Volume 73, No. 1
March, 2016

Print : ISSN 0972-8538
Online : ISSN 0974-0112

Indian Journal of Horticulture

The Horticultural Society of India
Indian Agricultural Research Institute
New Delhi-110 012
Website : www.hsi1942.in

Estd. 1942

Overseas distribution
IOS Press, The Netherlands

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[Founded in January 1942]

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Online version available at: www.indianjournals.com

DOI : 10.5958/0974-0112.2016.00009.8

Indian J. Hort. 73(1), March 2016: 1-7

Analysis and utilization of genetic diversity of '*Ambri*' apple (*Malus* × *domestica* Borkh.) in Jammu region

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ABSTRACT

Present investigation was conducted in erstwhile Doda district of Jammu & Kashmir state to study the genetic diversity among '*Ambri*' apple variants and its exploitation to revive this indigenous variety in the state for commercial cultivation and to utilize in apple improvement programme. Among the 34 collected variants, a wide range of diversity was observed in respect of fruit weight (86.90-334.97 g), fruit length (59.13-84.00 mm), fruit width (68.21-94.31 mm), flesh firmness (3.65-9.65 N), seed number (5.00-7.00), TSS (10.90-20.00ºBrix) and titratable acidity (0.31-1.78%). Highest coefficient of variation was recorded for titratable acidity (54.76%). Fruit weight showed significantly positive correlations with fruit length and stalk and number of seeds per fruit. First three factors having eigen value having >1 explained 70.04% of total variation observed in the collected population. Of the total collections, only seven variants attained the numerical rating, being accepted of consumer's acceptability. Variants BM2910 and BC3210 were identified as most acceptable variants for mass multiplication and to revive the '*Ambri*' apple cultivation and their further use in breeding programmes.

Key words: *Ambri* apple, fruit weight, genetic diversity, variants.

INTRODUCTION

Apple, the principal temperate fruit crop of India is being cultivated on 3.12 lakh ha area with an annual production of 9.12 million tonnes and productivity of 6.1 tonnes ha^{-1}, in general and 8.57 tonnes ha^{-1} in Jammu & Kashmir (J&K) state, in particular. Despite of limited growing region, 23806 tones apple worth of US $ 8.32 million, is exported from India annually (Anon, 1). In India, about 33 apple varieties are grown, however, the availability of Indian apple (fresh) in markets is only restricted between July to November, because of poor shelf life. '*Ambri*' (developed naturally either through chance seedling or bud mutation) an excellent dessert variety of apple is indigenous to Kashmir, and continues to keep its superiority by virtue of its crisp texture, sweet flesh and excellent aroma with prolonged storability (up to six months in ordinary storage under typical temperate areas). Despite of long gestation period, biennial bearing habbit and susceptibility to scab disease, '*Ambri*' apple is in great demand because of unparalleled flavour and prolonged shelf-life, which have also made '*Ambri*' variety the choicest parent to use in Indian apple breeding programme for improving the quality and shelf-life of Delicious apples with good success. With the introduction of early maturing cultivars like Starkrimson and Mollies Delicious, the demand of Indian consumers later in the season relies on imported apple at non-affordable price, causing decreased per capita consumption of this nutritious fruit. Hence, the revival of '*Ambri*' apple being a late maturing cultivar, is an excellent alternative for the sustainability of Indian apple industry. The scattered plantations of '*Ambri*' apple can be found in Kashmir valley, and Ramban, Doda and Kishtwar districts of Jammu region. The existence of seedling populations and its highly cross-pollinated nature have contributed towards the tremendous variability in shape, size and colour development thereby providing a platform for exploitation of vast gene pool of '*Ambri*' apple. Due to scant attention of researchers, meagre efforts have been made to analyse the genetic diversity of '*Ambri*' apple and for further exploitation for its revival in J & K state.

Apple germplasm and the maintenance of genetic diversity are important for future breeding because genetic diversity gives species the ability to adapt to changing environments and provide the raw material to breed new cultivars *via* hybridization or selection (Dhillon and Rana, 3). Estimating genetic diversity and determining the relationships among germplasm collections enhance efficiency of its management and genetic improvement (Rana *et al*., 11). Future of breeding programmes depends on the availability of genetic variability to increase productivity. Morphological characterization of trees

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and fruits is the first and the most important step for the description, classification and characterization of germplasm collections (Verma *et al.*, 14). Apple fruits are characterized using both, maturity indices including firmness, sugar, starch, acid content, and ethylene concentration as well as marketability indices such as flesh and background colour and fruit shape which consumer use to differentiate cultivars. In the present study, the attempt has been made to analyse the existing variability of '*Ambri*' apple and exploit the traits that contribute towards fruit weight and consumer acceptability of '*Ambri*' apple.

MATERIALS AND METHODS

The potential areas of erstwhile Doda district (now divided into three districts, *viz.*, Ramban, Kishtwar and Doda) were surveyed personally or through the help of extension personnel of State Department of Horticulture, Jammu (J&K) for selecting and marking the trees of '*Ambri*' populations during first year (2009-10). A random sample of 20 fruits, harvested at commercial maturity, from each tree was taken during second year for recording the data on fruit characters (Table 1). Data on fruit weight (g) was recorded on top pan balance, while fruit size (length and width), average cross diameter, and depth (mm) and width (mm) of stalk and eye basin were measured with Mitutoyo digital Vernier calipers. Locule aperture was described as per the method given in the TG/14/9 document (UPOV, 13). Fruit TSS (ºBrix) was measured with hand refractometer. Flesh firmness was measured with the help of Effegi penetrometer-FT 327(as force in N). Fruit colour was measured with the help of Hunterlab colour meter, and expressed as CIE Hunter colour values. Titratable acidity (as % malic acid) was determined by standard AOAC method (Horwitz, 5). Ascorbic acid was determined as per the method of Roe (12). To assess the consumer preference, organoleptic evaluation of fruits of different variants was done by the panel of judges as per the 9 point hedonic scale (Peryam and Pilgrim, 10).

Cluster and factor analysis was applied to study the relative contribution made by the different fruit characters. The factors having eigen value greater than or equal to one were extracted as variables. Also multiple linear regression equation was fitted to predict the fruit weight of '*Ambri*' apple on the basis of remaining fruit characters. The data were analysed using software SYSTAT-12.

RESULTS AND DISCUSSION

The proportions of different morphological characters of fruits observed in the collected variants of '*Ambri*' apple have been presented graphically in Fig. 1. Among different fruit shapes, conic type was dominant (44.12%) followed by broad conic (20.59%) and globose (17.65%), while for other shapes such as globose conic, broad globose and ovoid, they ranged between 2.94-8.82%. Majority of the variants (97.06%) had creamy-white flesh colour. Closed locule aperture was observed in 41.18% variants, while locule apertures were moderately closed and fully open each in 29.41% variants. The colour frequency for L^* value of variants was equal (29.41% in each) between 27-32 and 32-37 colour values ranges. The a^* value of 50% variants ranged between 37-42 followed by 42-47 (29.41%). In case of b* colour value, the highest frequency (47.05%) of variants ranged between 10-14 b^* value followed by 18.22 (26.47%) and 14-18 (23.53%).

Range, mean, standard deviation and coefficient of variation of different physico-chemical fruit characters of '*Ambri*' apple variants are presented in Table 2. Among the characters studied, fruit weight ranged between 86.90-334.97 g, whereas fruit length, fruit width, fruit diameter, stalk depth and stalk width ranged between 59.13-84.00 mm, 68.21-94.31

Table 1. Location of '*Ambri*' apple variants in Jammu region, J&K.

Variant*	Place	Elevation (m amsl)	Coordinates	
BS0110, BS0210, BS0310, BS0410, BS0510, BS0610, BS0710, BS0810, BC0910, BC1010, BC1110, BC1210, BC1310, BC1410, BC1510, BS1610, BS1710, BS1810, BS1910, BS2010, BD2110, BS2210, BS2310, BS2410, BC2510, BC2810, BM2910, BC3210, BC3310	Bhaderwah	1,618	32°58'58.95"N	75°42'39.45"E
BN3110	Banihal	2,832	33° 25' 0" N	75° 12' 0" E
BL3410	Bhalessa	1,644	33°01'55.96"N	75°54'36.11"E
DD2610, DD2710	Doda	1,131	33°08'38.12"N	75°32'46.73"E
GL3010	Galhar	2,046	33°20'18.66"N	75°56'02.33"E

*Tree age of variants ranged from 18-20 years

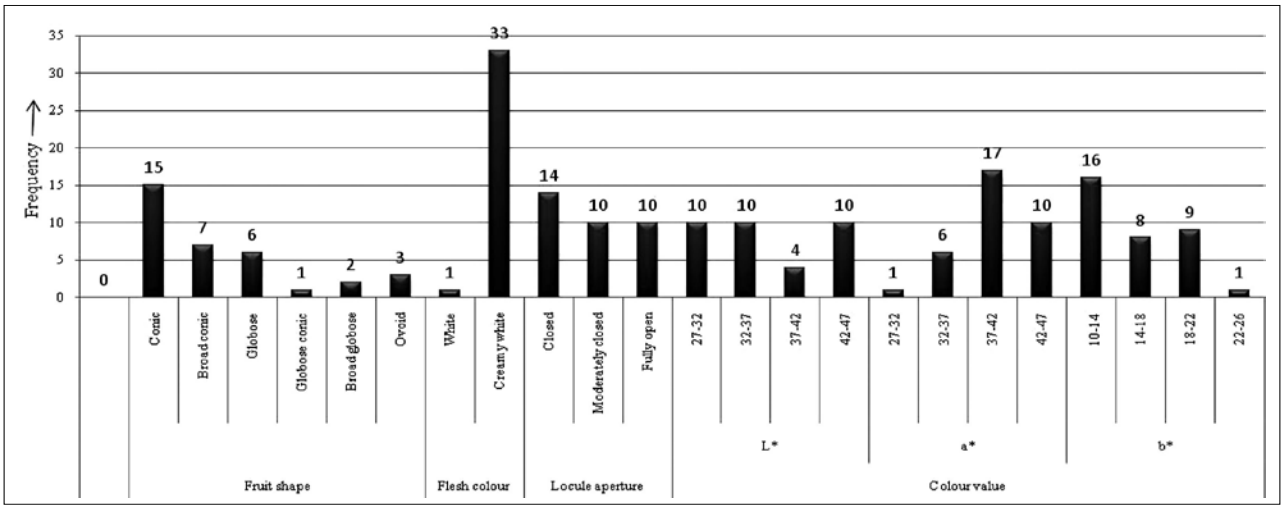

Fig. 1. Frequency distribution of morphological traits in *'Ambri'* variants.

Table 2. Range, mean standard deviation and coefficient of variation in physico-chemical fruit traits in '*Ambri*' variants.

Trait	Range	Mean	Standard deviation	Coefficient of variation
Fruit weight (g)	86.90-334.97	209.90	62.29	29.68
Fruit length (mm)	59.13-84.00	75.67	6.98	9.23
Fruit width (mm)	68.21-94.31	81.48	6.96	8.54
Fruit diameter (mm)	18.30-30.30	24.59	4.24	17.26
Stalk depth (mm)	1.10-2.80	2.06	0.44	21.56
Stalk width (mm)	1.40-4.00	2.71	0.62	22.71
Flesh firmness (N)	3.65-9.65	8.16	1.27	15.55
No. of seeds	5.00-7.00	5.38	0.60	11.22
TSS (°B)	10.90-20.00	14.47	2.13	14.68
Titratable acidity (%)	0.31-1.78	0.60	0.33	54.76
Ascorbic acid (mg/ 100 g)	2.10-6.80	3.72	1.38	37.11

mm, 18.30-30.30 mm, 1.10-2.80 mm and 1.40-4.00 mm, respectively. Values for flesh firmness, number of seeds, TSS, titratable acidity and ascorbic acid ranged between 3.65-9.65 N, 5.00-7.00/ fruit, 10.90-20.00ºBrix, 0.31-1.78% and 2.10-6.80 mg/100 g FW, respectively. Highest values for coefficient of variation was recorded as 54.76% for titratable acidity followed by 37.11% in ascorbic acid, 29.68% in fruit weight, 22.71% in stalk width, 21.56% in stalk depth, 17.26% in fruit diameter, 15.55% in flesh firmness, 14.68% in TSS and 11.22% in number of seeds. Fruit length and width registered lower values for coefficient of variation as 9.23 and 8.24%, respectively.

