separated,

Table 2. Fertilizer management in lime and sapotaorchard

Age (years)	Nitrogen (g/plant)	P_2O_5 (g/plant)	K_2O (g/plant)
Lime			
1	75	40	75
2	150	80	150
3	225	120	225
4	300	160	300
5	375	200	375
6	450	240	450
7	525	280	525
8	600	320	600
9	675	360	675
10	750	400	750
Sapota			
1	200	200	300
2	200	200	300
3	200	200	300
4	200	200	300
5	200	200	300
6	1000	500	500
7	1000	500	500
8	1000	1000	1500
9	1000	1000	1500
10	1000	1000	1500

shade dried and grounded to fine powder for nutrient analysis. For nutrient analysis, 1g of sample was digested with tri acid mixtures (nitric, sulphuric and perchloric acid at 9:2:1). Micronutrients were estimated by directly feeding the filtered tri acid extract of the plant sample to a calibrated atomic absorption spectrophotometer using respective hollow cathode lamps for each element (Fe, Mn, Zn and Cu). Micronutrient concentration was expressed in mg kg^{-1} on dry weight basis.

Soil samples were collected from four quadrants at two different depths (0-15cm and 15-30cm) of the lime and sapota orchard (15 composite samples from each depth). Soil samples were air dried, grounded and passed through 2mm sieve and subjected to analysis of available micronutrients, namely Fe, Mn, Zn and Cu. For the soil analysis 20g soil samples was shaken with 40ml 0.005M DTPA extractant for 2 hours (Linday and Norvell, 1978). The filtered extract was directly read on AAS (model Thermo M6 series; Thermo Scientific, Waltham, Mass.) for micronutrient analysis of iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn).

RESULTS AND DISCUSSION

Micronutrient concentration in soil samples

Micronutrient concentration of soil samples under sapota plantations are shown in Table 3. Among

micronutrients, highest concentration was observed in Mn, followed by Fe, Zn and Cu. Surface soil recorded higher micronutrient concentration compared to sub-surface soil except for Mn. The micronutrient concentration in soil was crucially interpreted considering the critical limit of soil availability of DTPA extractable Zn, Cu, Mn and Fe as 0.6, 0.2, 2 and 4.5 mg kg^{-1} respectively suggested by Lindsay and Norvel (1978) and Katyal(2018).

The available Fe content in lime and sapota orchard ranged from 5.3 to 7.7 mg kg^{-1} and 3.4 to 8 mg kg^{-1} with mean value of 6.1 and 5.19 mg kg^{-1} respectively. However, sub surface mean values of DTPA Fe content in lime and sapota orchard was 5.4 and 4.59 mg kg^{-1} respectively (Table 3). Most of the soil samples showed Fe concentration below the sufficiency range (6-8 mg kg^{-1}) suggesting that Fe deficiency might arise in future in sapota plantation. Higher bicarbonate concentration of irrigation water used in fruit orchard could result in Fe deficiency. In the medium black soils of study site, Fe deficiency in lime plantations owing to increased concentration of bicarbonate ions in irrigation water was reported by Somasundaram et al. (2011). Similar report of Fe deficiency in pomegranate orchard was also reported by Gathala et al. (2004). Considering the critical concentration of soil Mn (2 mg kg^{-1}), DTPA extractable Mn concentration in both lime and sapota orchard were above sufficiency range. In lime and sapota orchard, DTPA-Mn of surface samples varied from 13.7 to 27.8 mg kg^{-1} and 8.4 to 15.2 mg kg^{-1} with mean value of 20.2 and 12.11 mg kg^{-1} respectively. In sub surface soil Mn concentration varied from 12.7 to 24.6 and 6.57 to 16.03 mg kg^{-1} with a mean value of 18.8 and 11.3 mg kg^{-1} respectively in lime and sapota orchard. In vertisol, high concentrations of both total and DTPA extractable Mn had been reported earlier by few authors (Singh *et al.,* 2006; Kumar and Babel, 2011). However, Surwase et al.(2016) also found low status of Fe and Mn in silty clay loam soils under orange crop, although soils had optimum Zn and Cu. Available Zn concentration in lime orchard was higher than that of sapota orchard. The Zn content was low to marginal level in lime orchard. The DTPA extractable Zn concentration of lime and sapota orchard varied between 0.42 to 0.97 mg kg^{-1} and 0.17 to 0.74 mg kg^{-1} respectively in surface soil. Sub surface DTPA Zn concentration varied from 0.26 to 0.81 and 0.19 to 0.72 mg kg^{-1} respectively in lime and sapota orchard. Earlier study reported Zn deficiency in fruit orchard soils of south eastern Rajasthan (Kumar and Babel, 2011; Somasundaram *et al.* 2011). Among all the four micronutrients, Cu concentration was lowest in orchard soils. The DTPA extractable Cu concentration varied between 0.082 to 0.51 mg kg^{-1} and 0.02 to 0.35 mg kg^{-1} in surface soils of lime and sapota plantations. Sub surface samples recorded lower Cu content varying from 0.05 to 0.33 mg kg^{-1} and.02 to 0.25 mg kg^{-1} in lime and sapota orchard.Soils of orchard have pH >7.5, Zn forms negatively charged ions called zincate ions (ZnO_2^{2-}) which can reduce Zn availability in soils (Katyal, 2018). Except for Mn, Fe, Zn and Cu concentration were higher in surface compared to sub surface soil. Similar results were also reported by Surwase et al. (2016) who reported higher DTPA extractable micronutrients in surface soils of orange orchards due to higher soil organic carbon and biological activity in surface layer. Thus, balanced micronutrient fertilization is necessary to correct nutrient deficiency in soils of fruits crops for doubling farmer's income.

Micronutrient concentration in plant samples (leaves and petioles)

Plant analysis is known as adiagnostic tool for managing mineral nutrition and the total nutrient concentrationin the leaf tissue provide an accurate production potential of fruit crop which mostly depends upon the supply and uptake of particular nutrient (Srivastava and Singh 2006). Leaf micronutrient concentration, like soil micronutrient content, showed wide variation (Table 4).The mean Fe content in leaves and petioles of lime trees were 196.8 and 161 mg kg^{-1} whereas, in sapota plantations it was 127 and 120 mg kg^{-1}respectively. Leaf Fe concentrations was higher than the normal Fe concentration in plant tissues. However, 12% plant samples were deficient in Fe and in case of petioles 8, 54 and 33% samples were deficient, sufficient and high in Fe concentration. Considering the optimum level of total Fe concentration in plant tissue (50-100 mg kg^{-1}), more than 62% of samples were sufficient and 18% samples recorded excess of Fe concentration. During field examination for sample collection, some trees showed interveinal chlorosis and necrotic symptoms were observed in leaves of both lime and sapota crop.(Fig. 2). Considering the normal Mn content in plant tissues (15 to 50 mg kg^{-1}), most

Table 3. Micronutrient concentration and ranges (mg kg^{-1}) in soils of lime and sapotaorchard

Soil depth (cm)	Fe	Mn	Cu	Zn
		Lime		
Surface soil (0-15)				
Min	5.3	13.7	0.082	0.42
Max	7.7	27.8	0.51	0.97
Mean*	6.1±0.23	20.2±1.1	0.28±0.025	0.69±0.032
Sub surface (15-30)				
Min	3.8	12.7	0.05	0.26
Max	7.2	24.6	0.33	0.81
Mean*	5.4±0.30	18.8±0.81	0.18±0.02	0.53±0.031
		Sapota		
Surface soil (0-15)				
Min	3.4	8.4	0.02	0.17
Max	8.0	15.2	0.35	0.74
Mean*	5.19 ± 0.33	12.11± 0.5	0.19 ± 0.03	0.38±0.05
Sub surface (15-30)				
Min	2.8	6.57	0.02	0.19
Max	6.5	16.03	0.25	0.72
Mean*	4.59 ± 0.29	11.3 ± 0.72	0.10 ± 0.02	0.42 ± 0.05

**Mean of 15 samples, ± Standard error of mean*

Table 4. Leaf and petiole micronutrient concentration and ranges (mg kg^{-1}) in lime andsapotaplantations

	Fe		Mn		Cu		Zn	
			mg kg^{-1}					
	Leaf	**Petiole**	**Leaf**	**Petiole**	**Leaf**	**Petiole**	**Leaf**	**Petiole**
				Lime				
Range	45 – 470.3	61.2 – 313.5	18.6 – 84.9	5.64 – 55.3	45 – 1588	63 – 941	46.3 – 650.8	68 – 473.2
Mean*	196.8 ± 22.7	161.1 ± 20.1	42.87 ± 3.58	18.6 ± 2.2	869 ± 89.6	526 ± 57.9	411 ± 39.8	293 ± 21.3
				Sapota				
Range	89.41 – 231.24	33.52 – 284.51	22.2 – 97.61	11.31 – 69.26	4.35 – 19.78	2 – 18.48	0.62 – 48.12	0.54 – 36
Mean*	127.66 ± 6.56	119.8 ± 12.23	48.84 ± 3.98	33.46 ± 2.92	8.25 ± 0.77	9 ± 0.89	16.66 ± 2.25	13.01 ± 2.1

**Mean of 30 samples, ± Standard error of mean*

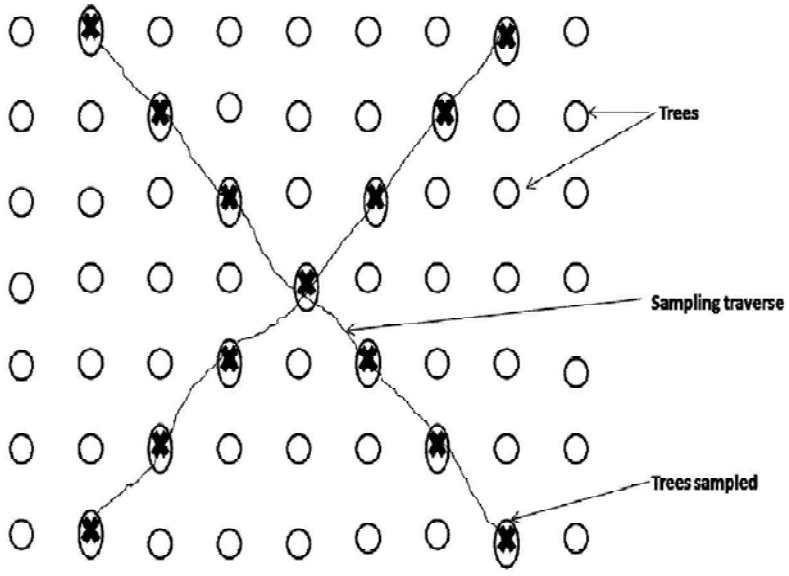

Fig. 1. Collection of representative plant samples from sapotafruit orchard

Fig. 2. Iron deficiency in lime (a) and sapota (b) plants

of the leaf samples showed deficient to sufficient status. The average Mn concentration in lime varied between 43 and 19 mg kg^{-1} and in sapota was 48 and 33 mg kg^{-1} respectively for leaves and petioles samples (Table 4). Leaf samples registered 64% sufficientand 36% excess concentrationof Mn, whereas in petiole samples 8% samples were deficient. Excessive Mnconcentration in plant tissues can alter various processes such as enzyme activity, absorption, translocation and utilization of other mineral elements (Ca, Mg, Fe and P), causing oxidative stress (Ducicand Polle, 2005; Lei *et al.,* 2007). Mean Cu concentration in lime leaf samples varied between 869 and 526 mg kg^{-1} in leaf and petiole sample. In contrast, lower Cu concentration values of leaf samples were recorded in sapota plants. Copper concentration of sapota leaf samples varied from 4.35 to 19.78 mg kg^{-1} with a mean value of 8.25 mg kg^{-1}. The Cu concentration of petiole samples varied from 2 to 18.48 mg kg^{-1} with a mean value of 9 mg kg^{-1} (Table 4). The Cu concentration range in plant samples vary from 5 to 16 mg kg^{-1}. Based on the normal range of