Correlation coefficients presented in Table 3 revealed that fruit weight had highly significant and positive correlation with fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds. Fruit length was correlated significantly and positively with fruit width, fruit diameter, stalk depth and width. Fruit width had positive and significant correlation with fruit diameter, stalk depth and width. Fruit diameter was correlated significantly with stalk depth and width. Stalk depth was correlated significantly with stalk width and number of seeds and stalk width was correlated significantly with number of seeds.

The dendrogram produced through multivariate analysis performed on 12 quantitatively measured traits showed that 34 accessions were grouped into two clusters in which cluster I had 20, while cluster II had 14 (Fig. 2). In cluster I accession, GL3010 showed high genetic distance (21.51) followed by BC1110 (11.14) and GL3010 (10.06), while in cluster II BS0510 showed high genetic distance (21.51) followed by BS1710 (11.14). The minimum genetic distance was showed by BS0610 (1.34) in cluster I and BS2210 in cluster II. Grouping of accessions into

Table 3. Correlations among different fruit characters of '*Ambri*' apple variants.

	FW	FL	FW	FD	SD	SW	FF	SN	TS	TA	AA
FW	1.000										
FL	0.490**	1.000									
FW	0.840**	0.648**	1.000								
FD	0.726**	0.476**	0.722**	1.000							
SD	0.667**	0.408*	0.732**	0.679**	1.000						
SW	0.667**	0.499**	0.599**	0.564**	.0752**	1.000					
FF	0.183	0.176	0.127	0.079	0.079	0.292	1.000				
SN	0.472**	0.066	0.394	0.354	0.479**	0.452**	0.139	1.000			
TS	-0.072	0.387	-0.031	0.117	-0.113	0.131	0.194	-0.327	1.000		
TA	0.115	0.221	-0.016	-0.084	-0.070	0.058	0.229	-0.202	0.432	1.000	
AA	-0.131	-0.414	-0.238	-0.086	-0.219	-0.158	-0.113	0.078	-0.328	-0.078	1.000

**Significant at 1%; FW = Fruit weight; FL = Fruit length; FW = Fruit width; FD = Fruit diameter; SD = Stalk depth; SW = Stalk width; SN = No. of seeds; FF = Flesh firmness; TS = Total soluble solids ; TA = Titratable acidity; AA = Ascorbic acid

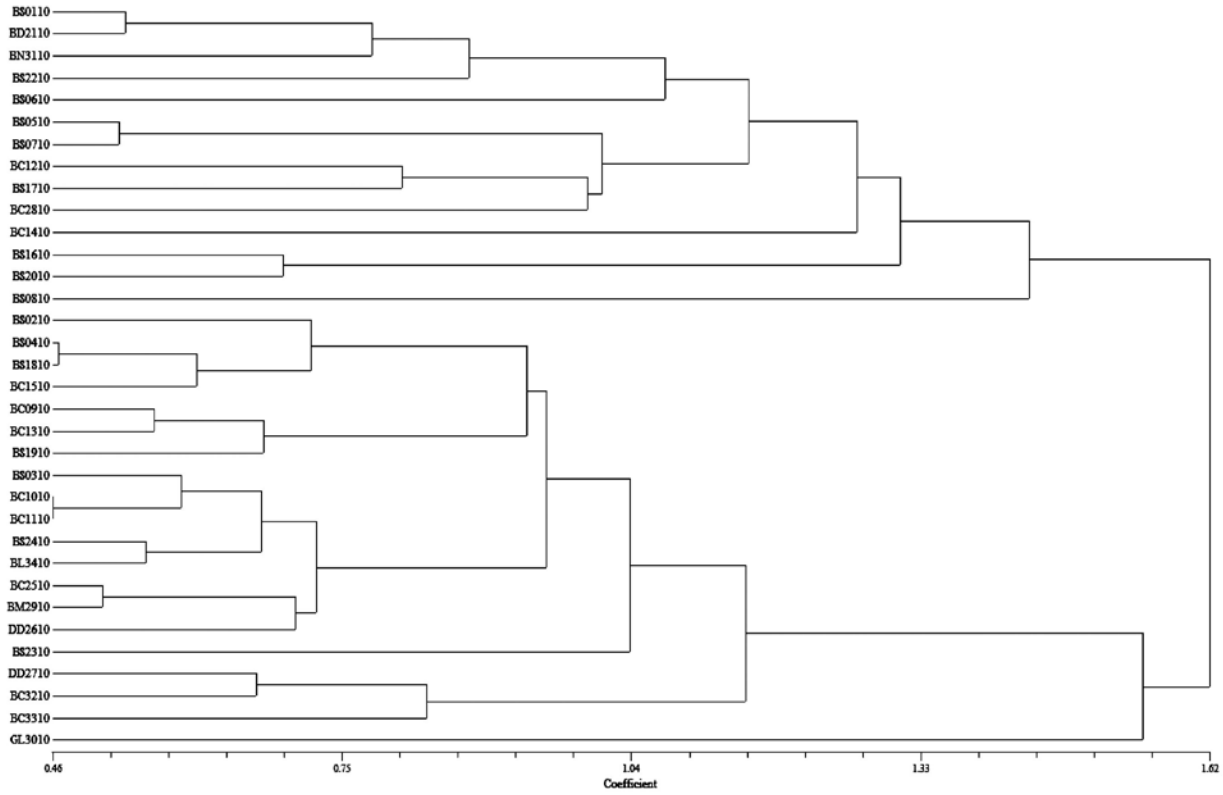

Fig. 2. Dendrogram of '*Ambri*' variants based on quantitative traits.

few numbers of homogenous clusters facilitate the selection of diverse parents and also permits precise comparison among all the possible pair of populations and provide an opportunity for bringing together gene constellation yielding desirable progenies.

Factor analysis technique was applied to extract the basic factors underlying the observed characters of '*Ambri'* apple and values pertaining to it (Table 4). The other factors corresponding to eigen value less than unity (λ<1), were not taken into further consideration, and ignored due to Guttmen's lower bond principle. After ignoring the non-significant correlation, orthogonal factors were extracted. The centroid method of analysis was used in arriving at

Table 4. Loadings of components (having λ<1) extracted through principal component analysis.

Trait	Loading		
	PC1	PC2	PC3
Fruit weight	0.88	-0.10	0.083
Fruit length	0.68	0.47	-0.224
Fruit width	0.90	-0.06	-0.148
Fruit diameter	0.82	-0.08	-0.133
Stalk depth	0.85	-0.20	-0.081
Stalk width	0.83	0.02	0.185
Flesh firmness	0.25	0.32	0.718
No. of seeds	0.53	-0.53	0.312
TSS	0.08	0.84	-0.042
Titratable acidity	0.05	0.66	0.397
Ascorbic acid	-0.29	0.50	0.435
Eigen value	4.55	2.06	1.10
% of total variance	41.34	18.71	9.98
% of cumulative variance	41.34	60.05	70.04

the factors. The following three factors were thus obtained:

Factor 1 : 0.88X1 + 0.68X2 + 0.90X3 + 0.82X4 + 0.85X5 + 0.83X6 + 0.53X8

Factor 2 : 0.84X9 + 0.66X10 + 0.50X11

Factor 3 : 0.72X7

These three factors having eigen values more than one explained 70.04% of the total variation. The first, second and third factors had variance of 4.55 (41.34% of total variation), 2.06 (18.37% of total variation) and 1.10 (9.98% of the total variation), respectively. The rotated factor matrix and the communalities were obtained through orthogonal transformation procedure. The first factor extracted was the combination of fruit weight, fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds in, which all the characters had positive loadings. The contributing characters of first principle component comprised of the characters which are responsible for yield. The second factor was the combination of TSS, titratable acidity and ascorbic acid, which pertained to the quality parameters. Third factor comprised of only one loading, *i.e.* flesh firmness, which was related to the storability quality of fruit.

For exploiting the existing variability to revive the '*Ambri*' apple in J&K, the organoleptic evaluation of all the variants was conducted as per the hedonic scale. Of the collected variants (34), only seven could score the numeric rating to the extent of consumer acceptability (Fig. 3). BM2910 was the most acceptable variant as it scored the highest numerical rating (9.5) followed by BC3210 (8.5). These two variants were found most promising and may be recommended for mass multiplication to revive the '*Ambri'* apple for the sustainability of apple industry of the state. Besides, these variants proved also found superior in respect of fruit length, TSS and good acid content (Table 2) over standard '*Ambri*' (having maximum fruit length upto 77.66 mm, TSS up to 15.90ºBrix and acidity up to 0.09%) as reported by Bisati (2), while studying the variation in '*Ambri'* in Kashmir region.

Variability refers to the differences which develop between individuals may be inherited (genetic) or

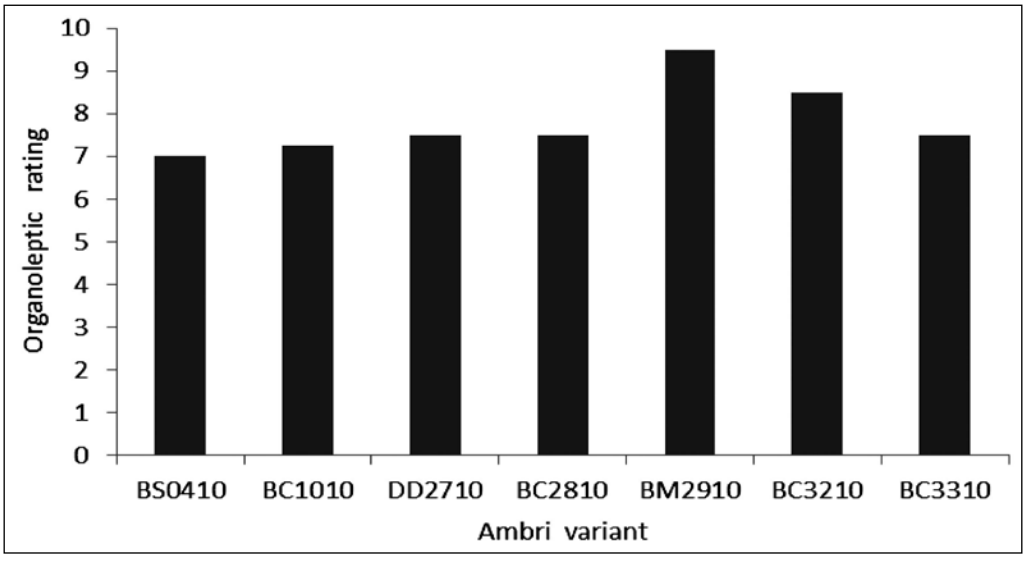

Fig. 2. Overall acceptability of '*Ambri'* apple variants.

un-inherited (environmental). The changes due to genetic causes can be inherited from one generation to the next generation, while the changes arise due to environmental causes cannot. Therefore, there must be genetic variability among the population from which better individuals are expected to be selected. Since naturally growing plants are generally of the seed origin, therefore variability present in them is mostly due to genetic causes. In light of above facts, it is evident that variability of the randomly selected samples of '*Ambri'* apple is due to genetic variation and is heritable. In the present study, considerable variation for different traits was observed particularly for titratable acidity, ascorbic acid content, fruit width, stalk width, stalk depth, fruit diameter, flesh firmness, TSS and number of seeds per fruit. Study undertaken by Kunihisa *et al*. (6) indicated that these traits are controlled by multiple alleles therefore, if variability is available, there is chance for their improvement through selection of these traits. Similarly, Mratinic and Aksic (7) while studying the local apple germplasm in Serbia found large variations for physico-chemical characteristics enabling the selection of some superior clones from the local apple types. Highest coefficient of variation in '*Ambri*' apple variants was recorded for titratable acidity among the studied population. Similarly, Mratinic and Aksic (7) also recorded the highest coefficient of variation (80.42%) for titratable acidity among different physico-chemical characters of apple. Least variable characters among the studied population pertained to fruit length and fruit width. Correlation coefficients revealed the relationship between the characters, and inference can be drawn regarding the indirect selection for a particular character. Correlation coefficients between almost all phenology traits were found significant. Fruit weight, an important character contributing towards yield was significantly and positively correlated with the dimensions of fruit and stalk and number of seeds. In general, fruit diameter proved to be a significant parameter for fruit weight and volume is correlated significantly with stalk depth and stalk width. Stalk depth was correlated significantly with stalk width and number of seeds and stalk width was correlated significantly with number of seeds. Principal component analysis (PCA) has been used previously to evaluate apple germplasm (Pereira-lorenzo *et al*., 9). A multivariate statistical tool, PCA is used to study correlations among fruit traits and to establish genetic relationships among different cultivars. Associations between different traits established by this method may correspond to genetic linkage between the loci controlling those traits or a pleiotropic effect (Oraguzie *et al*., 8). PCA coefficients are functions of the eigen values and eigen vectors of variance/ covariance matrix. Eigen values measure the variance accounted by a given principal component, and is useful for determining the number of significant factors. In the present study, three principal components having eigen value more than one were extracted. Thus, eleven characters of '*Ambri'* apple cultivar were classified into three basic factors by applying the factor analysis technique of multivariate analysis. The highest loading on first component were due fruit weight, fruit length, fruit width, fruit diameter, stalk depth, stalk width and number of seeds. TSS, titratable acidity and ascorbic acid contributed highest loading on second principle component. Flesh firmness contributed highest loading towards principle component 3. These factors could be used in further breeding programmes for getting the transgressive recombinants. Similarly, Farrokhi *et al.* (4) also reported that principle component analysis divided the yield contributing parameters into one principle component and fruit quality parameters into other. Present studies confirmed the utility of fruit weight as an important contributing trait for yield and accession identification. Principal component analysis has also been used previously to evaluate germplasm of apple (Pereira-lorenzo *et al*., 9). Further the prediction model for fruit weight worked through regression analysis is as under:

$$\text{Fruit weight} = -361.00 + 6.15\ (\text{Fruit width}) + 25.94\ (\text{Stalk width}) \quad (R^2 = 0.75)$$

Above equation reveals that with as unit increase in fruit width and stalk width, fruit weight increases 6.15 and 25.94 times, hence wider fruits have high fruit weight. No significant influence of fruit length has been observed on fruit weight, even though they were positively and significantly correlated.

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Received : September, 2015; Revised : January, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00010.4

Indian J. Hort. 73(1), March 2016: 8-12

Effect of rootstock and age of seedling on success of *in vitro* shoot tip grafting in Kinnow mandarin

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ABSTRACT

The *in vitro* shoot tip grafting (STG) is a method employed for production of virus-free plants. The present investigation was carried out to study the effect of rootstock and age of seedling on success of *in vitro* shoot tip grafting in Kinnow mandarin. Four citrus rootstocks, *viz*., sour orange (*Citrus aurantium* L.), Carrizo citrange [*Citrus sinensis* (L.) Osbeck x *Poncirus trifoliata* (L.) Raf.], rough lemon (*Citrus jambhiri* Lush.) and Cleopatra mandarin (*Citrus reshni* Hort. ex. Tan.) were selected for present study. The maximum STG success was observed in Carrizo citrange on 12-day-old seedlings. STG on rough lemon rootstock took minimum number of days for bud sprout followed by Carrizo citrange. The highest number of leaves per graft was recorded on 12-day-old Carrizo citrange seedlings followed by rough lemon. The highest scion shoot length was observed on 12-day-old seedlings of Carrizo citrange followed by that of 16-day-old rough lemon seedlings. The maximum number of roots per graft was produced in Carrizo citrange. The highest per cent transplanting success of STG plantlets was observed on Carrizo citrange followed by rough lemon. It was observed that the Carrizo citrange was found best for most of STG parameters, *viz*. per cent STG success, number of leaves per graft, scion shoot length, number of roots per graft, stock root length and transplanting success.

Key words: Citrus rootstocks, *in vitro* shoot tip grafting, Kinnow mandarin.

INTRODUCTION

Kinnow is a hybrid mandarin (*Citrus nobilis* Loureiro × *C. deliciosa* Tenore) developed by H.B. Frost in 1915 (Hoa *et al*., 3), which is widely grown in North India. Virus and virus-like diseases are the major production impediments in Kinnow growing area. There is no way to an increase in the existing yield levels and securing the citrus industry from graft transmissible diseases which causing decline, until the planting material is made free from important virus and virus-like pathogen in the country. The viral infection cannot be managed under field conditions. In *Citrus* species, the technique of *in vitro* shoot tip grafting (STG) has been well documented for the production of healthy planting material (Navarro, 7). The standard procedure of shoot-tip grafting technique as described by Navarro *et al.* (9) was used which consisted of grafting of *in vitro* generated etiolated seedling at early stage (2-3 weeks) under aseptic conditions, with a small shoot tip (0.1-0.2 mm). However, few reports have mentioned its application in Kinnow. Taking this in account, the present investigation was conducted to find out the effect of rootstock and age of seedling on success of *in vitro* shoot tip grafting.

MATERIALS AND METHODS

The present experiment was conducted in Department of Horticulture, College of Agriculture, CCS HAU, Hisar and Center for Plant Biotechnology, Department of Science and Technology, Government of Haryana, Hisar during 2011-12. Four rootstocks, *viz*. sour orange (*Citrus aurantium* L.), Carrizo citrange [*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.], rough lemon (*C. jambhiri* Lush.) and Cleopatra mandarin (*C. reshni* Hort. ex. Tan.) were selected for experiment. Shoot tips of scion cultivar Kinnow were taken from *in vitro* proliferated cultures on Murashige & Skoog (6) medium. A stereoscopic microscope was used for *in vitro* shoot tip grafting.

Fresh fruits of different rootstocks were collected in the month of January-February. Seeds were extracted and stored in juice in refrigerator. The stored seeds were washed with 2-3 drops of Tween-20® per hundred ml of water for 10 min. followed by washing under running tap water for 30 min. to remove the effect of detergent. These washed seeds were treated with 0.2% carabendazim and 0.1 per cent streptocycline for 20 min. on a magnetic stirrer at 50°C and then washed 3-4 times with double distilled water. Thereafter, seeds were decoated and surface sterilized with 0.1% $HgCl_2$ for 5 min. followed by 3-4 times washing with autoclaved double-distilled water. The seeds were treated with ethanol 70% for 30 sec followed by 3-4 times washings with autoclaved double-distilled water. The surface-sterilized seeds were inoculated individually in culture tubes containing Murashige and

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Skoog (1962) medium supplemented with BAP 0.5 mg/l and NAA 1.0 mg/l for germination. The culture tubes were incubated at 25 ± 2°C temperature in continuous darkness for 2-4 weeks. Seedlings recovered by *in vitro* seed germination were used as rootstocks. Two-to-three week (12 to 18 days) old etiolated seedlings were removed from the test tubes under aseptic conditions and decapitated leaving about 2-4 cm of epicotyl under the laminar air-flow. Cotyledons were detached and root tips cut back to about 1-2.5 cm. An inverted-T incision was made on each epicotyl through the cortex with a sterile razor blade. Decapitated rootstock was kept moist by putting a drop of liquid media at cut surface to avoid desiccation.

In shoot tip grafting, scion apical meristem was approximately 0.2-0.3 mm in length. The shoot tips of Kinnow mandarin were excised from *in vitro* raised shoots. These shoot tips (0.2-0.3 mm) along with 1-2 leaf primordial were dissected under stereoscopic microscope in laminar air-flow. Scions were kept moist by putting a drop of liquid medium to avoid desiccations at the time of grafting. An inverted-T cut was made on decapitated apical portion of rootstock. For preparation of inverted-T cut, a vertical cut of about 3-4 mm was made at apical end of decapitated seedling, after that a horizontal cut was made at half of the rootstock diameter at the base of vertical cut. The flaps of cut were opened and excised shoot tip of 0.2-0.3 mm was inserted in the cortex of cut end. All operations were carried out under aseptic conditions. The STG plants were cultured in a liquid medium composed of MS (Murashige and Skoog, 6) macro-and micro-elements fortified with the vitamins medium (WM) and sucrose @ 7.5 per cent. A folded Watman No. 4 filter paper platform was placed in the culture tube. The culture tubes were capped to ensure high relative humidity inside the tube. The micro-grafts were kept at 25 ± 1°C and exposed daily to 16/8 h photoperiod (2000 lux florescent tubes).

Five weeks after grafting, the scion of successful grafts produced 2-5 expanded leaves. The STG plants were then kept inside the culture tubes, for 1 to 2 weeks, containing half-strength MS medium. These STG plants were carefully taken out from the culture tubes and washed with sterilized distilled water to remove any adhering medium. Then, the STG plants were treated with 0.2 per cent carbendazim solution for 5 min. to prevent fungal infection. The STG plants were transferred to plastic pots containing autoclaved mixture of soil, sand and vermi-compost in the ratio of 1:1:1 and kept in greenhouse by covering pots with polyethylene bags to maintain humidity, temperature. In green house, STG plants were fertigated with half-strength of MS salts for three times during acclimatization at weekly interval. After 10-12 days, polyethylene bags were removed initially for a short duration (1-2 h) daily for 4-5 days. Gradually, the daily exposure time was increased 1 h for each day. Polyethylene bags were completely removed after 20 days. The observations were recorded for number of STG survival after 15, 30 and 45 days of transplanting in pots under greenhouse.

In order to test the significance of variation in experimental results obtained from different parameters of STG were statistically analyzed by using completely randomized block design.

RESULTS AND DISCUSSION

The maximum grafting success was recorded in Carrizo citrange followed by the rough lemon. Among the age of seedlings, mean success was higher on 16-day-old seedlings. The highest STG success was observed in Carrizo citrange on 12-day-old seedlings (38.23%). The 12 to 14 day-old seedlings in Carrizo citrange, 16-days-old in rough lemon and Cleopatra mandarin and 18-day-old seedlings in sour orange were found better for STG, respectively (Table 1).

These findings are in agreement with those reported by Navarro (8). Dass *et al*. (2) reported that the overall success of *in vitro* grafts was more in Troyer citrange followed by Carrizo citrange and rough

Table 1. Effect of rootstock and age of seedling on STG success (%) in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	16.67 (24.04)*	18.33 (25.30)	23.33 (28.84)	28.33 (32.13)	21.67 (27.58)
Carrizo citrange	38.33 (38.23)	33.33 (35.24)	26.67 (31.06)	16.67 (24.04)	28.75 (32.14)
Rough lemon	26.67 (31.06)	31.67 (34.22)	36.67 (37.24)	18.33 (25.30)	28.33 (31.95)
Cleopatra mandarin	16.67 (24.04)	23.33 (28.84)	31.67 (34.22)	18.33 (25.30)	22.50 (28.10)
Mean	24.58 (29.34)	26.67 (30.90)	29.58 (32.84)	20.42 (26.69)	
CD at 5%	Rootstock (R) = 1.64		Seedling age (D) = 1.64		R × D = 3.27

*Figures in parenthesis indicate the angular transformed values.

lemon. Karunakaran *et al.* (4) found maximum per cent success of STG when Coorg mandarin was used as scion with Rangpur lime and Troyer citrange rootstock seedlings. The results of the present study are in conformity with those of Hoa *et al*. (3) and Kumar (5). The diameter of seedling is also a deciding factor in success of STG. It was also observed that the Carrizo citrange seedlings were thicker than other seedlings. According to Navarro (8), Troyer citrange seedling reached to a size of 3-5 cm with a diameter of 1.6-1.8 mm at the point of grafting within 12 days. This study indicated that the success of *in vitro* grafting depended on type of rootstocks used because of compatibility differences between rootstock and the scion. The reduction in successful grafts with older rootstock may be due to harder stem, which was difficult to cut and insert the scion on it. The lower success in younger seedlings appears to be due to precocious callus formation that may bury the scion within it.

The sprouting in successful grafts was faster in Rough lemon followed by Carrizo citrange. It was least on 16-day-old seedlings. However, as per interaction (Table 2), STG on rough lemon seedling of 14-day age sprouted earliest. This might be due to graft compatibility differences between species. These findings are in line with those reported by Singh *et al.* (10) in *Desi* mandarin on rough lemon. Similar results were also reported by Kumar (5) in Kinnow.