Cu (100 mg kg^{-1}) in plants, lime plants samples showed excessive total Cu content. This was mainly attributed to the spray of Cu based fungicide to control fungal disease in orchard. Some plants with young leaves showed chlorosis symptoms due to Cu toxicity. However, in sapota crop, 13 and 21% of leaf and petiole samples were recorded as deficient and 79% were sufficient in Cu. However, Cu deficiency symptoms (dieback of apical buds) in sapota were observed during plant sampling in some sapota trees. Some common symptoms included premature defoliation and die back of twigs occurred. The tip of the twigs developed multiple buds which died soon. Zinc concentration of lime crop for leaf and petiole varied from 46.7 to 650.8 mg kg^{-1} and 68 to 473.3 mg kg^{-1} respectively with a mean value of 411 and 293 mg kg^{-1}. In sapota crop, the Zn concentration of leaf and petiole samples varied between 0.62 - 48.12 and 0.54- 36.0 mg kg^{-1} with a mean value of 16.7 and 13.0 mg kg^{-1} respectively (Table 3). Wide difference between Fe content in sapota and lime was observed in the study. Based upon the Zn concentration (<20 mg kg^{-1}), more than 73% samples were sufficient in Zn content in lime orchard. However, in sapota crop 50% of leaf samples were deficient where as 42% recorded optimum to highand 8% had excess Zn status.In petioles, 67 and 33% samples recorded deficiency and sufficiency of Zn respectively in sapota plants. High Zn concentration in lemon orchard was also reported by Somasundaram et al. (2011) where more than 88% leaf samples recorded higher Zn content. They suggested accumulation of excess of Cu in leaf resulted in greater accumulation of Zn to maintain nutrient balance.

Soil and foliar method of fertilizer application is utilized for sapota crop.Foliar application of micronutrients is considered as quickest means to correct nutrient deficiency in fruit trees. In sapota crop, Fe, Mn, Zn and Cu deficiency can be corrected by foliar spray ferrous sulfate (0.2 to 0.4%), manganese sulphate (0.3%), zinc sulphate (0.2 to 0.5%) and copper sulphate (0.1%) (Satyagopal*et al.,* 2015).Copper based fungicide (copper oxychloride with 3g l^{-1} of water) sprays will be helpful in correcting the Cu deficiency of sapota. Possibility of micronutrient response to its application in crops could be as high as 90% for very low, 60 to 90% for 'low' and 30 to 60% for 'optimum' levels of extractable micronutrients (Cooper and Abi-Ghanem, 2017). Thus,identifying the deficiencies of micronutrients timely and application of balanced fertilizers at correct time can enhance crop production and quality of fruits.

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Original Research Paper

Identification of NBS-LRR Resistance Gene Analogues (RGA) from Rose (IIHRR13-4) Resistant to Powdery Mildew (*Podosphaera pannosa* (Wallr.:Fr.) de Bary)

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ABSTRACT

Resistance is the best strategy to manage powdery mildew (*Podosphaera pannosa* (Wallr.:Fr.) de Bary) of rose. Identification of resistant genes (R genes) from plant species will help in breeding programs. Nucleotide Binding Site - Leucine Rich Repeats (NBS-LRR) is a major class of R gene family in plants. This study reports the identification and molecular characterization of resistance gene analogues from roses maintained at ICAR-Indian Institute of Horticultural Research (IIHR). The powdery mildew resistant line IIHRR13-4 was compared with the susceptible commercial cultivar, konfetti. PCR based approaches with degenerative primers based on different conserved motifs of NBS-LRR were employed to isolate resistance gene analogues (RGAs) from rose. Eleven RGAs (IIHRR13-4R1, IIHRR13-4R2, IIHRR13-4R3, IIHRR13-4R4, IIHRR13-4R5, IIHRR13-4R6, IIHRR13-4R7, IIHRR13-4R8 IIHRR13-4R9 and IIHRR13-4R10) were identified from powdery mildew resistant germplasm line, IIHRR13-4, based on the sequence and similarity to RGAs from rosaceae family and other crops. The major similarity to rose RGAs reported are from *Fragaria vesca, Rosa* hybrid cultivar*, Prunus* and *Rosa chinensis.* RGAs isolated from IIHRR13-4 belonged to Toll Interleukin Receptor (TIR)-NBS-LRR and Non-TIR-NBS-LRR RGAs (Lecine Zipper (LZ) type). Different motifs of RGAs identified were P-loop, RNBS A, kinase 2, kinase 3a, RNBS-D and GLPL of NBS domain. This study reports the existence of resistance at genetic level in powdery mildew resistant genotype IIHRR13-4. These RGAs will be useful for mapping and characterization of R genes in IIHRR13-4 and breeding for improved powdery mildew resistance in roses.

Key words: Nucleotide Binding Site-Leucine Rich Repeats (NBS-LRR), *Podosphaera pannosa,* Powdery mildew, Resistance Gene Analogues (RGA) and Rose.

INTRODUCTION

Two major events involved in defense mechanism are recognition of pathogen attack and induction of defense responses from plants against the pathogen.The defense response against particular pathogen is triggered by an interaction between R gene from the host and avirulence gene product of pathogen which restricts the pathogen invasion (Flor, 1971; Holt *et al.,*2000). R genes form a diverse group of related sequences that are widely distributed in plant genome. There are five classes of *R* genes based on their structural characteristics of predicted protein structure and majority of these *R*-genes belong to nucleotide-binding site (NBS) and leucine-rich repeats (LRRs) groups (Ellis and Jones, 1998; Hammond-Kosack and Jones, 1998; Hattendorf and Debener, 2007a; Hattendorf and Debener, 2007b). Putative NBS domains are concerned with signalling and they are characterized by several highly conserved motifs, *viz.* P-loop, Kinase-2 and Gly-Leu-Pro-Leu (GLPL) motifs. Structural domains of LRRs are involved in protein-protein interactions and pathogen recognition (Belkhadir *et al.,* 2004; Ellis *et al.,* 2003; Yung, 2000).

The NBS-LRR domain is classified into two groups based on their N-terminus. First is the amino terminal toll interleukin receptor (TIR)-NBS-LRR and the second is without TIR region known as Non-TIR-NBS-LRR class. TIR-NBS-LRR is characterized by their similarity to toll receptor in *Drosophila* and interleukin 1 receptor of mammals and Non-TIR groups has a leucine zipper (LZ) or coiled coil(CC) motif instead of TIR. Non-TIR group is widely distributed in both monocots and dicots while TIR group is rare in cereals and grasses. So far several R genes have been cloned in different crops(Collier and Moffett, 2009; Dangl and Jones, 2001; Ellis *et al.,* 2003; Hammond-Kosack and Jones, 1997; Jones, 2001; Liu *et al.,* 2007).

Resistance gene analogues are putative derivatives of R genes. The highly conserved domains provide unique identity to R genes in plant genome (Hammond- Kossack *et al.,* 1996). The conserved motifs of NBS-LRR can be used to isolate resistance genes from plants by PCR based approach with oligonucleotide degenerate primers. RGAs have been isolated from wide varieties of plant species (Hattendorf and Debener, 2007a). Characterization of RGA is an effective strategyto identify R genes and develop markers for disease resistance (Mayer *et al.,* 1999; Hattendorf and Debener, 2007a; Biber *et al.,* 2010; Backiyarani *et al.,* 2013; Lei *et al.,*2014; Sekhwal *et al.,* 2015).

Rose is one of the economically important ornamental crops from Rosaceae family and it has the highest economic impact in the world. Rose flower industry comprises of local and international marketing of cut flowers, loose flowers, scent, oil and medicines. The worldwide estimated production of rose is 18 billion cut stems, 60-80 million potted-rose and 220 million for landscape purposes (Debener and Byrne, 2014). Apart from roses, other economically important members of Rosaceae family are apples (*Malus*), strawberries (*Fragaria*), stone fruits like peach, plum, apricots (*Prunus*) and pears (*Pyrus*). Most of the species of Rosaceae family are woody perennials. There is a wide range of pathogens *viz.,* fungi, bacteria, virus, phytoplasma and nematodes that attacks rose plants causing its death and thereby reducing the marketability of roses. Powdery mildew is one of the most damaging diseases of Rosaceae family (Xu *et al.,* 2007). *Podosphaera pannosa* (Wallr.:Fr.) de Bary is an obligate (biotrophic) pathogen (order Erysiphales, phylum Ascomycotina) that inhabits numerous economically important plants.Severe powdery mildew infection reduces greenhouse cut flower production (Leus*et al.,* 2003; Xu *et al.,* 2005, Debener and Byrne, 2014).

Characterization of R genes from wild varieties will help in obtaining disease resistant cultivars of roses (Hattendorf *et al.,* 2004; Hattendorf and Debener, 2007b). So far,only two R genes have been characterized in rose *viz*. Rdr 1 for black spot resistance (Von Malek *et al.,* 2000; Ayana *et al.,* 2011) and RPP1 for powdery mildew resistance (Linde and Debener, 2003; Linde *et al.,* 2004) from Institute for Ornamental Plant Breeding, Germany. Diseaseresistance loci have been identified and mapped in apple (Calenge*et al.,* 2005; Perazzolli *et al.,* 2014; Pessina *et al.,* 2014), strawberries (Zamora *et al.,* 2004), peach (Dirlewanger *et al.,* 1996, 2004; Quarta *et al.,* 2000; Dettori *et al.,* 2001; Lalli *et al.,* 2005), *Arabidopsis thaliana* (Aarts *et al.,* 1998; Mayer *et al.,*2003) and soybeans (Yu *et al.,*1996).