The number of leaves per graft on different rootstocks varied after 45 days of grafting. The maximum number of leaves per graft was observed on Carrizo citrange followed by rough lemon. Among age of seedlings, maximum number of leaves developed per graft, when 16-day-old seedlings were used for grafting. The interaction between rootstock and age of seedling was found significant. Highest number of leaves per graft (3.44) was recorded on 12-day-old Carrizo citrange seedlings (Table 3). These differences for number of leaves per graft in different rootstocks might be due to the growth habit of rootstocks, nutrient uptake, graft compatibility and time taken to establish connection between the stock and scion. Similar results were reported by Ali *et al.* (1), while grafting of Kinnow on sour orange rootstock. The above results are in concurrence with those of Vijayakumari *et al.* (11), who also found varied response in Nagpur mandarin on rough lemon rootstock.

The length of new shoot on different rootstocks varied after 45 days of STG. The maximum length of shoot was observed on Carrizo citrange followed by that on rough lemon. Among the age of seedling, the scion shoot length was maximum on 16-day-old seedlings. The interaction was found significant. Longest shoots (1.48 cm) were observed on 12-day-old seedlings of Carrizo citrange. In rough lemon, the maximum shoot length (1.27 cm) was recorded on 14-day-old seedlings and in Cleopatra mandarin, the maximum shoot length (1.08 cm) was noted on 16-day-old seedlings. In sour orange, maximum scion shoot length (1.05 cm) was recorded on 18-day-old seedlings (Table 4). These differences for length of scion shoot in different rootstocks might be due

Table 2. Effect of rootstock and age of seedling on days taken for bud sprouting after STG in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	24.22	22.89	20.78	20.44	22.08
Carrizo citrange	19.56	19.89	20.89	22.22	20.64
Rough lemon	20.33	18.11	19.11	21.33	19.72
Cleopatra mandarin	21.44	20.78	20.11	24.56	21.72
Mean	21.39	20.42	20.22	22.14	
CD at 5% Rootstock (R) = 0.25	Seedling age (D) = 0.25			R × D = 0.49	

Table 3. Effect of rootstock and age of seedling on number of leaves per graft* in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	1.56	1.89	2.44	3.00	2.22
Carrizo citrange	3.44	3.11	2.78	2.44	2.94
Rough lemon	2.11	3.22	2.89	2.22	2.61
Cleopatra mandarin	1.78	2.44	2.78	2.56	2.39
Mean	2.22	2.67	2.72	2.56	
CD at 5% Rootstock (R) = 0.18	Seedling age (D) = 0.18			R × D = 0.37	

*Observations were recorded after 45 days of STG

Table 4. Effect of rootstock and age of seedling on scion shoot length* (cm) after STG in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	0.53	0.70	0.80	1.05	0.77
Carrizo citrange	1.48	1.27	1.17	1.00	1.23
Rough lemon	0.72	1.27	0.92	0.89	0.95
Cleopatra mandarin	0.58	0.64	1.08	0.79	0.77
Mean	0.83	0.97	0.99	0.93	
CD at 5% Rootstock (R) = 0.03	Seedling age (D) = 0.03			R × D = 0.06	

*Observations were recorded after 45 days of STG

to the growth habit of rootstock, nutrient uptake, graft compatibility and time taken in establishing connection between the stock and scion. These findings are in line with those reported by Kumar (5). Similar results were also reported by Ali *et al*. (1) on sour orange rootstock.

The maximum number of roots per graft was noted in Carrizo citrange followed by rough lemon. These differences in number of roots per graft may be due to the growth habit of rootstocks. Among age of seedlings, there was an increasing trend regarding number of roots per graft with age of seedling, which was maximum on 18-day-old seedlings (Table 5). Root length at the time of transplanting on different rootstocks also varied. The maximum root length was observed in Carrizo citrange followed by rough lemon. Among age of seedlings, there was an increasing trend regarding length of stock roots with age of seedling, which was maximum on 18-day-old seedlings (Table 6). These differences in root length may also be due to variation in growth habit of rootstocks.

The maximum percentage of transplanting success of STG plants was observed when Kinnow shoot tip was grafted on Carrizo citrange followed by 63.07 per cent on rough lemon at 15th day after transfer in sterilized soil mixture (sand: soil: vermi-compost :: 1:1:1) under greenhouse conditions (Table 7). The higher survival of grafts on Carrizo citrange and rough lemon might be due to comparatively better graft union and compatibility between stock and scion. Similar results were also reported by Karunakaran *et al.* (4) in Coorg mandarin and Kumar (5) in STG plants of Kinnow on Carrizo citrange rootstock.

Among different rootstocks, Carrizo citrange and rough lemon gave better success of *in vitro* shoot tip grafting in Kinnow mandarin (Fig. 1). It was observed that the Carrizo citrange was best for most of STG parameters, *viz.*, STG success, number of leaves per graft, scion shoot length, number of roots per graft, stock root length and transplanting success.

Table 5. Effect of rootstock and age of seedling on number of roots per graft* in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	1.44	1.78	2.44	3.00	2.17
Carrizo citrange	2.44	2.33	3.22	3.56	2.89
Rough lemon	1.56	2.11	2.44	2.78	2.22
Cleopatra mandarin	1.56	1.89	2.22	2.67	2.08
Mean	1.75	2.03	2.58	3.00	
CD at 5%	Seedling age (D) = 0.19			R × D = NS	
Rootstock (R) = 0.19					

*Observations were recorded at the time of transplanting

Table 6. Effect of rootstock and age of seedling on stock root length* (cm) after STG in Kinnow.

Rootstock	Seedling age (days)				Mean
	12	14	16	18	
Sour orange	3.73	3.98	4.20	4.43	4.09
Carrizo citrange	5.68	5.88	5.99	6.16	5.93
Rough lemon	4.87	5.21	5.48	5.73	5.32
Cleopatra mandarin	4.20	4.50	4.81	5.10	4.65
Mean	4.62	4.89	5.12	5.36	
CD at 5% Rootstock (R) = 0.05	Seedling age (D) = 0.05			R × D = 0.09	

*Observations were recorded at the time of transplanting

Table 7. Transplanting success (%) of STG plants on different rootstocks under greenhouse conditions.

Rootstock	Days after transfer in soil mixture		
	15	30	45
Sour orange	58.33 (49.81)	45.83 (42.57)	45.83 (42.57)
Carrizo citrange	83.33 (66.17)	70.83 (57.39)	70.83 (57.39)
Rough lemon	79.17 (63.07)	66.67 (54.80)	66.67 (54.80)
Cleopatra mandarin	62.50 (52.39)	54.17 (47.39)	54.17 (47.39)
CD at 5%	10.95	8.21	8.21

*Figures in parenthesis indicate the angular transformed values.

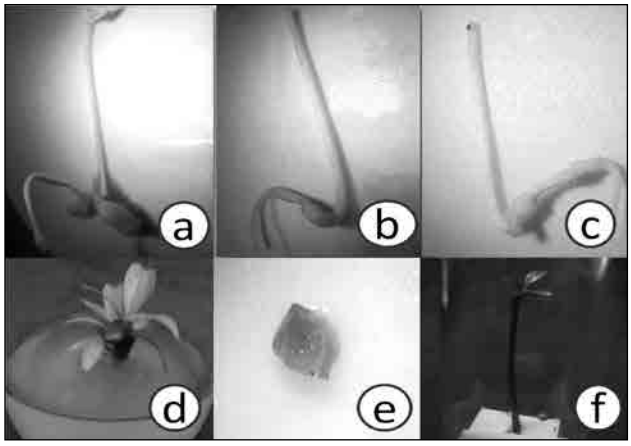

Fig. 1. Stages of *in vitro* shoot tip grafting in Kinnow mandarin; a & b = rootstock preparation; c = newly grafted plant, d & e = scion preparation; f = STG plant after 45 days of grafting.

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Received : March, 2013; Revised : December, 2015; Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00011.6

Indian J. Hort. 73(1), March 2016: 13-18

Analysis of genetic diversity among Indian Ocean coconut accessions through microsatellite markers

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ABSTRACT

The extent of genetic diversity among nineteen coconut accessions comprising collections from the Indian Ocean Islands were characterized with eight polymorphic microsatellite primers. The fixation index (Fst) was found to be higher (0.78) between Laccadive Micro (LMT) and Chowghat Orange Dwarf (COD) population and the lowest Fst value (0.04) was found among the population Guelle Rose Tall (GLT) and Sri Lankan Tall (SLT). An average Fst value of 0.48 was observed for the accessions indicating higher level of population differentiation among the accessions. The maximum genetic distance (2.29) was observed between Laccadive Green Tall (LGT) and Chowghat Orange Dwarf (COD). The minimum genetic distance (0.04) observed between Laccadive Micro (LMT) and Srilankan Tall (SLT). Overall, the within population variation was found to be higher (67%) than among the population variation (33%) for these coconut accessions. The clustering pattern distinguished two main groups among the Indian Ocean Islands population. The control population COD formed the first group and the remaining populations form the second group. The clustering within the second group revealed the relationship among the accessions under study and the information on possible migration of coconut types within the region which could be useful for planning future collections as well the utilization of conserved types.

Key words: *Cocos nucifera* L., genetic diversity, microsatellite markers.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an important pan-tropical crop grown in the islands and in the countries lining the Atlantic, Pacific and Indian oceans. The Indian Ocean is the third largest ocean with islands such as Madagascar, Zanzibar, Pemba, Sri Lanka, Andaman and Nicobar, Comoros, Lakshadweep, Seychelles and Mauritius scattered over a vast surface area of 73 million km² with wide presence of coconut mostly as cultivated and as natural stand in few places. Coconut as one of the main cultivated crops in these islands, play a major role in livelihood security of large number of families in these countries. While the history of coconuts is said to be relatively recent in some of these islands, the coconuts were ought to have been grown naturally in many islands such as Seychelles even before human arrival, which needs to be confirmed through further investigations.

While the exact origin of coconut is unknown, the centre of domestic diversity is generally agreed to be South or South East Asia. It is believed to be endemic in the Indo-Malayan region and dispersed by floating on the ocean currents to sandy and tropical coasts of the Indian and Pacific oceans, where it naturally established (Harries, 4). These naturally disseminated coconuts have characteristics such as long angular fruit, high husk to nut ratio and slow germination that favour seed dispersal by floating. Human selection in the Malesian region between South East Asia and the Western Pacific is considered as the most likely region for coconut domestication (Harries, 5, 6). The ancient sea faring Austronesian were believed to have visited as far as Madagascar and spread domesticated coconuts in Indian Ocean islands where the wild type would be naturally established already. Introgression, or introgressive hybridisation, accounts for the two groups that have been identified by fruit component analysis (Harries, 4) and by molecular analyses by various workers. The recent claim for independent origins for Indian Ocean and Pacific centres of diversity (Gunn *et al*., 3) is also consistent with the hypothesis that introgressive hybridisation between the facultatively cross-pollinated palms from these two groups resulted in the phenotypic variability within and between modern cultivated populations (Harries, 4). The present study was undertaken to understand the extent of genetic variability and relationship of eighteen coconut populations belonging to eight groups of the Indian Ocean islands.

MATERIALS AND METHODS

The experimental material consisted of eighteen exotic collections made by ICAR-CPCRI, Kasaragod

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from islands of Indian Ocean region and one indigenous germplasm conserved at the national gene bank. All the nineteen accessions used in the present study are now being conserved at the International Coconut Gene bank for South Asia under CPCRI. The details of the accessions and their place of collection are given in the Table 1. DNA was extracted from the spear leaf of the palms following the procedure of Upadhyay *et al*. (11), whereby 5 g of spear leaf tissue was ground in liquid nitrogen and transferred to extraction buffer containing 10% sodium dodecyl sulfate **(**SDS). The contents were heated at 65°C, cooled and extracted with an equal volume of 24:1 chloroform: isoamyl alcohol mixture. The supernatant was transferred to a new tube and DNA was precipitated with 70% ethanol. For microsatellite analysis, 12.5 ng of DNA template, 200 µM deoxynucleotide triphosphate (dNTPS), 1 unit of *Taq* polymerase (Bangalore Genie, India) and 1 µM

Table 1. Details of Indian Ocean coconut accessions studied.