Study of R gene and its locus can help to reveal their exact function in pathogen recognition followed by defense and their evolution among particular plant species. This can be used to develop novel disease management strategies (McHale *et al.,* 2006). In this context, the best desirable strategy for disease management is development of resistant varieties as it can be a cost effective alternative for chemical method of disease management. Powdery mildew resistance was observed in rose genotype (IIHRR13-4) during field evaluation but mechanism of disease resistance was unknown. The objective of this study was to identify and characterize resistance gene analogues from IIHRR13-4.

MATERIALS AND METHODS

Rose genotypes used in this study were obtained from Division of Ornamental crops, ICAR- Indian Institute of Horticultural Research (IIHR). Eight rose genotypes (Table 1) were used to identify resistant gene analogues based on earlier reports. Among those genotypes selected, IIHRR13-4 was found to be resistant, *Rosa indica* was immune and remaining were highly susceptible to powdery mildew.

Genomic DNA was isolated from coppery red rose leaves by CTAB method (Doyle and Doyle, 1987). Six sets of degenerative primers (Table 2) were used

Table 1. Rose genotypes maintained at IIHR used in the study

S.No.	Rose genotypes used in the study	Field assessment of Powdery mildew disease
1	R1 - IIHRR13-4 (PMR)	Resistant
2	R2 - Rosa indica	Resistant
3	R3 - 11-3	Susceptible
4	R4 - First Red	Susceptible
5	R5 - Dean De Pointers	Susceptible
6	R6 - Fantasy	Susceptible
7	R7 - Konfetti	Susceptible
8	R8 -13-24	Susceptible

Table 2. List of primers used in this study for the amplification of RGAs

S.No.	Primer name	Sequence (5'-3')	Motif
1	RS1 F	GGIGGIATIGGIAAAACIAC	GGMGKTT
	RS1 F	RAARCAIGCDATRTGIARRAA	FLHIACF
2	RS3 F	GGIGTIGGIAAIACI	GGVGKTT
	RS3 R	RAARCAIGCDATRTGIARRAA	FLHIACF
3	RS4F	GGIGGIATIGGIAAAACIAC	GGMGKTT
	RS4R	RAARCAIGCSATRTCIARRAA	FLDIACF
4	RS10F	GGIGGIATIGGIAAAACIAC	GGMGKTT
	RS10R	YTCIGGRAAIARIGCRCARTA	YCALFPE
5	RS11F	GGIGGIYTIGGIAARACIAC	GGLGKTT
	RS11R	YTIIGGRAAIARIGCRCARTA	YCALFPE
6	RS12F	GGIGGIGTIGGIAAIACI	GGVGKTT
	RS12F	YTCIGGRAAIARIGCRCARTA	YCALFPE

for amplification of RGAs. RS1 RS2 and RS3 primer pairs were specific for TIR-NBS-LRR type and RS10, RS11 and RS12 were for LZ type (Hattendorf and Debener, 2007a). PCR assays were performed with genomic DNA in a total volume of 25 µl containing10µM of forward and reverse primers (Sigma Aldrich, India), 3 units of *Taq* polymerase and 2.5 mM*Taq* buffer (Genei, Bengaluru, India).PCR reaction was performed in Eppendorf thermal cycler with initial denaturation at 94^{0}C for 3 min, 35 cycles of 94^{0}C for 1 min, 42^{0}C for 1 min, 72^{0}C for I min followed by final extension step at 72^{0}C for 10 minutes (Hattendorf and Debener, 2007a).

Agarose gel (1.5%) electrophoresis was carried out to view and purify the PCR products.The amplified products were further purified by Nucleospin gel and purification kit by Macherey-Nagel GmbH & Co. KG, Germany. The purified products were ligated into P$^{TZ\ 57R/T}$vector. Cloning was done with Thermo-Fisher Scientific InsTA clone PCR cloning kit (Thermo-Fisher Scientific Baltics UAB, Lithuania). Transformed colonies were selected for plasmid isolation and presence of insert was confirmed by plasmid PCR. Cloned plasmids were sequenced for further identification (Hattendorf and Debener, 2007a).

RGA sequences were analysed using NCBI Vecscreen (https://www.ncbi.nlm.nih.gov/tools/vecscreen/) and BioEdit (Hall, 1999). Sequence similarity search was done using NCBI Blast (https:/

/blast.ncbi.nlm.nih.gov) for RGA sequences. Amino acid sequences were generated using Expert Protein Analysis System (ExPASy) (https://web.expasy.org/translate/) translating tool and conserved motifs were identified by amino acid sequence alignment. Phylogenetic tree was constructed with MEGA-6 (Tamura*et al.,* 2013) with bootstrap analysis with 1000 replications. Sequences of selected RGAs were deposited at NCBI-Gen Bank database.

RESULTS AND DISCUSSION

Genomic RGAs were identified from rose genotypes with various degenerate primer sets with an amplified product of 550-700 bp length (Fig.1).From the different primer sets used only RS1 and RS10 primer combinations amplified in rose plants irrespective of susceptibility or resistance. The PCR amplified products were cloned and 41 colonies were picked for confirmation and sequence analysis. Finally ten RGA clones were selected from IIHRR13-4 by RS1 primer combination and three RGAs identified respectively from IIHRR13-4, Konfetti and First red by RS10 combination. These RGAs were finalised based on the sequence length and similarity to RGAs from Rosaceae family and other plant RGAs. The RGAs sequences with internal stop codons were eliminated. Total eleven RGAs were confirmed from IIHRR13-4 after sequence analysis and similarity to other plant RGAs. Other two RGAs were confirmed

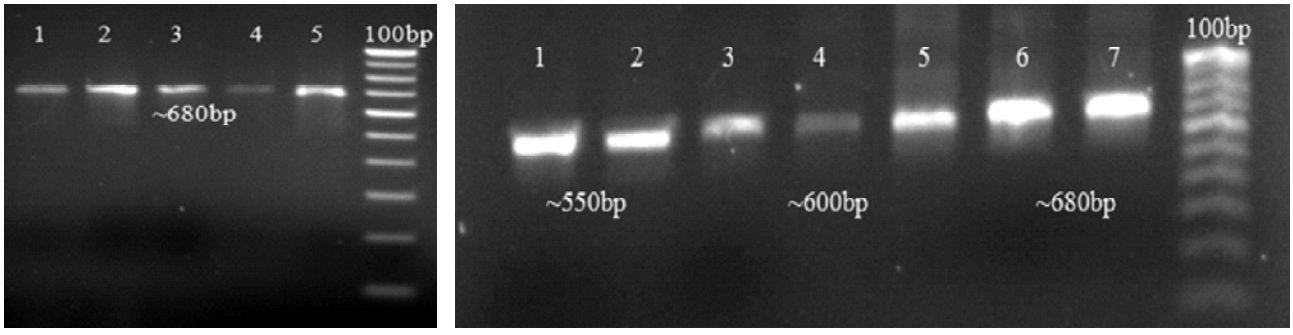

Fig. 1. Agarose gel electrophoresis confirming the amplification of Rose RGAs fragments, 1-5, 1 -7 - Rose RGAs. RGA fragments amplified at 550-700bp.

from powdery mildew susceptible First red and konfetti. All the amplified PCR products were purifiedand cloned in P^{TZ} vector. The sequence homology of rose RGAs to other plant proteins and other known R genes was confirmed by NCBI BLAST search. The list of proteins present in other plants belonging to rosaceae family to which close similarity was observed for the RGAs identified in the present study is given in Table 3.

The R gene sequences retrieved from NCBI database used in the phylogenetic analysis are listed in the Table 4. RGAs identified in the present study showed similarity to both TIR class of NBS-LRR RGAs and Non-TIR (LZ) class of NBS-LRR. RGAs identified from susceptible varieties showed similarity to RGAs of *Rosaceae* family but some of them excluded after amino acid translation because of the presence of internal stop codons. Finally thirteen RGA sequences *viz.,* IIHRR13-4R1, IIHRR13-4R2, IIHRR13-4R3, IIHRR13-4R4, IIHRR13-4R5, IIHRR13-4R6, IIHRR13-4R7, IIHRR13-4R 8, IIHRR13-4R9, IIHRR13-4R10, IIHRR13-4RS10 (IIHRR13-4), IIHRSFRR10 (First Red) and IIHRRRIRS10 (*Rosa indica*) were identified in this study.

Multiple sequence alignment identified highly conserved amino acid motifs present in the RGAs of IIHRR13-4. Multiple sequence alignment of IIHRR13-4 RGAs was performed with other R genes of Rose, *Arabidopsis, Solanum*, *Nicotiana*, *Malus*, *Prunus, Fragaria* and apoptotic protease activating factor(APAF) gene (Fig. 2). Six highly conserved amino acids motifs of NBS domain identified were P-loop, RNBS (Resistance nucleotide binding Site)-A, Kinase-2, Kinase-3a, RNBS-D, and GLPL. NCBI CD-search (Conserved Domain software) was used to find and confirm the conserved domains of RGAs and presence of nucleotide binding domain (NBARC domain) and LRR3 super family domain. The selected RGAs were further analysed for their phylogenetic relationships among Rosaceae family and other plant R genes.

Table 3. List of RGAs identified from the present study, their GenBank accession numbers and sequence similarity with RGAs of other Rosaceae family

Rose RGAs	NCBI Accession number	Protein to which closer similarity observed	Plant species	Identity (%)
IIHRR13-4R1	MG958641	TMV resistance protein N-like isoform X2	*Fragaria vesca* sub sp. *vesca*	77
		Putative NBS-LRR resistance protein	*Rosa* hybrid cultivar	80
IIHRR13-4R2	MG970527	Putative NBS-LRR resistance protein	*Rosa* hybrid cultivar	98
		Putative winged helix-turn-helix DNA-binding domain, leucine-rich repeat domain	*Rosa chinensis*	
IIHRR13-	MG970528	Putative transcription factor WRKY family	*Rosa chinensis*	94
4R3		Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	82
IIHRR13-	MG970529	Putative transcription factor WRKY family	*Rosa chinensis*	100
4R4		Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	83
IIHRR 13-4R5	MG970530	TMV resistance protein N-like	*Fragaria vesca* subsp. *vesca*	67
		TMV resistance protein N-like	*Prunus avium*	62
IIHRR13-	MG970531	Putative NBS-LRR resistance protein	*Rosa* hybrid cultivar	87
4R6		Putative toll-like receptor, P-loop containing nucleoside triphosphate hydrolase	*Rosa chinensis*	88
IIHRR13-	MG970532	Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	100
4R7		TMV resistance protein N-like	*Fragaria vesca*su bsp. *vesca*	73
IIHRR13-	MG970533	Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	83
4R8		Putative transcription factor WRKY family	*Rosa chinensis*	72
IIHRR13-	MG970534	Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	88
4R9		Putative transcription factor WRKY family	*Rosa chinensis*	75
IIHRR13-4R10	MG970535	NBS-LRR resistance protein	*Rosa* hybridc ultivar	88
		Putative TIR-NBS-LRR resistance protein	*Rosa* hybrid cultivar	84
IIHRR13-4RS10	MK704433	Putative disease resistance protein RGA3 and RGA4	*Rosa chinensis*	74
IIHRSFR R10 (First	MK704434	Putative disease resistance protein RGA1, RGA2, RGA3 and RGA4	*Rosa chinensis*	86, 85, 86 and 90
Red)		Isolate F11P2-4F NBS-LRR resistance protein gene	*Rosa* hybrid cultivar	87
IIHRRRI RS10	MK689860	Putative disease resistance protein RGA2 RGA3 and RGA4	*Rosa chinensis*	97, 88 and 87
(*Rosa indica*)		NBS-LRR resistance protein gene	*Rosa* hybrid cultivar	88

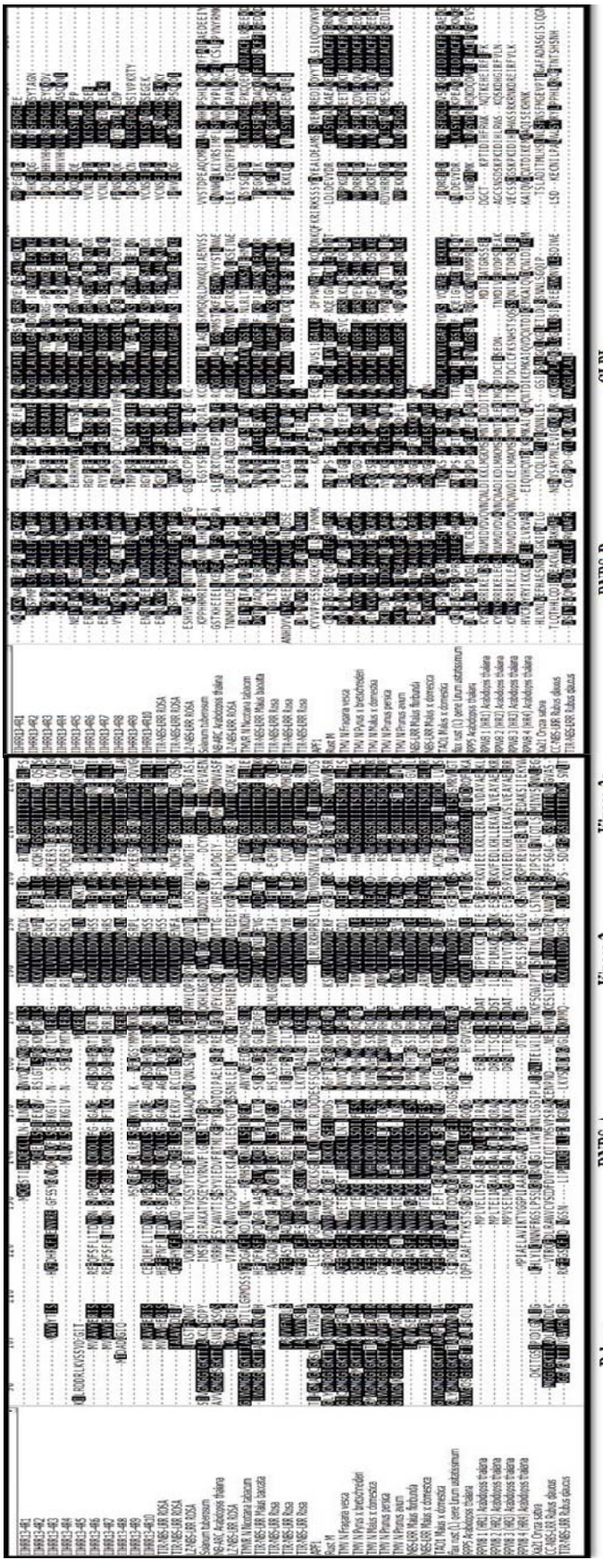

Fig. 2. Multiple sequence alignment showing conserved motifs in the amino acid alignment of IIHRR13-4 RGAs with other R genes of Rose, *Arabidopsis*, *Solanum*, *Nicotiana*, *Malus*, *Prunus*, *Fragaria* and APF gene. Highly conserved amino acids of motifs (P-loop, RNBS-A, Kinase-2, Kinase-3a, RNBS- D, and GLPL) are shaded

Table 4. List of R genes retrieved from NCBI database used in the phylogenetic analysis that were compared with RGAs of rose

Sl.No.	R gene	Host	Accession No.
1	RGC1	*Solanum tuberosum*	AF266747.1
2	NB-ARC domain disease resistance protein	*Arabidopsis thaliana*	NP187360.1
3	Virus resistance (N) gene	*Nicotiana glutinosa*	U15605.1
4	TIR-NBS-LRR type R protein 7	*Malus baccata*	AAQ93075.1
5	TIR-NBS-LRR resistance protein	*Rosa* hybrid	AM075235.1
7	Rust resistance protein M gene	*Linum usitatissimum*	U73916.1
8	RPP5	*Arabidopsis thaliana*	NM114316.3
9	L6	*Linum usitatissimum*	U27081.1
10	RPW8.1, RPW8.2	*Arabidopsis thaliana*	AF273059.1
11	Xa21	*Oryza sativa*	AY885788.1
12	LZ-NBS-LRR resistance protein	*Rosa hybrid*	AM075248.1
13	TMV resistance protein N-like	*Fragaria vesca* subsp. *vesca*	XM011459053.1
14	TMV resistance protein N-like	*Prunus avium*	XM021944693.1
15	Apoptotic protease activating factor 1 (APAF1) mRNA (out group)	*Homo sapiens*	AF149794.1

Phylogenetic tree was constructed using MEGA-6 software to identify genetic relationship and diversity among rose RGAs and other known plant R genes from *Rosaceae* and other species (Table 3). Different R genes from different crops available in GenBank were selected to analyse the phylogenic relationship among R genes. The phylogeny was constructed using Neighbour Joining method with1000 boot strap replications (Fig. 3). The two distinct groups of RGAs, TIR and LZ types were clearly separated in phylogram. Apoptotic protease activating factor 1 (APAF1) related to human cell death was used as out-group to construct phylogentic tree because of its NBS domain with greater protein sequence similarity to NBS-LRR proteins of plants. Highest degree of similarity of IIHRR13-4 RGAs was observed with *Malus domestica*, *Rosa chinensis*, *F. vesca* and *Rosa* hybrid cultivar. Comparative sequence analysis specifies the clustering of rose RGAs with certain Rosaceae RGAs. IIHRR13-4R2, IIHRR13-4R3, IIHRR13-4R4, IIHRR13-4R8 andIIHRR13-4R9 clustered together with TIR-NBS-LRR *Rosa* hybrid cultivar. IIHRR13-4R7 clustered with TMV resistance N like protein of *Fragaria vesca*. IIHRR13-4R5 clustered with TMV resistance N like protein of *Prunus avium* and TIR-NBS-LRR *Rosa* hybrid cultivar. IIHRR13-4R1 grouped with TMV of *Nicotiana tabaccum*. Other RGA IIHRRCONRS10 grouped with LZ-NBS-LRR of Rosa hybrid cultivar, NBARC of *Arabidopsis thaliana*, RGC1 of *Solanum* and RGA 4 of *Rosa chinensis*. RGA identified from First Red IIHRSFRRS10 were grouped with Xa21 of *Oryza sativa*. Neighbour Joining phylogenetic tree confirms the similarity of TIR and LZ type of RGAs from IIHR rose genotypes to RGAs of Rosaceae family.

Identification of RGAs can assist in breeding program for superior disease resistance because of the specific gene feature. RGA fragments are generated from different motifs of conserved domains of R genes that code for resistance against particular pathogen. RGA based markers that linked to R genes are more specific and facilitate selection of desirable disease resistant lines (Ellis *et al.,* 2000; Biber *et al.,* 2010, Hattendorf and Debener, 2007a). PCR based approach with degenerate primer is an efficient method for identification and cloning of RGAs from plants (Hattendorf and Debener, 2007a; Vossen *et al.,* 2013; Yu *et al.,* 1996).

PCR based approach was used in the study to identify potential RGAs linked to powdery mildew resistance in IIHR line of rose (IIHRR13-4) for which molecular basis for mechanism of disease resistance had not been earlier identified. Based on previous observations under field evaluation, genotype IIHRR13-4 that wasfound resistant was selected along with wild rose species *R. indica* which was

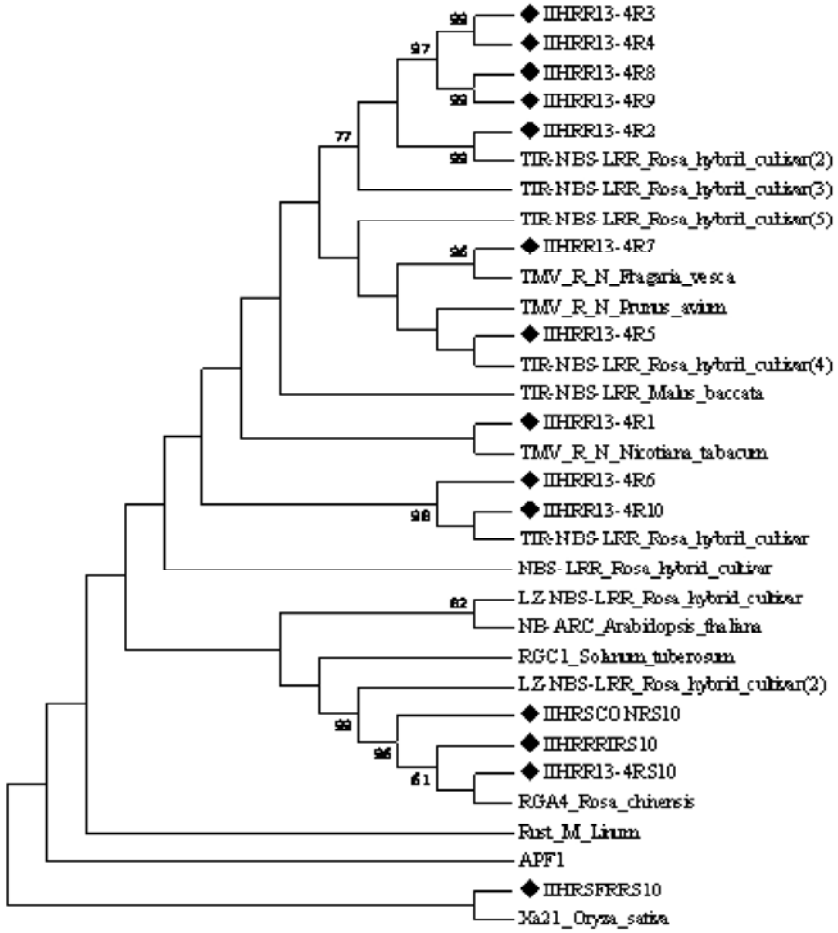

Fig. 3. The phylogenetic tree constructed with Neighbor-Joining method based on the amino acid sequences of rose RGAs, along with RGAs and R genes from *Rosaceae* and other plant species. The bootstrap values obtained from 1000 replications Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed.