Sl. No.	Name of Accn. (Abbrv. and No.)	No of palm(s)/ population	Place of collection	% Polymorphic loci	Observed hetero-zygosity	Observed gene diversity	Inbreeding coefficient
1.	Sambava Tall - ST-(IND139)	3	Sambava, Madagascar	87.50	0.25	0.46	0.48
2.	Sambava Green Tall - SGT-(IND141)	4	Sambava, Madagascar	75.00	0.25	0.38	0.34
3.	Pemba Orange Dwarf - POD-(IND133)	1	Pemba Island, Zanzibar	37.50	0.25	0.13	0
4.	Pemba Red Tall - PRT-(IND 136)	2	Pemba Island, Zanzibar	25.00	0.13	0.13	0
5.	Comoros Tall - CT-(IND142)	2	Comoros	75.00	0.31	0.39	0.15
6.	Comoros Moheli Tall - CTT-(IND177)	3	Comoros	62.50	0.38	0.28	0.08
7.	Coco Belu Dwarf - CBD-(IND145)	3	Seychelles	75.00	0.21	0.37	0.33
8.	Sri Lankan Tall - SLT-(IND015)	5	Sri Lanka	100.00	0.58	0.48	-0.13
9.	Sri Lankan Yellow Dwarf SLYD-(IND063)	3	Sri Lanka	100.00	0.25	0.49	0.56
10.	King Coconut - KCD-(IND052)	3	Sri Lanka	25.00	0.17	0.13	-0.06
11.	Laccadive Dwarf - LD-(IND337)	3	Lakshadweep Islands	50.00	0.13	0.23	0.25
12.	Laccadive Micro Tall - LMT-(IND030)	4	Lakshadweep Islands	50.00	0.15	0.23	0.27
13.	Laccadive Green Tall - LGT-(IND336)	7	Lakshadweep Islands	75.00	0.22	0.36	0.43
14.	Gonthembili Tall II - GT-(IND 156)	7	Sri Lanka	87.50	0.38	0.38	*0.07*
15.	Andaman Ranguchan Tall - ART-(IND017)	7	Andaman	100.00	0.43	0.51	0.28
16.	West African Tall II - WAT-(IND140)	2	Madagascar	50.00	0.31	0.23	-0.06
17.	Guelle Rose Tall - GLT-(IND138)	4	Mauritius	100.00	0.38	0.53	0.30
18.	Zanzibar Tall - ZBT-(IND037)	3	Zanzibar	75.00	0.35	0.37	0.16
19.	Chowghat Orange Dwarf COD-(IND007) (Control)	2	Kerala	0.00	0.00	0.00	0.00
	Total	68	Mean	65.79		0.32	0.18

of each primer were used as PCR reaction mixture. The polymerase chain reaction (PCR) conditions were identical to those of Perera *et al*. (10) for CAC primers and 51°C annealing temperature was set for the CnCIRG11 primer. The amplified products were resolved in a 5% denaturing polyacrylamide gel and the bands were visualized by silver staining (Bassam and Caetano-Anollés, 1). The microsatellite bands were scored manually and the alleles were sized with reference to a 30-330 bp ladder (Gibco Brl).

The calculation of genetic diversity values and construction of the unweighted pair group method with arithmetic mean (UPGMA) dendrogram using Nei's genetic distance (Nei *et al*., 8) was carried out using the POWERSSR v 1.2. Software (Liu, 7). The analysis of molecular variance was done using GENALEX software (Peakall and Smouse, 9) with a significance setting permutation value of 999.

RESULTS AND DISCUSSION

A total of 68 individual coconut palms from eighteen Indian Ocean coconut accessions and an indigenous dwarf coconut accession Chowghat Orange Dwarf (COD) from the Indian mainland were analyzed. The number of individuals varied from one for the Pemba Red dwarf (PRD) to seven each for Laccadive Green (LGT), Gonthembili (GLT) and Andaman Ranguchan (ART). A total of 31 alleles with an average of 3.9 alleles/ locus were detected across 19 Indian Ocean coconut accessions. The highest numbers of five alleles were detected for the CAC2 and CAC4 loci and lowest numbers of three alleles were detected for the CAC11, CAC13 and CAC 8 loci. The CAC2 loci showed the highest average of 2.68 alleles, while the CAC11 loci showed the lowest average of 1.21 among all the 19 accessions studied. The mean number of alleles detected for all the eight loci were highest (3.25) for the Gonthembili Tall (GLT), while it is lowest (1.13) for the Pemba Red Dwarf (PRD). The control sample COD showed a mean value of 0.75 for the alleles detected in all the loci. The highest percentage of polymorphic loci (100%) was observed for the Srilankan Yellow Dwarf (SLYD), Guelle Rose Tall (GLT), Andaman Ranguchan Tall (ART) and Sri Lankan Tall (SLT). The maximum mean observed heterozygosity value was found to be in SLT population and lowest mean observed heterozygosity were found in Pemba Red (PRD) and Laccadive Dwarf (LD) populations. The overall mean heterozygosity among all the 19 accessions for the eight loci studied was observed to be 0.27 (Table 1). The mean gene diversity value (Ho) ranged from 0.13 (Pemba Orange Dwarf (POD), Pemba Red (PRT) and King coconut (KCD)) to 0.53 (Guelle Rose Tall (GLT) among the 19 populations studied with an average of 0.32 (Table 1). Among the 8 loci studied, the primer CAC3 shows the highest gene diversity value of 0.73, while the primer CAC11 and CAC10 had a gene diversity value of 0.22.

The coefficient of inbreeding (F) is the probability that two alleles at a randomly chosen locus are identical by descent. This is an important measure since inbreeding depression results in lower performance and viability, reproductive fitness is particularly affected due to loss of dominance arising from increased homozygosity. The coefficient of inbreeding ranged from -0.13 to 0.56. The Sri Lanka Yellow Dwarf (SLYD) showed the highest inbreeding coefficient of 0.56. The Sri Lanka tall (SLT), King coconut Dwarf (KCD) and West African Tall (WAT) showed the lowest inbreeding coefficient of -0.13, -0.6 and -0.6, respectively among the populations studied. The only private allele of 172 bp was found in the CAC11 loci for the Sri Lankan Tall population (data not shown).

Wright's F-statistics was used to characterize population genetic structure. These statistics allow the partition of genetic diversity (~heterozygosity) within and among populations. Among the eight loci studied, CAC13 showed the maximum F_is, while the minimum was observed at the CAC8 and CAC10 loci. The overall mean value was observed to be 0.19. The Fit value was found to be higher in CAC13 loci and lower in CAC10 loci. The mean value was observed to be 0.58. The Fst value was found to be higher in CAC11 loci and lower in CAC2 loci. The mean value was observed to be 0.48. The Nm value was higher in CAC2 loci and lower in CAC11 loci. The mean value was found to be 0.29. The results indicate the potential of the primers to elucidate the genetic diversity among the coconut accessions under study (Table 2). Among the 19 coconut populations studied, the Fst value was found to be higher (0.78) between Laccadive Micro (LMT) and Chowghat Orange Dwarf (COD) population and the lowest Fst value (0.04) was found among the population Gonthembili (GLT) and Sri Lanka Tall (SLT). The average Fst value of 0.46 indicates the higher level of population differentiation among the accessions (Table 3). The maximum genetic distance (2.50) was observed between Laccadive Micro Tall (LMT) and Chowghat Orange Dwarf (COD) followed by Laccadive Green (LGT) and COD (2.29). The minimum genetic distance (0.04) was observed between Laccadive Micro (LMT) and Sri Lanka Tall (SLT). Generally, the genetic distance between COD and other accessions was higher. The Laccadive Green tall (LGT) and Laccadive Micro Tall (LMT) are closely related (Table 4). The within population variation was found to be higher (67%) than between the population variation (33%) of 19

Table 2. F-Statistics and estimates of Nm over all populations for each locus.

All Popln.	Locus	Fis	Fit	Fst	Nm
	CAC2	0.38	0.57	0.31	0.57
	CAC3	0.18	0.60	0.52	0.23
	CAC4	0.26	0.66	0.54	0.21
	CAC6	0.16	0.56	0.48	0.27
	CAC8	-0.01	0.44	0.45	0.31
	CAC10	-0.08	0.37	0.42	0.35
	CAC11	0.10	0.61	0.58	0.19
	CAC13	0.54	0.80	0.56	0.20
	Mean	0.19	0.58	0.48	0.29
	SE	0.07	0.05	0.03	0.05

F_{IS} = Inbreeding coefficient, F_{IT} = Overall fixation index, F_{st} = Fixation index.

coconut population accessions from Indian Ocean islands (data not shown). This indicate the need to evaluate the accessions further for effecting selection for desirable traits.

The unweighted pair group mean arithmetic (UPGMA) dendrogram was drawn for nineteen coconut populations in Indian Ocean Islands using the Nei's genetic distance. The clustering pattern distinguished two main groups among the Indian Ocean Islands population studies. COD formed the first group and the remaining populations formed the second group. The second group was further clustered into two sub-divisions. West Africa Tall (WAT), Comoros Moheli Tall (CTT), Andaman Ranguchan (ART), Zanzibar Tall (ZBT), Sri Lanka Yellow Dwarf (SLYD), Guelle Rose Tall (GLT), Laccadive Green (LGT), Laccadive Micro Tall (LMT) and Sri Lanka Tall (SLT) formed the first division and Coco Belu (CBD), Pemba orange (POD), Pemba red (PRD), King coconut (KCD), Laccadive Dwarf (LD), Comoros Tall (CT), Sambava Green (SGT), Gonthembili Tall (GT) and Sambava Tall (ST) formed the second division (Fig. 1).

Among the dwarf accessions, the Sri Lankan Yellow Dwarf (SLYD) alone separated from the rest of the dwarfs and grouped with the first cluster of talls indicating the level of out crossing in the accession. Other dwarf accessions CBD, POD, KCD and LD were grouped together within the first division along with PRT. The accession PRT though it is clustered with dwarfs, the clustering pattern indicate that it may be an out crossed POD as both the collections were from the same location and it possesses the traits of POD. The low level of observed heterozygosity and low level of gene diversity (0.13) in PRT was also substantiating the conclusion. The dwarf populations *viz.*, Sri Lankan Yellow Dwarf, Laccadive Dwarf, Pemba Orange Dwarf, King Coconut Dwarf and Coco Belu Dwarf clustered with the Tall populations and this shows the long history of cultivation in their respective regions and intercrossing with the Talls. The information stress the need to purify these accessions before using them in breeding programmes which needs further evaluation and selection.

In the present study, the coconuts as far as from Andaman in the east to the Madagascar in the western Indian Ocean showed less differentiation. The low gene diversity and microsatellite polymorphism in dwarfs is in consistent with the previous findings and the autogamous nature of the dwarfs. The Sambava Tall showed a high inbreeding coefficient (0.48) indicating the presence of an increased number of homozygotes and thus inbreeding as compared to the other populations. The Sri lankan Tall exhibited 100% polymorphic loci and a low inbreeding coefficient in contrast to the Sri lankan Yellow Dwarf, which despite a high polymorphic loci exhibited a high inbreeding coefficient (0.56) (Table 1). This is because the Sri Lankan dwarf exhibits more homozygotes in all the loci, whereas in comparison the SLT shows more heterozygotes. Similarly, the ART and GT also show more number of homozygotes despite having a polymorphism in all the loci studied. In case of other dwarfs such as COD, PRT and POD, there is complete absence of heterozygotes and hence the

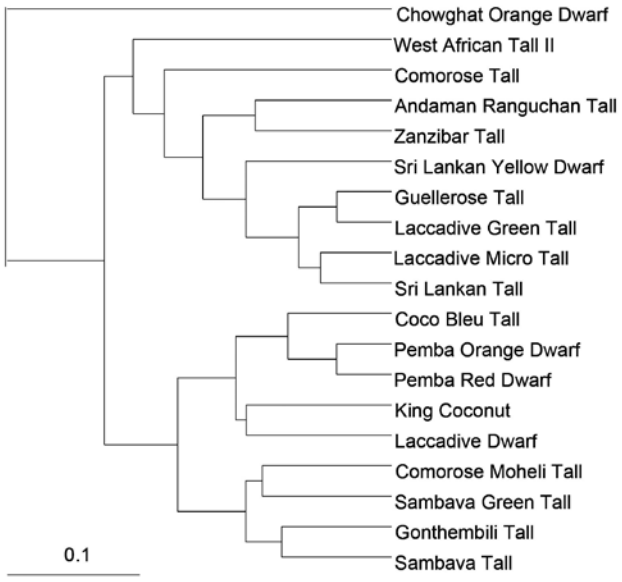

Fig. 1. UPGMA dendrogram showing the relationship between the 19 Indian Ocean coconut accessions.