immune to powdery mildew. Molecular profiling of RGAs with NBS conserved motifs helps in diversity studies of R gene families and identification of molecular markers for disease resistance (R) genes (Vossen *et al.,* 2013). Six degenerate primers of conserved NBS motifs used in the present study were selected from Hattendorf and Debener (2007) for isolation of genomic RGAs from rose. RS1, RS2 and RS3 primers helped in identifying TIR class of genomic RGAs and RS4, RS5 and RS6 aided amplification of non-TIR class (LZ) of RGAs from rose.The difference in each primer set relies on single amino acid in the motif sequence. Primers of P-loop motif sequence (GG.GKTT) differed in the third amino acid of the motif (GG (M/V/L) GKTT). In the same way, primers designed on NBS-IX motif also differed in single amino acid change. Motif sequence of NBS-IX was FL.IACF and change was on the third amino acid (FL(H/D)IACF). These variation in primer sequences helps to identify complete set of RGAs present in the rose genome.

Genomic RGAs isolated from rose belonged to TIR and non - TIR class (LZ) of NBS-LRR resistance genes. Non-TIR classes of RGAs are present in monocotyledons (wheat, rice, maize) and di-cotyledons but TIR class of RGAs mostly found in dicotyledonous plants. Hattendorf and Debener (2007a) reported that rose genome contained more TIR class of RGAs rather than non-TIR class with respect to number and diversity of RGAs in rose genome. The clear distinction between TIR and LZ class is based on the motif sequences of NBS domain (Xu *et al.,* 2005).

RGAs identified from all genotypes of rose (Table 1) irrespective of powdery mildew resistance and susceptibility. Previous investigations showed constitutive expression of RGAs but their transcription was induced by different factors in different crops

(Hammond-Kosack and Jones, 1997). Expression of RGAs specifically after pathogen attack explains their role particularly in defense response. Hattendorf and Debener (2007b) explained relative expression of RGAs in rose by checking the expression of RGAs in *Diplocarpon rosae* (black spot disease) inoculated and control rose leaves. Enhanced expressions of TIR-RGAs were observed in rose after black spot inoculation than untreated control, indicating direct function of TIR-RGAs in disease resistance against black spot. Genomic RGAs isolated from powdery mildew susceptible lines of rose (Table 1) were excluded because of presence of premature stop codons. RGA isolated from First Red (IIHRRSFRRS10) was without stop codons and that grouped with Xa21 of *O. sativa* in the phylogram. The genomic RGA of First Red probably may not express during powdery mildew infection process to prevent the disease and leads to the susceptibility to powdery mildew. NCBI blast results indicate that IIHRSFRR10 was showing similarity to *Rosa* hybrid cultivar NBS-LRR resistance protein pseudogene by 86.59%. Therefore, the RGA was identified from First Red may be pseudogene. These results gave information regarding the expression levels of RGAs in rose with respect to disease resistance. Some of the RGAs were identified as pseudogenes in many crops (potato, *Arabidopsis,* cotton, lotus and tomato). Non-functional pseudogene paralogs of R-genes (*Xa21*, *Cf9*, *Pto* and *Dm3*) were identified and have strong identity with other NBS protein but their sequences are short and presence of premature stop codons was observed. Pseudogenes are assumed to be involved in the *R* gene evolution process (Sekhwal *et al.,* 2005; Song*et al.,* 1997).

The NCBI blast results showed that IIHRR13-4R4 were 100% similar to putative transcription factor WRKY family of *R. chinensis*. WRKY super family of plant transcription factors plays important role in plant defense. The plant immune receptors detect pathogen effector proteins through WRKY transcription factors and activate defense (Phukan*et al.,* 2016). The NBS-LRR usually connects with other protein domains. *Arabidopsis* RRS1-RNB-LRR protein carries C-terminal WRKY DNA binding domain that enables formation of receptor complex with another NBS-LRR protein, RPS4 that helps in detection of bacterial effectors. This ligand-receptor binding initiates activation of defense mechanisms this indicates that the plants defense depends on intra-cellular immune receptors.

The phylogenetic tree showed clear separation of two different classes of NBS-LRR RGAs (TIR and LZ). Resistance gene analogues identified in the present study were closely related to *Rosa* hybrid cultivar, *R. chinensis* and other species of *Rosaceae* family (*Fragaria, Prunus* and *Malus)*. The conserved motifs identified from RGAs of IIHR13-4were similar to *Rosa multiflora* hybrid (Hattendorf and Debener, 2007a), chestnut rose (Xu *et al.,* 2005), strawberry (Zamora *et al.,* 2004) and other plant NBS-LRR R genes. These conserved motifs of NBS domain codesfor ATP or GTP binding proteins and hydrolysis activity (Phukan *et al.,* 2016; Saraste *et al.,* 1997; Xu *et al.,* 2005). IIHRR13-4 RGAs were showing more homology with TIR-NBS-LRR disease resistance gene of *Rosa hybrid* and *TMV*- N like disease resistance gene of *F. vesca*. The two major clades observed were TIR group and non-TIR group (LZ-NBS-LRR) of RGAs. TIR group of NBS-LRR genes of *Rosa* hybrid was clustered together with IIHRR13-4 RGAs. Phylogenetic studies revealed the relationship between RGAs identified from rose and other R genes/RGAs of Rosaceae and other family also.

Multiple sequence alignment by ClustalW showed that different motifs of rose RGAs were P-loop, RNBS-A, kinase 2, kinase 3a, RNBS-C and GLPL of NBS domain. Rose TIR - RGAs carried an aspartic acid residue (D) at the end of kinase 2 region (NBS III). Usually LZ-RGAs (Leucine Zipper) possess tryptophan residue (W) instead of aspartic acid residue. Other R genes with LRR3 super family domain reported earlier were NBARC domain of putative rp3 protein from *Zea mays*, RPP5 disease resistance protein of *A. thaliana* and NBARC domain of R genes of Solanaceae, *O. sativa*, Rosids*, Vitis vinifera* (RX-CC-NBARC), *Malus domestica*, *Capsicum annuum* and *Citrus* sp. LRR domain of known R genes (rp3, RPP5, NBARC) was present in IIHRR13-4 RGAs. The presence of conserved domain LRR 3 super family will give unique identity to RGAs identified from the genome of powdery mildew resistance germplasm line IIHRR13-4.

Several RGAs present in each plant genome may or may not link to resistance. The IIHRR13-4 was found resistant to powdery mildew in field evaluations. The

powdery mildew resistant line IIHRR13-4 was studied along with other resistant and susceptible rose lines. The RGAs were identified from all rose genotypes and some were excluded because of stop codons. Finally eleven RGAs selected from the IIHRR13-4 that might be linked to resistance mechanisms. This is the initial study on resistance mechanisms in the rose line IIHRR13-4 against powdery mildew disease. The expression analysis studies (N. K Chandran, Personal communication) revealed more expression of RGAs and resistance related transcription factors in IIHRR13-4 compared to susceptible cultivar konfetti upon powdery mildew infection. The comparison between IIHRR13-4 and konfetti revealed that expression level of RGA transcripts might not be sufficient to elicit resistance in konfetti. This indicates the importance of proper and required expression of disease resistance gene against particular pathogen. This study indicates that several R gene candidates (RGAs) are present in rose plants but only few are linked to disease resistance. These RGAs identified from IIHRR13-4 might be putative derivatives for R gene(s) against powdery mildew and may help in future research on mapping and characterization of R genes from IIHRR13-4.

Map based cloning approach is used to isolate R genes and that requires high-density genetic maps. Genome-wide RGA identification would assist to develop markers and mapping resistance genes and further possible cloning.

CONCLUSION

The present study explains the putative molecular mechanism behind resistance to powdery mildew resistance in IIHRR13-4 through different motifs present in the NBS domain of NBS-LRR group of R genes. This can be used as a basis for further studies related to molecular mechanism of resistance since RGAs are potential candidates for functional resistance gene and marker development in various breeding programs. The results of present study will help to develop RGA based markers linked to powdery mildew resistance in rose and this will help in rose resistant breeding and disease resistance screening programs using R gene profiling. Further study related to expression level of RGAs will provide more insight into molecular basis of disease resistance.

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Short Communication

Evaluation of Novel Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.) Hybrids for Flower Quality Traits under Polyhouse Condition

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ABSTRACT

The present study was carried out to evaluate the performance of two gerbera hybrids IIHR15-7 and IIHR16-8 along with their parents and a commercial check, for flower quality traits under polyhouse condition in completely randomized block design, during 2016-17 to 2018-19. The hybrids IIHR15-7 and IIHR16-8 had been developed through the half-sib method of breeding with IIHR9 and Arka Ashwa, respectively, as parents. Data for three years were pooled and analyzed statistically. In both hybrids IIHR15-7 and IIHR16-8, all the quantitative traits were found to be on par with the respective commercial checks. They had novel flower colour (as per RHS Colour Chart) *i.e.* NN155A, White Group for IIHR 15-7 and 65A Red Purple Group for IIHR16-8, with semi-double and double forms of flowers, respectively. These hybrids are suitable for cut-flower and flower arrangement purposes. Further, these hybrids will be useful for developing new gerbera hybrids with novel traits.

Key words: Cut-flower, Evaluation, Gerbera, Novel hybrids, Polyhouse

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.),of the family *Asteraceae*, is one of the important cut-flowers grown for domestic and export markets. The total area of floriculture in India is 275000 ha and cut flower production of 783000 MT. Gerbera grown under 870 ha with productivity of 21300 t/ha, and stands fourth most important cut flower in India. Highest production of gerbera comes from Uttarakand with 7.80 (000' MT), while share of Karnataka is 6.2 (000, MT) (Anon., 2017). There is a great demand for gerbera, particularly in European markets during the winter season and almost around the year in India. In view of importance of the crop and to bring down the high cost of imported gerbera, two indigenously gerbera hybrids i.e. IIHR15-7 and IIHR16-8 were developed and evaluated with their parents and commercial check for flower quality traits under polyhouse condition.

Half sib method of crossing was employed to develop novel gerbera hybrids involving parents IIHR9 and Arka Ashwa during 2014-15 which were crossed with mixed pollen of different varieties. The hybrid seeds thus obtained were raise *in vitro*. The plants obtained from these single seeds were sub-cultured many times till the sufficient suckers are produced *in vitro*. The hardened plants were planted in polyhouse with 50% shade for evaluation. Two hybrids, IIHR15-7 and IIHR16-8, were selected on the basis of flower quality traits. Both the hybrids, along with their parents and the respective commercial check varieties Susan and Bismark, were evaluated in replicated trial in Completely Randomized Block Design under naturally-ventilated polyhouse for three consecutive years 2016-17, 2017-18 and 2018-19. Observations were recorded on flower diameter (cm), flowerstalk length (cm), flowers talk diameter (mm), number of flowers/plant/month, vase life (days),flower colour from RHS Colour Chart and flower form. Data of three years were pooled and analyzed statistically using OPSTAT.

Data presented in Table 1 showed that hybrid IIHR15-7 found to be on par with parent IIHR9 and

commercial check Susan for flower quality traits. It recorded flower diameter of 11.89 cm, which was on par with the parent IIHR9 (11.46 cm), Arka Ashwa (11.86 cm) and commercial check Susan (12.04 cm); flower stalk length (61.39 cm) which was on par with parent IIHR9, Arka Ashwa and commercial check Susan; flower stalk diameter (5.79 mm) and number of flowers/plant/month (2.87) recorded were on par with the parent IIHR9 (2.43), Arka Ashwa (2.56) and commercial check Susan (2.69). The hybrid IIHR 15-7 recorded vase life of 7.74 days which was also on par with the parent IIHR9, Arka Ashwa and commercial check Susan. Hybrid IIHR 15-7 recorded novel flower colour (RHS Colour Chart) NN155A, White Group, with semi-double form of flowers. Kumar (2013), Singh *et al.* (2017) and Soni and Godara (2017) evaluated ten genotypes under naturally ventilated polyhouse at different locations and recommended Kyllian, Vilassar, Partrizia, Szantal, Feliks and Dana Ellen for getting better cut flower yield and quality flowers.

Table 1. Evaluation of gerbera hybrid IIHR 15-7 with parent and commercial check for flower quality traits under polyhouse (pooled data of three years)

Hybrid/ Genotype	Flower diameter (cm)	Flower stalk length (cm)	Flower stalk diameter (mm)	No. of flowers/ plant/ month	Vase life (days)	Flower colour (RHS colour chart)	Flower form
IIHR15-7	11.89	61.39	5.79	2.87	7.74	White group NN155A	Semi-double
IIHR9 (parent)	11.46	61.19	5.80	2.43	7.29	Red purple group 69A	Semi-double
Arka Ashwa (check)	11.86	61.10	6.64	2.56	7.42	Red purple group 68D	Semi-double
Susan (commercial check)	12.04	62.81	6.62	2.69	7.59	White group NN155C	Semi-double
SEm±	0.47	0.44	0.38	0.49	0.51	-	-
C.D. at 5%	NS	NS	NS	NS	NS	-	-

Data presented in Table 2 showed that hybrid IIHR16-8 also found to be on par with parent Arka Ashwa and commercial check Bismark for flower quality traits.The hybrid IIHR 16-8 recorded flower diameter of 12.89 cm,which was on par with its parent Arka Ashwa (11.86 cm) and the commercial check Bismark (12.12 cm); flower stalk length (65.64 cm) was also found to be on par with parent Arka Ashwa (61.10 cm) and commercial check Bismark (62.93 cm); flower stalk diameter (5.77 mm) was on par with parent Arka Ashwa (6.64 mm) and the commercial check Bismark (6.21 mm) and, number of flowers/plant/month (2.85) recorded was on par with the parent Arka Ashwa (2.56) and commercial check Bismark (2.73). The hybrid IIHR 16-8 recorded vase life of 7.00 days which was also on par with the parent Arka Ashwa and commercial check Bismark. The hybrid IIHR16-8 also recorded novel flower colour (RHS Colour Chart) 65A Red Purple Group, withdouble form of flowers. Aswath *et al.* (2016) also evaluated two novel gerbera hybrids with check for flower quality under naturally ventilated polyhouse. Mahender *et al.* (2017), Deepa *et al.* (2019) and Jangde *et al.* (2019) evaluated different gerbera varieties for flower quality traits and found that cultivars Marinella, Bonnie, Ambra, Sciella and Fredi recorded more number of flowers per plant under polyhouse condition.

On the basis of three years of evaluation undernaturally-ventilated polyhouse, gerbera hybrids IIHR15-7 and IIHR 16-8 were found to be promising for novel flowercolour, flower form and flower quality traits.

Table 2. Evaluation of gerbera hybrid IIHR 16-8 with parent and commercial check for flower quality traits under polyhouse (pooled data of three years)

Hybrid/ Genotype	Flower diameter (cm)	Flower stalk length (cm)	Flower stalk diameter (mm)	No. of flowers/ plant/ month	Vase life (days)	Flower colour (RHS colour chart)	Flower form
IIHR16-8	12.89	65.64	5.77	2.85	7.00	Red purple group 65A	Double
Arka Ashwa (parent and check)	11.86	61.10	6.64	2.56	7.42	Red group group 68D	Semi-
Bismark (commercial check)	12.12	62.93	6.21	2.73	7.26	Red purple 45B	Semi-double
SEm±	0.50	0.47	0.43	0.41	0.52	-	-
C.D. at 5%	NS	NS	NS	NS	NS	-	-

IIHR 15-7 (Arka White)

IIHR 16-8 (Arka Pink)

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Short Communication

Studies on Fruit Development in Pink and White types of Wax Apple (*Syzygium samarangense* Merr. & Perry) in Goa, India

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ABSTRACT

Fruit development studies were taken up in white and pink types of wax apple trees aging twelve years old at Goa, India. The study was initiated with the onset of flowering in November during the year 2018. After tagging the flowers on anthesis, samples were drawn periodically to record parameters like fruit weight, fruit volume, fruit length and diameter (upper, middle and lower), quality or biochemical parameters like total acids and sugars. Relative growth rate (RGR) was calculated for all parameters and graphs were generated. In both the types, fruit weight, fruit volume, fruit length and diameter increased in a sigmoidal pattern. The quality characters like TSS, total acids and total sugars also showed a sigmoidal pattern of increase whereas the increase in reducing sugars exhibited a double sigmoidal pattern of increase. It was evident from the curves that there was pronounced peak in growth rate between 21 and 28 days after anthesis for fruit weight, fruit volume, fruit length and diameter, in both pink and white types of wax apple.

Key words: Fruit development, Goa, Wax apple, White and Pink types

INTRODUCTION

Wax apples (*Syzygium samarangense* Merr. & Perry) (syn. *S. Javanicum* Miq.; *Eugenia javanica* Lam. in part; *E. alba* Roxb.) are watery crunchy tropical fruits, found commonly in South East Asian countries like, Malaysia, Indonesia, Thailand etc. These fruit trees are called after many vernacular names like, samarang rose apple, djamboesemarang (Indonesia); jambuayerrhio (Malaya); pinijambu (Ceylon); jumrool, jamrul, or amrool (India); chompukao, or chompukio (Thailand); makopa (Philippines); cashu di Surinam, or Curacaoseappel (Curacao); wax jambu and water apple, generally. The tree is indigenous from Malaya to the Andaman and Nicobar Islands where there are wild trees in the coastal forests. It was introduced into the Philippines in prehistoric times and is widely grown throughout islands. It is common in Thailand, Cambodia, Laos, Vietnam and Taiwan, frequently cultivated in India and in Zanzibar and Pemba, but primarily as an ornamental in earlier days. But now, realizing the scope of the processing potentialities, progressive farmers, and agro-ecotourism units of coastal India are keen in cultivating such exotic fruits. The wax apple is extra-tropical, growing only at the lower altitudes–up to 4,000 ft (1,220m) in India. The waxy fruit, usually light-red, sometimes greenish-white or cream-colored, is pear-shaped, narrow at the base, very broad, and flattened, indented and adorned with the 4 fleshy calyx lobes at the apex. The skin is very thin, the flesh, white, spongy, juicy, sub-acidic to sweet, with ild flavours. There may be 1 or 2 some what rounded seeds, or none. The fruits are reported to have total acids content varying from 0.6 % to 0.9% and TSS from 5.6 to 12.8 % (Moneruzzama *et al*. 2015 and Rosnah *et al,* 2012), It has been reported that, in Ceylon, the fruits are ripe from March to May; in India, the flowering spans from Nov-Dec to Mar-April and fruits are available from March to June. in Java, flowering occurs from April to June and fruiting from June to August (Morton 1987). In Tripura (North Eastern part of India), these trees flower in March-April and fruit in June-July (Sankaran *et al*, 2006). In West coast of India (Goa), the trees initiate to flower in October-November and give fruits in summer (i.e.) during Feb-April. The spans of flowering and fruiting overlap, due to continuous

flushes of flowering in the tree. Even after the peak bearing and harvest in April, the trees put forth some flowers and yield few fruits in June, with the onset of South West Monsoons of India. The pattern of fruit development with respect to morphological and biochemical changes during the growth phase has not yet been studied systematically in this region. Therefore, this study was undertaken in order to study the growth and development of wax apple fruits right from anthesis to harvest.