Table 3. Pair-wise population Fst values of 19 coconut germplasm accessions.

ST	SGT	POD	PRT	GT	CT	CTT	CBD	WAT	KCD	LD	SLYD	LMT	LGT	GLT	ART	ZBT	COD	SLT	
0.00																			ST
0.17	0.00																		SGT
0.34	0.30	0.00																	POD
0.40	0.35	0.29	0.00																PRT
0.11	0.15	0.27	0.30	0.00															GT
0.13	0.15	0.34	0.38	0.22	0.00														CT
0.22	0.28	0.45	0.50	0.38	0.21	0.00													CTT
0.20	0.15	0.15	0.26	0.19	0.18	0.26	0.00												CBD
0.35	0.23	0.47	0.56	0.30	0.34	0.41	0.29	0.00											WAT
0.36	0.23	0.46	0.54	0.28	0.32	0.48	0.20	0.45	0.00										KCD
0.32	0.25	0.29	0.35	0.23	0.24	0.39	0.16	0.30	0.30	0.00									LD
0.15	0.19	0.37	0.42	0.25	0.15	0.18	0.25	0.33	0.41	0.31	0.00								SLYD
0.31	0.27	0.54	0.61	0.37	0.29	0.33	0.43	0.41	0.58	0.47	0.18	0.00							LMT
0.23	0.20	0.42	0.47	0.29	0.17	0.19	0.29	0.30	0.44	0.34	0.12	0.09	0.00						LGT
0.15	0.14	0.31	0.35	0.20	0.13	0.18	0.21	0.21	0.31	0.23	0.06	0.09	0.06	0.00					GLT
0.14	0.18	0.34	0.39	0.25	0.17	0.22	0.22	0.27	0.31	0.34	0.14	0.29	0.21	0.12	0.00				ART
0.16	0.27	0.44	0.49	0.32	0.16	0.19	0.29	0.37	0.43	0.34	0.12	0.24	0.16	0.10	0.12	0.00			ZBT
0.55	0.54	0.74	0.84	0.49	0.59	0.66	0.50	0.69	0.77	0.65	0.58	0.78	0.70	0.57	0.51	0.65	0.00		COD
0.16	0.15	0.30	0.36	0.19	0.16	0.19	0.22	0.24	0.33	0.27	0.10	0.07	0.08	0.04	0.17	0.15	0.59	0.00	SLT

Table 4. Pair-wise population matrix of Nei's Genetic Distance (1972) of 19 coconut germplasm accessions.

ST	SGT	POD	PRT	GT	CT	CTT	CBD	WAT	KCD	LD	SLYD	LMT	LGT	GLT	ART	ZBT	COD	SLT	
0.00																			ST
0.32	0.00																		SGT
0.59	0.41	0.00																	POD
0.56	0.36	0.09	0.00																PRT
0.22	0.24	0.38	0.30	0.00															GT
0.23	0.25	0.49	0.39	0.41	0.00														CT
0.38	0.48	0.72	0.71	0.91	0.35	0.00													CTT
0.38	0.23	0.13	0.18	0.32	0.32	0.42	0.00												CBD
0.77	0.34	0.55	0.55	0.56	0.56	0.66	0.46	0.00											WAT
0.60	0.24	0.40	0.29	0.35	0.39	0.76	0.17	0.45	0.00										KCD
0.62	0.36	0.30	0.20	0.33	0.34	0.66	0.20	0.32	0.22	0.00									LD
0.40	0.46	0.88	0.95	0.76	0.32	0.32	0.66	0.90	1.03	0.72	0.00								SLYD
0.58	0.43	0.94	0.89	0.71	0.46	0.43	0.89	0.52	0.90	0.72	0.25	0.00							LMT
0.51	0.39	0.85	0.84	0.70	0.34	0.28	0.74	0.51	0.86	0.70	0.18	0.07	0.00						LGT
0.43	0.33	0.81	0.78	0.55	0.30	0.40	0.65	0.40	0.67	0.51	0.16	0.08	0.07	0.00					GLT
0.38	0.40	0.72	0.70	0.73	0.40	0.45	0.53	0.57	0.52	0.83	0.47	0.67	0.51	0.40	0.00				ART
0.33	0.55	0.95	0.88	0.80	0.27	0.29	0.62	0.66	0.70	0.60	0.22	0.36	0.27	0.19	0.24	0.00			ZBT
1.22	0.94	1.03	0.88	0.74	1.18	1.37	0.68	0.99	0.57	0.60	1.45	2.50	2.29	1.69	1.07	1.55	0.00		COD
0.42	0.36	0.62	0.62	0.47	0.39	0.40	0.59	0.46	0.68	0.57	0.26	0.04	0.12	0.10	0.55	0.36	1.61	0.00	SLT

observed gene diversity and inbreeding coefficient is nil. The Coco Belu Dwarf exhibited all the values for tall indicating the possibility of this sample as being an out crossed one.

The accessions COD and PRT showed the maximum population differentiation measure (0.84) as compared to any other talls (0.49-0.78) included in this study. This may indicate that the PRT had an extensive out crossing in the past and selection towards more homozygosity. Moreover, PRD has been classified as belonging to the South pacific group of dwarfs in contrast to COD, which is classified with the South East Asian Dwarfs (Baudouin, and Lebrun, 2). The lowest Fst value (0.04) was observed for the SLT and the GLT followed by Yellow Dwarf and the GLT. The LMT showed low differentiation of 0.07 with SLT and 0.09 with both LGT and GLT. The genetic distance measure was the highest for the coconut populations of the Laccadive Islands (2.29-2.50), followed by the coconut populations of the western Indian Ocean (0.88-1.69) and the Srilanka Tall populations (0.74-1.61). The lone coconut population from the Seychelles showed the least genetic distance measure (0.68) and the Andaman population recorded a higher genetic measure as compared to it. The low genetic distance measure of the Seychelles can be attributed to the complete absence of the human intervention in these Islands till recently and possibly represents the naturally established coconuts that have floated from South East Asia during pre historic times. The Srilankan Tall populations are closer to the Lakshadweep and distantly related to the Andaman and Seychelles coconut population. Moreover, the cluster diagram of the Indian Ocean coconut populations did not reveal any region-wise grouping pattern.

Assessment of genetic diversity through morphological tools alone is insufficient as it is always influenced by environmental interaction. Application of DNA-based markers is complimentary to the morphological markers in identifying the genotypes in genetic resources management. The present study has revealed the interrelationships among the accession in terms of genetic diversity, which will be useful in planning new germplasm explorations for targeted traits for further enriching the gene pool.

ACKNOWLEDGMENT

The authors acknowledge the research facilities provided by the Director, ICAR-CPCRI, Kasaragod.

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Received : March, 2013; Revised : December, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00012.8

Indian J. Hort. 73(1), March 2016: 19-24

Morphological-biochemical-physiological traits assisted selection for *kusmi* lac production on *ber* (*Ziziphus mauritiana* Lam.) varieties

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ABSTRACT

***Ber* (*Ziziphus mauritiana* Lam.) is a common lac host for *kusmi* and *rangeeni* strain of *Kerria lacca*. In the present study, morphological, biochemical and physiological traits of 23 *ber* cultivars / varieties were analyzed during 2011-12 and 2012-13 to identify traits for high lac production under rainfed growing areas. *Ber* varieties were significantly different for morphological, biochemical and physiological traits. Canopy, shape, branching pattern of tree (morphological traits); initial settlement density, mean settlement length and sex ratio (among lac attributing traits); total sugars, soluble protein and chlorophyll content index (among biochemical and physiological traits) had significant association with scrapedlac. Best sub-set regression using R^2 for scrapedlac revealed that only sex ratio has predicted 62% of scrapedlac yield. Four traits, *viz*., canopy and branching pattern of tree, sex ratio of lac insect and soluble protein in leaves of *ber* varieties were able to assess it up to 67% and these traits may be used as marker, while selecting host *ber* varieties for *kusmi* lac production. Based on these parameters, out of 23 *ber* varieties four (Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karaka) were identified as promising for lac cultivation in rainfed regions of Jharkhand.**

Key words: *Ber*, *Ziziphus mauritiana* Lam., *Kerria lacca*, *kusmi* lac.

INTRODUCTION

Jujube or Indian plum or *ber* (*Ziziphus mauritiana* Lam.) is an economically important tropical fruit tree grown all over the drier parts of the Indian sub-continent for its fresh fruits (Awasthi and More, 1). It is a fast-growing and hardy tree that can bear extremes of temperatures and thrives well under dry conditions (Pareek, 7). It plays an important role in supporting livelihood income, employment, folk medicine, timber and livestock fodder. Genetic diversity of *Ziziphus* spp. in India is high and about 20 species are found between 8.5-32.5° North and 69-84° East (Azam *et al*., 2). The genetic relationship among fruit cultivars of *Z. mauritiana* Lam. (*ber*) had been realized for utilization of some genotypes in fruit as well as lac production (Saha *et al*., 8).

Ber tree is commercially exploited for lac cultivation in India extensively along with other host trees *palas* (*Butea monosperma*) and *kusum* (*Schleichera oleosa*). It is a very good host for both *rangeeni* and *kusmi* biotypes of bivoltine, *Kerria lacca*, (Kerr). Lac is the scarlet resinous secretion of scale insect which yields three useful materials resin, dye and wax. Thousands of these tiny insects colonize the suitable branches of host trees and secrete the resin as a protective covering. Only wild *ber* varieties were exploited for commercial lac cultivation in India till date. Therefore, the present study was carried out to identify morphological-biochemical-physiological traits for winter *kusmi* lac production on *ber* plants.

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MATERIALS AND METHODS

The experiment was conducted at ICAR-IINRG, Ranchi, Jharkhand, India during 2011-12 and 2012-13. Twenty three cultivars / varieties of *ber* were procured from ICAR-Central Arid Zone Research Institute, Jodhpur and planted in July 1996. Each genotype had ten trees in a row and spaced at 4 m. Trees were arranged in diagonal system of planting. Integrated nutrient management on growth and yield in *ber* was adopted as per recommended schedule except foliar spray of thiourea. All trees were pruned in February 2011 to ensure proper shoot development at inoculation time. Adequate new branches emerged at inoculation time in July. *Kusmi* broodlac was inoculated for the first time in few branches of all the cultivars/varieties of *ber* used in this study to raise winter *kusmi* crop (July 2011-Feb 2012). Broodlac was harvested at maturity in February 2012 coinciding with pruning. These *ber* varieties were again inoculated in July 2012 to raise another winter crop (July 2012 - Feb 2013). Data were recorded for tree morphology, lac yield attributing traits and biochemical-physiological traits in leaves in three replications.

Initial settlement density was measured by counting number of crawlers settled in one square centimeter area at three levels of branches (lower, middle and

upper). Mean settlement length was calculated by taking an average of settlement length of crawlers on ten branches per tree. Per cent initial insect mortality was taken at 30 days after lac inoculation by counting dead crawlers in settled length of one square centimetre. Female to male sex ratio was counted during sex differentiation stage. Harvested broodlac was weighed for each *ber* variety. The resinous cover was scraped off from the twigs, weighed as scrappedlac. Fresh leaf samples were taken from inoculated and uninoculated (control) trees of each *ber* variety for biochemical analysis. The sugars were determined by Nelson's arsenomolybdate method (Nelson, 5). Non-reducing sugar was calculated by subtracting reducing sugar from total sugars. Soluble protein was estimated by Lowrey's method (Lowrey *et al.*, 3). Chlorophyll content index (CCI) was measured by chlorophyll content meter (CCM 200, Opti-Sciences). Observations were recorded from mature leaves of three plants of each variety in inoculated and un-inoculated trees at 11 to 12 noon and average was considered for analysis. Data were analyzed for genetic variability in all traits under investigation, interaction of lac insect with *ber* varieties on biochemical and physiological traits, correlation coefficient of traits with lac yield and best subset regression as per standard biometrical approaches. CAZRI Gola is well established fruit variety and susceptible to lac insect and thus considered as check in this studies.