The study was conducted in ICAR-Central Coastal Agricultural Research Institute, located at Old Goa, Goa, India. Well grown and yielding trees of white (variety Krystal Taiwan) and pink types (variety Pink) of *Syzygium samarangense*, those are twelve years old were selected for the study. The study was initiated in November, when the onset of flowering was noticed. The flower buds open in the morning hours. The freshly opened flower buds were tagged on the day of anthesis. During the fruit development, the samples of minimum ten fruits were drawn once in seven days for analyses. Likewise, the samples were drawn till the harvest stage. At every stage (*i.e.*) on 7, 14, 21, 28, 35, 42, 49, 56, 63 and 70 DAA (days after anthesis) morpho-physical parameters like, fruit weight, fruit volume, fruit diameter (as the fruit is bell shaped, diameter readings were recorded in three places *viz*., upper, middle and lower designated as D1, D2 and D3 respectively in the graphs) and fruit length were recorded.Fruit weight was recorded using electronic balance; fruit volume was measured using water displacement method; diameter readings were measured using Vernier Caliper. Besides, biochemical parameters like, total acids (titration against 0.1 N NaOH using phenolphthalein indicator), total sugars (Phenol-Sulphuric acid method) and reducing sugars (Nelson-Somyogi method) were also estimated in every stage of development. TSS was observed using digital refractometer. Growth curves were generated using RGR values using the formula, RGR= (ln2-ln1)/ (tn2-t1), where ln are natural logarithmic values of readings recorded at regular time intervals and t represents the time taken (say seven days in this study)

Goa experiences monsoon season from June to September or mid-October. Only after the complete cessation of monsoons, do the wax apple trees start showing fruit buds on the branches. During the period of study, the flowering phase in the trees under study was recorded from mid-October to mid-February. The period taken from anthesis to harvest was 63 and 70 days in pink and white wax apple respectively. It has been reported that, the formation of flower buds does not mean early flowering in *Syzygium samarangense*. In the dry season, wax apple commonly flowers early or late and even protocols have been developed by Shu *et al* (1998) in Taiwan to trigger the flowering period depending upon the site of flower panicles appearance *viz*., leaf axils, shoot tip, new flush etc. Usually, shoot growth proceeds in flushes which are more or less synchronous, depending on the climate. There are definite flowering seasons, often two, sometimes three in a year, but the timing varies from year to year. Wax jambu commonly flowers early or late in the dry season; the flowers appear to be self-compatible and the fruit ripens 30-40 days after anthesis (Orwa *et al*. 2009). He has also reported that, flowers fall on the ground in 2-3 days, leaving behind the tiny fruits to mature and ripen in about 2 months. In favourable conditions, a healthy tree can produce abundant fruits and has two fruiting seasons annually, May-September and November to March as reported from Kenya. When mature, the tree is considered a heavy bearer and can yield a crop of up to 700 fruits. Sankaran *et al*. (2006) have reported that the wax apple trees have two flushes of flowering during March-April and June-July under climatic conditions of North Eastern Hill region, especially in Tripura. Moneruzzaman *et al*. (2012a), have reported after a detailed study on different varieties of wax apple, that, 'Giant Green' cultivar had creamy white flower colour,'Masammanis Pink' had white colour flower, while 'Jambumadu Red' had the creamy white to light yellow flower andthe number of days between anthesis and the harvest maturity (DAA) were as 41, 46, and 55 days for 'Masammanis Red', 'Jambumadu Red' and 'Giant Green' under Malaysia climatic conditions. It is observed that the pink or red types take shorter duration for attaining maturity, when compared to green or white types, which is in corroboration with the current study. In a different study Moneruzzaman *et al*. (2015) has reported that 'Masam Manis Pink' cultivar had the earliest fruit development and maturity approximately 38 days after anthesis followed by 'JambuMadu Red' cultivar with nearly 45 days. On the other hand, 'Giant Green' cultivar had late maturity with about 50 days to reach harvest stage from anthesis. It has been noted that *Syzygium pycnanthum* required 80-89 days

after anthesis to attain harvest stage (Mudiana and Ariyanti, 2010).

In wax apple, variety 'Pink', the average fruit weight recorded seven DAA was 1.28 g, whereas during harvest (70 DAA), it was recorded as 47.16g .The21stday average fruit weight of 5.59 g spurted to 24.05 g on 28th DAA. The fruit volume was negligible on 7 and 14 DAA and recorded a value of 2.55 cc on 21st DAA, however, increased to 22 cc on 28 DAA, in a trend similar to increase in fruit weight. The fruits harvested 70 DAA recorded fruit volume of 46.60 cc.

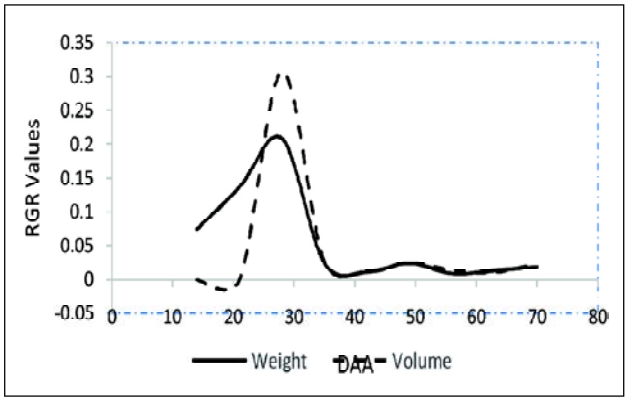

Fig. 1. RGR for fruit weight and volume in wax apple variety Pink

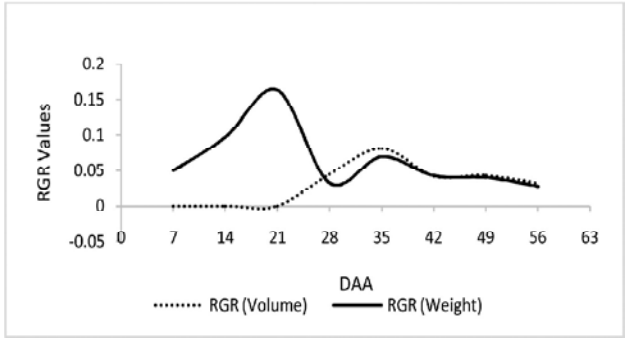

Fig. 2. RGR for fruit weight and volume in white wax apple variety Krystal Taiwan

The RGR curve (Fig 1) shows that there was a rapid development from 7th to 35th day and then, the growth rate slowed down towards the 49th DAA, which later slightly increased (between 49 and 56 DAA) then decreased towards the harvest. The second increase recorded can't be considered as a peak, whereas the initial or first increase is definitely a peak, therefore depicting a sigmoid curve.

In white wax apple, variety 'Krystal Taiwan', the initial fruit weight was recorded to be 0.9 g, which increased to 35.07 g during harvest, i.e., 63 DAA. The fruit volume values were negligible on 7, 14 and 21 DAA, whereas it was found to be 6.20 cc on 28 DAA and increased to 34 cc during harvest. The increase in fruit weight was steady from 7 to 21 DAA, and later on attained a jump size of 7.87 g. The RGR curve pattern depicting the increase in fruit weight also showed a trend similar to that in Pink variety, expressing a well renowned peak 21 DAA and a less pronounced peak on 35th DAA. The only peak in RGR curve for fruit volume in Krystal Taiwan, white wax apple coincided the second peak of fruit weight RGR curve (Fig. 2).

It has been reported by Moneruzzaman*et al* (2012 a) that, 'Giant Green' cultivar had the largest fruit (89 g) weight followed by 'Jambumadu Red' cultivar with a value of 85 g while'Masammanis Pink' cultivar produced the minimum fruit (78 g) weight. In another study by Moneruzzaman *et al* (2012 b), the Pink variety wax apples recorded fruit weight of 38 g. In a study conducted on different types of wax apples it was found that, the fruit weight varied significantly among 5 accessions of wax apples, ranging from 50.88 g in thered or dark pink type to 28.35 g in the light pink bell shaped type with a mean value of 35.66 g (Risvy, 2013). The fruit weight values recorded in the study are in corroboration with these reports.

The related species guava also showed a sigmoidal growth pattern, with one peak flanked by two slag phases (Mukherjee and Datta, 1967), whereas Salunkhe and Desai (1984) have reported in guava a sigmoidal growth pattern but with an unusual behaviour of rapid increase in fruit weight in the initial phase. Another fruit species *Eugenia stipata* (Hernandez *et al*. 2007) from the same family Myrtaceae also showed single sigmoidal growth pattern similar to the current study. Similarly, the growth curve of Pomegranate also showed a single sigmoidal pattern (Shulman, 1984).

In Pink variety wax apple, the average initial length seven DAA was observed to be 1.50 cm, which increased to 6.60 cm during harvest stage *ie*.,70 DAA. The fruit equatorial diameter was measured upper side (D1), middle (D2) and lower side (D3), as the fruit is nearly pear shaped. D1 was 0.40 cm seven DAA and increased to 2.80 cm 70 DAA. However, the increase was from 1.03 to 4.00 cm and

1.38 to 4.20 cm for D2 and D3 respectively from 7 to 70 DAA. The fruit length increased from 1.50 cm to 6.60 cm during the development. Similar previous studies also show that, fruits are pear-shaped and 1.5-2 inches long(Orwa*et al*. 2009). 'Giant Green' and 'Masammanis Pink' cultivars had bell shaped fruits while 'Jambumadu Red' cultivar had a pear shaped fruit. (Moneruzzaman*et al*, 2012 a). Fruit length was 5.23 cm in Pink variety wax apple and fruit diameter was nearly 3.5 cmduring harvest. (Moneruzzaman*et al*, 2012 b)

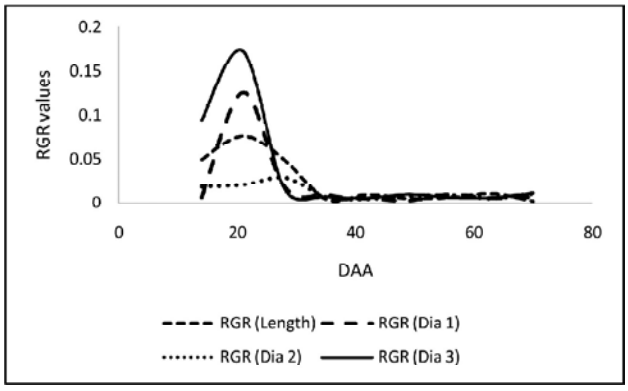

Fig. 3. RGR for fruit dimension parameters in wax apple variety 'Pink'

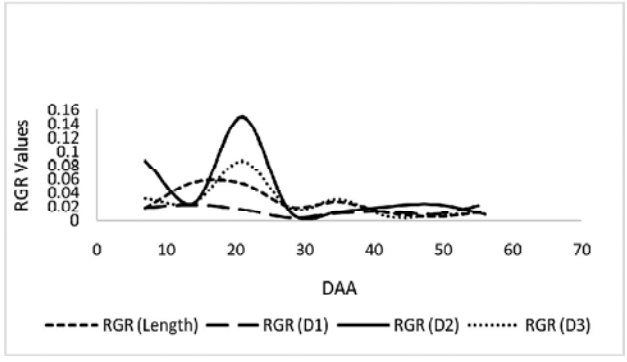

Fig. 4. RGR for fruit dimension parameters in white wax apple, variety Krystal Taiwan