RESULTS AND DISCUSSION

Initial settlement density (ISD), mean settlement length (MSL), sex ratio (SR), % initial mortality (IM%), broodlac yield (LY) and scrapedlac yield (SY) were significantly different among *ber* varieties (Table 1). Initial settlement density ranged from 64.8 (Sanaur 5)

Table 1. Lac attributing characteristics on *ber* varieties for *kusmi* lac production.

Variety	ISD	MSL	SR	IM%	LY /plant	Output ratio	SY/ plant
Dandan	85.0	51.5	37.2	25.7	377.5	3.8	184.9
Aliganj	85.6	45.0	39.9	38.6	439.7	4.4	111.9
Seb × Katha F_1	113.8	54.3	34.7	18.5	265.8	2.7	84.5
Bagwadi	99.9	46.7	31.0	30.2	501.3	5.0	208.6
Illaichi	89.4	44.0	34.2	16.7	487.7	4.9	333.8*
Thornless	134.0	45.2	29.4	15.8	631.5*	6.3*	263.1
Maharwali	124.8	34.4	35.7	10.0	324.2	3.2	118.4
Kali	151.1	49.8	26.2	11.2	495.7	5.0	343.4*
CAZRI Gola	87.6	32.7	33.3	20.0	433.3	4.3	270.2
Reshmi	111.3	34.8	38.4	18.1	207.0	2.1	47.6
Katha	155.2	58.6	24.0	12.3	617.2*	6.2*	298.6
Seb × Gola F_1	120.9	55.9	34.8	21.3	670.3*	6.7*	326.5*
Seb × Tikadi BC_1	119.1	48.4	27.3	17.4	460.3	4.6	169.8
Chhuara	141.9	54.7	26.2	19.5	486.7	4.9	318.0
Umran	110.8	53.2	24.8	21.9	472.5	4.7	294.1
Jogia	167.6	58.4	22.0	30.8	690.8*	6.9*	419.3*
Banarsi Karaka	114.7	60.7	21.2	21.3	648.3*	6.5*	438.0*
ZG-3	86.1	48.7	24.3	15.6	536.3	6.4*	297.8
Seb	76.7	30.3	27.3	25.5	280.8	2.8	184.7
Sanaur 5	64.8	22.1	30.0	19.9	268.3	2.7	96.8
Kaithali	129.0	42.5	22.3	16.3	731.3*	7.3*	496.6*
Banarsi Pebandi	90.4	38.2	24.4	25.6	595.0	6.0	478.7*
Mundia	174.3	37.1	25.8	7.0	421.7	4.2	341.4*
Range	64.8-174.3	22.1-60.7	21.2-39.9	7.0-38.6	207.0-731.3	2.1-7.3	47.6-496.6
Mean	114.03	45.52	29.3	20.0	480.1	4.9	266.4
CD at 5%	41.19	19.99	4.52	13.6	176.39	1.76	51.45

ISD = initial settlement density, MSL = mean settlement length (cm), SR = sex ratio, IM% = initial mortality percent, LY = broodlac yield (g), Output ratio = broodlac output ratio, SY = scrapedlac yield (g), * = significantly higher than check, CAZRI Gola

to 174.3 (Mundia). MSL varied from 22.1 cm (Sanaur 5) to 60.7 cm (Banarasi Karaka) with mean of 45.5 cm. The lowest value of SR was recorded in Banarsi Karaka (21.0), whereas, its highest value (40.0) in Aliganj. Aliganj had highest IM% (38.6) followed by Jogia and Bagwadi. Six cultivars/varieties, *i.e.*, Thornless, Katha, Seb × Gola F_1, Jogia, Banarsi Karaka and Kaithali were having significantly higher brodlac yield than CAZRI Gola. Eight cultivars/ varieties (Illaichi, Kali, Seb × Gola F_1, Jogia, Banarsi Karaka, Kaithali, Banarsi Pebandi and Mundia) had significantly higher scrapedlac yield than CAZRI Gola. An increase of 50 to 69% in broodlac yield and 17 to 84% in scrapedlac were recorded as best varieties (Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karka) over check (CAZARI Gola).

Sucrose ingested by the phloem sap feeding insects is utilized both as respiratory substrate and also for synthesis of rehalose, mannitol etc. Trehalose has been reported as the main sugar in other phloem sap sucking insects such as aphids, followed by glucose and small amount of sucrose and fructose (Moriwaki *et al*., 4). *Ber* varieties were significantly different with respect to reducing sugars (RS), non reducing sugar (NRS), total sugars (TS) and soluble protein (SP) and chlorophyll content index (CCI). RS in the leaf was found decreased in all *ber* varieties upon inoculation with lac insect. RS varied from

Table 2. Biochemical and physiological traits in *ber* varieties and their interaction.

Variety	RS-I	RS-C	NRS-I	NRS-C	TS-I	TS-C	SP-I	SP-C	CCI-I	CCI-C
Dandan	9.6	10.1	38.1	52.6	47.7	62.7	70.4	83.1	14.1	15.9
Aliganj	10.6	10.8	34.4	40.9	45.0	51.7	81.6*	91.00	16.4	19.57
Seb × Katha F_1	9.8	10.1	17.6	27.2	27.3	37.4	62.4	63.7	12.8	15.7
Bagwadi	15.4*	16.1	55.7	55.2	71.1*	71.3	58.3	62.0	17.9	20.7
Illaichi	15.6*	16.4	49.2	52.1	64.8	68.5	47.5	54.0	21.3*	25.6
Thornless	11.5	12.2	27.4	34.6	38.8	46.8	93.0*	110.9*	16.2	20.5
Maharwali	10.0	13.0	42.2	46.6	55.3	59.6	109.0*	122.5*	18.6	21.9
Kali	9.6	14.5	19.8	33.8	29.4	48.3	77.8*	92.1	12.5	28.3
CAZRI Gola (check)	9.2	12.5	47.1	74.0	56.3	86.5	61.9	76.6	14.8	39.2
Reshmi	6.5	6.6	32.5	32.4	39.0	40.0	103.6*	115.2*	17.5	21.4
Katha	9.5	14.3	54.8	57.6	64.3	71.9	85.3*	101.0*	18. 7	24.5
Seb × Gola F_1	7.4	11.9	45.6	46.6	53.1	58.5	107.8*	131.5*	12.5	13. 7
Seb × Tikadi BC_1	6.3	6.8	44.9	55.6	51.2	62.4	127.1*	145. 9*	17.7	18.6
Chhuara	6.1	9.0	37.1	38.1	43.3	47.2	124.3*	149.9*	13.4	35.4
Umran	11.5	12.2	68.8*	69.9	80.3*	82.1	142.4*	163.2*	18.0	37.7
Jogia	6.8	8.1	34.3	44.3	41.4	52.4	103.5*	119.7*	11.7	12.7
Banarsi Karka	10.9	16.7	46.1	57.2	57.0	73.9	73.9	83.3	15.6	21.4
ZG-3	10.1	11.9	34.7	35.7	45.8	48.7	144.2*	151.1*	14.6	19.7
Seb	11.3	12.3	34.8	35.4	45.1	49.7	128.1*	128.8*	14.0	16. 7
Sanaur 5	13.9	14.8	37.9	41.3	51.8	56.0	141.9*	142.3*	8.2	12.8
Kaithali	15.2*	15.6	31.1	32.8	46.3	48.4	136.4*	137.4*	12.1	12.7
Banarsi Pebandi	11.4	11.7	38.8	43.9	50.3	55.6	180.2*	180.7*	12.8	14.5
Mundia	13.7	14.3	32.9	33.6	46.6	47.9	161.1*	164.2*	11.00	14.8
Range	6.1-15.6	6.6-16.7	17.6-68.8	27.2-69.9	27.3-80.3	37.4-86.5	47.5-180.2	54.0-180.7	8.2-21.3	12.7 -39.2
Mean	10.6	12.3	39.3	45.3	50.0	57.7	105.3	116.1	14.9	21.0
CD at 5%	4.9	5.4	12.0	14.9	12.5	15.4	14.6	16.5	4.4	6.5
Cultivar (A)	3.34		8.99		8.64		11.89		3.83	
Inoculation (B)	N/A		2.65		2.55		3.51		1.13	
A × B	4.72		N/A		12.21		16.81		5.42	

RS = reducing sugar, NRS = non-reducing sugar, TS = total sugars, SP = soluble protein, CCI = chlorophyll content index, C = control, I = inoculated, * = significantly higher than check, CAZRI Gola

6.1 (Chhuara) to 15.6 (Illaichi) mg/g fresh weight in inoculated condition. Varieties Bagwadi, Illaichi and Kaithali were having significantly higher RS than check CAZRI Gola (Table 2); however none of them had significantly higher RS than check in control condition. There was no significant difference in RS due to inoculation condition, but interaction between variety and inoculation condition was significant. Umran had significantly higher NRS-I and TS-I in inoculated condition than check. None of varieties surpassed CAZRI Gola for NRS and TS in control condition. Inoculation condition affected significantly on NRS and TS but interaction between cultivars and inoculation condition was observed significant only in case of TS. Obeed *et al*. (6) observed significant varietal differences for total soluble solids (TSS) and total, reducing, and non-reducing sugars in five *ber* varieties grown in Saudi Arabia.

The lac infestation activates the protein synthesis pathway in the plant to produce more protein for defense as well for development of both, lac insect and plant. This is due to the fact that amount of essential amino acids made available by phloem sap is insufficient to meet the insect's requirement. This shortfall is partly compensated for by the endosymbiotic proteobacterium which biosynthesizes lacking essential amino acids from sucrose and aspartate present in the phloem sap. Soluble protein (SP) ranged from 47.5 and 54.0 mg/g fresh weight (Illaichi) to 180.2 and 180.7 mg/g fresh weight (Banarasi Pebandi) in inoculation and control conditions, respectively. In our study, Thornless, Mahrawali, Reshmi, Katha, Seb × Gola, Seb × Tikadi (BC_1), Chhuara, Umran, Jogia, ZG 3, Seb, Sanour 5, Kaithali, Banarasi Pewandi, and Mundia recorded significantly more protein than check CAZRI Gola, thus finding their suitability for lac cultivation. Protein plays an important role as macromolecules in biological system, made up of different types of amino acids forming the building block, working as signal molecule in various pathways. It has been suggested that aphids can break protein, including senescence like changes, and take advantage of the increased translocation. Illaichi was the only variety having significantly higher CCI than check in inoculated condition; however CAZRI Gola had highest CCI in control condition (Table 2). These parameters were also significantly different in lac inoculated vs un-inoculated (control) conditions irrespective of variety, except for reducing sugar. The interaction between variety and inoculation *vs* control were significant for SP, CCI, RS and TS also and not significant for NRS in these *ber* varieties. As a whole sugar, protein and CCI decreased in inoculated conditions as compared to control due to the stress imposed by lac insect on plants.

Canopy, shape, branching pattern of tree (morphological traits); initial settlement density, mean settlement length and sex ratio (among lac attributing traits); total sugars, soluble protein and chlorophyll content index (among biochemical and physiological traits) had significant association with scrapedlac (Table 3). These traits may be considered while selecting *ber* varieties for raw lac. Multiple regression coefficients of all 24 *ber* varieties were estimated to find out the maximum contribution of various traits towards raw lac. Estimates provided eight best subset regressions in *ber* varieties with scrapedlac as dependent variable and eight traits as independent variables ignoring broodlac yield (Table 4). Scrapedlac yield has been predicted up to 62% by sex ratio only (model 1). Considering low Cp value, four traits, *viz*., canopy of tree, branching pattern, sex ratio and soluble protein have assessed scrapedlac up to 67%. However, it could be estimated up to 73% by all traits under investigation (model 8). Sushil *et al*. (9) developed a multivariate equation derived from 4 independent variables, *viz*., resin per female, dry cell

Table 3. Correlations coefficient among quantitative traits in *ber* varieties for lac production.

Trait	Morphological-biochemical-physiological trait	Broodlac yield	Scrapedlac yield
Morphological	Canopy of tree	**	**
	Shape of tree	*	*
	Branching pattern	**	*
Lac attributing	Initial settlement density	**	**
	Mean settlement length of insect	**	**
	Female to male sex ratio	*	*
Biochemical	Total sugars	NS	**
	Soluble protein	*	**
	Chlorophyll content index	NS	**

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

Table 4. Best subsets regression using R square for scrapedlac yield.