The RGR curve shows that, there were pronounced peaks during increase in the polar and equatorial diameter (D1 and D3)between 21 and 28 DAA. But, the peaks in RGR curves of fruit weight and fruit diameter D2 occur between 28 and 35 DAA.Therefore, it can be concluded that the fruit growth follows a single sigmoid growth curve in wax apple variety 'Pink'. (Fig. 3)

In white wax apple, variety 'Krystal Taiwan', the initial average length seven DAA was 1.10 cm, which increased to 4.60 cm on 63rd DAA. D1, D2 and D3 were 0.40, 1.00 cm and 1.23 cm respectively seven DAA and increased to 2.85, 4.25 and 4.60 cm during harvest i.e., 63 DAA.There was a single prominent peak noticed in the RGR curves for D2 and D3 during fruit development between 21 and 28 DAA.However, RGR for D3 showed a less prominent peak, coinciding with second less dominant peak in RGR curve for fruit length. The increase of length showed two peaks during 7-14 DAA and 35-42 DAA. (Fig 4)

In general, fruit size is a genetic characteristic of the cultivars and is also used for identification of cultivars. Previous studies show that the fruit of'Jambumadu Red' cultivar was the longest (8.2 cm) followed by 'Giant Green' cultivar with a fruit length of 6.3 cm, whilst least fruit length was observed in 'Masammanis Pink' cultivar with a value of 5.5 cm. Fruit size is an inherent factor associated with different cultivars. 'Masammanis Pink' cultivar had the highest (5.5cm) fruit diameter while, 'Jambumadu Red' cultivar fruit had the least (4.6cm) diameter and 'Giant green' cultivar was intermediate at about 5.2 cm.They also found that the number of cells per fruit was higher for large fruited cultivars than for small fruited cultivars (Moneruzzaman *et al*. 2012b). In the evaluation study on different cultivars of wax apples conducted by Risvy (2013), it was noticed that the length of observed fruits varied significantly and ranged from 7.04 cm to 4.23 cm, with a mean value of 4.92 cm. Similarly, the diameter also varied from 4.23 cm to 2.67 cm, with a mean of 3.09 cm. These values are in corroboration with the current study.

The length of wax apples increased from 1.5 to 5 cm, the width from 1.2 to 6 cm, fruit weight from 2 to 150 g during 80 days growing period from anthesis, all following a single sigmoidal growth pattern as reported by Shu *et al* (1998)

Wax apples, both pink and white are majorly composed of moisture, sugars and acids. They are crunchy, sweet and relishing for table purpose. In South East Asian countries, various products likejam, jelly, candy, syrup, flakes etc are prepared from these fruits. The mild sugar acid blend renders the fruits suitable for value addition. The immature fruits are mildly acrid and acidic and towards maturity, the acidity decreases and sugar concentration increases.

In Pink variety, the percentage of total titrable acids decreased from 0.42 (7 DAA) to 0.11 per centduring

harvest. The rate of decrease in total acids touched a lowest peak 28 to 35 DAA and after being stable for one more week, it steadily decreased till harvest stage (70 DAA).In white wax apple, the total titrable acids reduced from 1.43 per cent (7 DAA) to 0.19 per cent (63 DAA) during harvest. The RGR curve showed that, the decrease in acidity was steady and uniform from seven to 35 DAA, whereas, it reached a negative peak during 35-42 DAA, again followed by a steady decline in acidity percentage till harvest, therefore, depicting a single sigmoidal pattern (Fig 5). In similar studies, it was reported that the decrease in fruit acidity coincided with an increase in sugar content of the fruits.The lowest amount of titrable acidity (0.78%) was observed in the 'JambuMadu Red' cultivar, followed by 'Giant Green' (0.83 %) and 'Masam Manis Pink' (0.90%). It has also been recorded that the soluble solids content in 'JambuMadu Red' was wide-ranging from 5.63 to 12.5% Brix (Moneruzzaman *et al*, 2015). In a different study, Moneruzzaman *et al* (2012 c) reported that titrable acidity of wax apple was 0.78 %.While comparing chemical composition changes in two types of wax apples, Rosnah*et al* (2012) found that, the total acidity of Kristal Taiwan (green white type) ranged between 0.2-0.25%, while the total acidity of Semarang Rose (pink type) were between 0.07-0.1%. These results are in corroboration with the results of this study.

In pink variety, Total Soluble Solids (TSS) increased gradually from 5.10 to 13.90°Brix (7 to 70 DAA). The increase rate was gradual from 7 to 35 DAA, and then, there was a spurt in increase from 35 to 49 DAA. Later on, the raise in TSS was gradual up to 60 DAA and then, there was a sharp increase towards harvest.In white wax apple, TSS increased from 4.1 (seven DAA) to 15.6°Brix during harvest (63 DAA). The rate of increase has a peak value during 35 to 42 DAA, coinciding the negative peak of reduction in acidity during fruit development. Therefore, it was observed that during fruit development, the significant increase in sugars and significant decrease in acids occur from 35 to 42 DAA. The above-mentioned peak for increase in TSS was flanked by one less prominent peak during initial period of development i.e., 14-21 DAA and the other one towards the harvest stage i.e., 56-63 DAA (Fig 6). It has been noted by Orwa *et al* (2009) that, of the different types of wax apples, the reddest fruits are the sweetest and superior varieties of excellent quality available. Total soluble solids (TSS) percentage significantly varied and ranged from 10.39% to 13.96% with the mean value of 12.05% in five different types of wax apples evaluated in Bangladesh (Risvy, 2013). On comparing a green and Pink type, Rosnah*et al* (2012) has reported the total soluble solid (TSS) in Kristal Taiwan (5.9-9.6°Brix) and Semarang Rose (5.2-9.0°Brix). Moneruzzaman *et al.* (2012 b) have reported that pink wax apples have 5.6° Brix TSS and 3.63 mg/10 g of total sugars.

In the present study, the total sugars content in Pink variety wax apple increased from 2.11 (7 DAA) to 5.95 (70 DAA) in a gradual manner. However, the RGR curve showed a peak in increase in total sugars,49-56 DAA followed by a sharp decline in rate and then a final spurt in the concentration of total sugars towards harvest stage (70 DAA) in a trend similar to that of increase in TSS content of the fruit. The concentration of reducing sugars in the fruit increased from 0.18 % (seven DAA) to 1.98 % (70 DAA). The trend in increase of reducing sugars also had an initial peak during 35 to 42 DAA, followed by a second peak during 49-56 DAA, thus showing a double sigmoid curve (Fig 6).

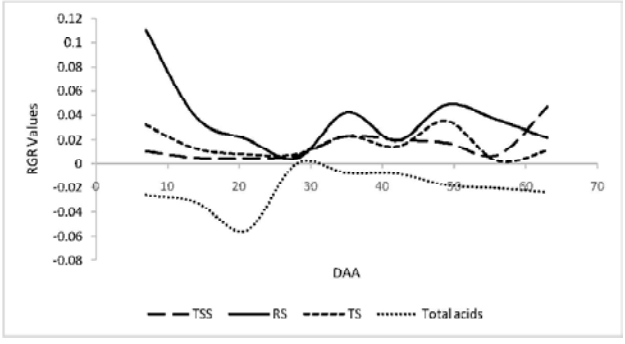

Fig. 5. RGR for quality characters in wax apple variety 'Pink'

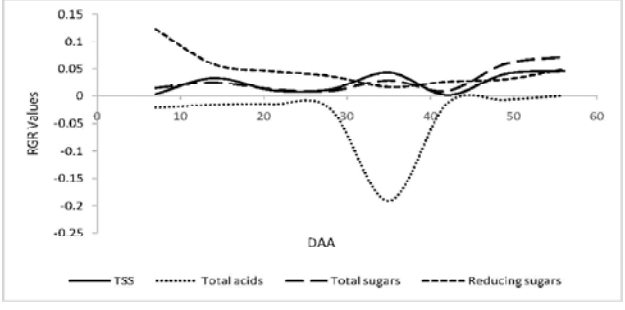

Fig. 6. RGR for quality characters in 'white' wax apple variety Krystal Taiwan

In white wax apple variety Krystal Taiwan, total sugars content in fruit increased from 2.8 per cent (seven DAA) to 13.87 per cent (63 DAA) at harvest. The rate of increase in total sugars showed a trend just similar to that of TSS with a peak during 35 to 42 DAA, flanked by one less prominent peak during initial period of development i.e., 14-21 DAA and the other one towards the harvest stage i.e., 56-63 DAA. The reducing sugars in the white wax apple fruits increased from 0.62 per cent (seven DAA) to 9.27 per cent (63 DAA) at harvest. The RGR curve showed that the trend in rate of increase in reducing sugars was stable without any peak, except during the initial period i.e., seven to 14 DAA.

In a study on compositional changes in two cultivars of wax apple in Malaysia, it was found that, the fructose content was highest in the water apple juice in the range of 7.05 to 9.15% (Kristal Taiwan) and 4.77 to 9.25% (Semarang Rose) indicating that, the fructose content is the major sugar contributing to water apple sweetness followed by glucose and sucrose. The glucose content varied between 6.74 to 8.37% (Kristal Taiwan) and 3.53 to 8.26% (Semarang Rose) while the sucrose content varied between 0.19 to 0.36% (Kristal Taiwan) and 0.38 to 1.51% (Semarang Rose). Fructose, being sweeter than sucrose and glucose, has a desirable influence on the taste of fruits. (Rosnah *et al,* 2012)

Sugar concentration of the fruit increased with fruit growth with fructose and glucose as the main components; starch content reached a maximum value of 11 % and decreased towards ripening (Shu *et al,* 1998).

Similarly, in pomegranate fruit development, a significant increase in TSS, total sugars and reducing sugars was observed 80 DAA, with a maximum values on 140 DAA. The equilibrium concentration of these biochemical on 100 DAA mark optimum maturity and the further increase is during phase of ripening (Kulkarni and Aradhya, 2005). Likewise, in both types of wax apples studied, there was a peak in increase of sugars between 30 and 40 DAA and then after 50 DAA.

This systematic study, conducted in *Syzygium samarangense* (pink and white types) indicates that the fruit development, comprising various aspects of the fruit follow sigmoid and double sigmoid growth pattern, which is in corroboration with similar studies conducted in fruits like wax apples in different places like Malaysia, Taiwan and Bangladesh and also in strawberry and apple.

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INFORMATION TO CONTRIBUTORS

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