Model#/ Variable	Cp	Rsqr	Adj Rsqr	Tree shape	Branch	Canopy	ISD	SR	TS	SP	CCI
1	0.811	0.621	0.603					*			
2	0.444	0.666	0.633		*	*		*		*	
3	0.927	0.695	0.647		*	*	*	*		*	
4	2.177	0.710	0.645		*	*	*	*		*	
5	3.550	0.722	0.640			*	*	*		*	*
6	5.328	0.726	0.623	*	*	*	*	*		*	*
7	7.148	0.729	0.603	*	*	*	*	*	*	*	
8	9.000	0.732	0.579	*	*	*	*	*	*	*	*

Shape of tree = Compact, Semi spreading, Spreading; Branching pattern = Short, medium, Long; Canopy of tree = Closed, semi open, open; ISD = initial settlement density, SR = sex ratio, TS = total sugars, SP = soluble protein, CCI = chlorophyll content index.

weight, density at crop maturity and life period and stated that this was the most efficient and convenient model for estimating lac productivity with 97.76% accuracy.

This is for general information that we cannot get lac yield as well as fruit yield simultaneously from *ber* varieties. It was evident from previous work at our institute that fruit yield reduced drastically up to 70% after lac inoculation in local wild *ber*. Moreover, in case of failure of lac crop, we get fruit value from these cultivars. The average fruit yield of *ber* varieties at the age group of 10 to 20 years is 50-72 kg fruit/ year in rainfed situation, whereas average broodlac output ratio is 6.2 to 7.3 from 2 kg broodlac inoculated per tree in recommended *ber* cultivars. Comparing fruit and lac yield, we get Rs 1,500 to 2,160 per tree per year by selling fruit and Rs 1,980 to 2,420 by selling broodlac per tree per year. With plant density of 600 trees/ ha (4 m × 4 m). Thus, we can get Rs 1,86,000 to 3,18,000 more income from lac cultivation in one hectare plantation.

In the light of above, 23 *ber* varieties known for its fruit value were screened for lac production through morphological, biochemical and physiological markers. Four traits of morphological (canopy and branching pattern), lac attributing (sex ratio) and biochemical (soluble protein) characters may be used as marker for winter *kusmi* lac production. Four *ber* varieties known for fruit purpose, *viz.,* Kaithali, Jogia, Seb × Gola (F_1) and Banarasi Karaka were identified as potential lac hosts (Fig. 1). These *ber* cultivars/varieties may be promoted at farmers' field to enhance the economy through lac production and in case of failure of lac crop, the fruit production thus, extending lac production in *ber* growing belts.

ACKNOWLEDGEMENTS

Thanks are due to Director, ICAR-IINRG, Ranchi for the facilities and help received from the technical staff Mr S. Tripathi and Mr M.L. Rabidas.

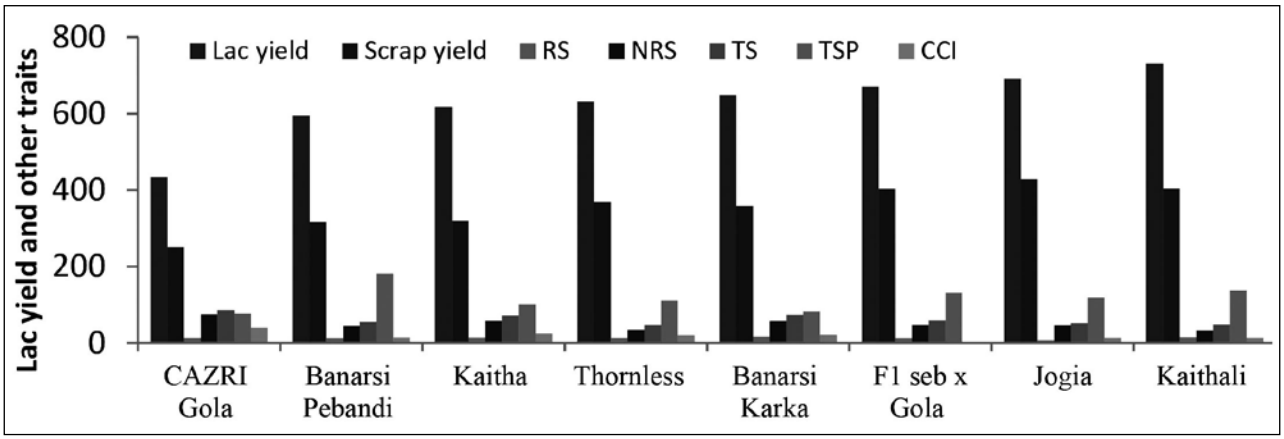

Fig. 1. Promising *ber* cultivars/varieties for *kusmi* lac production.

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Received : March, 2015; Revised : February, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00013.X

Indian J. Hort. 73(1), March 2016: 25-29

Effect of indole-3-butyric acid, putrescine and benzyladenine on rooting and lateral bud growth of *Ficus elastica* Roxb. ex Hornem leaf-bud cuttings

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ABSTRACT

An investigation was conducted to study the effects of indole-3-butyric acid (IBA), putrescine (Put) and benzyladenine (BA) on rooting and bud sprouting of leaf-bud cuttings of *Ficus elastica* Roxb. ex Hornem.. The highest number of roots per cutting (19.00) was obtained with Put 4000 ppm + IBA 4000 ppm and the lowest (4.17) was observed in control. Root length was increased with increasing Put concentrations and decreased with increasing IBA concentration. The highest length (12.17 cm) was observed with Put 4000 ppm and the lowest root length (7.1 cm) with Put 1000 + IBA 4000 ppm. The highest weight of roots (3.49 g) was obtained with Put 4000 + IBA 1000 ppm, and the lowest weight (1.2 g) was observed with control. In spite of the positive effects of IBA on rooting, higher concentrations produced thick and brittle roots, but application of Put improved the roots and led to more acceptable roots. The highest shoot length (30.5 cm) was obtained on cuttings treated with IBA 4000 + Put 4000 + BA 1000 ppm and the lowest shoot length (14.0 cm) was observed at BA 250 ppm treatment that was not significantly different compared to control. The highest number of leaves (6.0) was obtained with IBA 1000 + BA 4000 ppm and the lowest (3) obtained with control and BA 250 ppm. It was concluded that BA positively affected the number of leaves but IBA and Put had no significant effects on this parameter.

Key words: Bud sprouting, leaf-bud cuttings, polyamine, rubber tree, rooting.

INTRODUCTION

The growth hormones, regulate the growth of the plant species, which play an important role in root induction and growth of cuttings. Auxins, are the most effective in rooting in many plant species (Hartmann *et al*., 9). Polyamines (PAs), including diamine putrescine (Put), triamine spermidine (Spd) and tetramine spermine (Spm) are organic compounds with two or more primary amino groups that exist in plant cells. They play important roles in regulation of DNA replication and cell division, controlling of morphogenesis, senescence and resistance to environmental stresses (Kaur-Sawhney *et al*., 12; Couée *et al*., 2). It is documented that PAs have profound effects on plant growth and development (Farooq *et al*., 6). Cytokinins are important plant hormones that regulate various processes of plant growth and development including cell division and differentiation, enhancement of leaf expansion and nutrient mobilization (Hassan and El-Quesni, 10). The response of plants to cytokinins have been also discussed in more papers. For examples, Eraki (5) mentioned that application of BA on *Hibiscus sabdariffa* L. plants significantly increased plant height, number of branches, fresh and dry weights of leaves than the control.

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Ficus elastica (rubber tree) is a broadleaf evergreen shrub or tree that widely grown in the tropics as an ornamental tree. In colder climates, this is an extremely popular houseplant. This plant is usually propagated by stem or leaf-bud cuttings (Hartmann *et al*., 9). One of the problems in propagation with leaf-bud cuttings is no growth or slow growth of lateral buds to produce shoots. The purpose of this study was to investigate the effects of putrescine, IBA and BA on rooting and growth of lateral buds in *F. elastica* leaf-bud cuttings.

MATERIALS AND METHODS

An experiment was conducted using *F. elastica* plants in the greenhouse of Horticultural Science Department of Shiraz University. Healthy and uniform leaf-bud cuttings including the lamina, petiole and a segment of stem length of 3 to 4 cm with the lateral buds were taken from the middle portion of one -year-old shoots in January. Cuttings were dipped in 2% benomyl fungicide for 5 min. and subsequently washed with double distilled water before application of hormonal treatments. Cuttings were divided into 16 groups of 30 cuttings each. Group 1 was treated with distilled water as a control. Others were treated with indole-3-butyric acid (IBA) (1000, 2000 and 4000 ppm), putrescine (1000, 2000 and 4000 ppm) and their combinations. Cuttings were treated by submersing the stem portion in each treatment solution for 10 sec. Thereafter, cuttings were planted on a bed of sand

equipped with bottom heat facility and kept under intermittent mist system.

After 1 month of treatment, six cuttings of each treatments were removed from the medium and rooting percentage, root fresh weight, root length (length of the longest root) and root number were measured. To determine the quality of the roots, rooting index was calculated (Criley, 3). The cuttings in each category were given the following scores for their rootings: 5 for heavy rooting, 4 for medium rooting and 3 for light rooting. Alive, but not rooted cuttings and dead cuttings receive a score of 2 and 1, respectively.

When the cuttings were rooted, they were transplanted, and two cuttings were planted in a plastic pot 30 cm in diameter that were filled with a medium containing disinfected soil, leaf mold and sand (1:1:1) by volume. After the establishment of the plants in February, different concentrations of BA (0, 250, 500 and 1000 ppm) applied on each six cuttings (in three pots) of previous treatment as foliar sprays. Treatments were arranged as a factorial experiment in a completely randomized design with three replications for each treatment. After 5 months, growth indices of bud growth percentage, developed shoots (branch length), internode length and leaf number were measured.

Statistical analysis of data was performed using SAS software and the means were compared at 5% level using Duncan's multiple range test.

RESULTS AND DISCUSSION

The results showed that the all cuttings were rooted, but the best rooting index obtained from IBA 2000 ppm + Put 4000 ppm treatment (data not shown). High concentrations of auxin led to thick and brittle roots, but putrescine application improved the roots quality and increased the number of secondary thin roots. The highest number of roots per cutting (19.00) was obtained with Put 4000 ppm + IBA 4000 ppm that did not significantly differ with other concentrations of putrescine. The lowest root number (4.17) were obtained in control. Roots increased with increasing concentration of auxin and putrescine. Root length increased with increasing putrescine concentrations and decreased with increasing auxin concentration. The maximum length (12.17 cm) obtained with Put 4000 ppm and the lowest root length (7.1 cm) was related to Put 4000 ppm + IBA 4000 ppm. Root fresh weight increased with increasing auxin and putrescine concentrations. Maximum weight of roots per cutting (3.49 g) was obtained with Put 4000 ppm + IBA 1000 ppm, which was not significantly different with IBA 4000 ppm (3.44 g). Minimum root fresh weight (1.2 g) was recorded in control (Table 1).

Overall, the results indicated that although auxin had the positive effects on root formation, higher concentrations produced thick and brittle roots, but

Table 1. Interaction between IBA and putrescine on No., length and fresh weight of roots of *Ficus* cuttings.

Put (ppm)	IBA (ppm)				Mean
	Control	1000	2000	4000	
Root No.					
Control	4.16 h*	9.83 d-f	10.83 d-f	17.67 ab	10.62 B
1000	6.00 gh	10.83 d-f	11.68 c-e	17.17 ab	11.42 B
2000	8.00 fg	10.33 d-f	13.33 cd	17.17 ab	12.21 B
4000	9.50 ef	13.00 c-e	14.83 bc	19.00 a	14.08 A
Mean	6.92 D	11.00 C	12.67 B	17.75 A	
Root length (cm)					
Control	9.72 ac	8.20 bc	8.52 bc	8.58 bc	8.75 AB
1000	10.42 ab	8.17 bc	8.53 bc	7.12 c	8.56 B
2000	10.58 ab	8.85 bc	9.58 a-c	10.58 ab	9.90 A
4000	12.16 a	10.42 ab	8.75 bc	7.50 c	9.71 AB
Mean	10.72 A	8.91 B	8.85 B	8.45 B	
Root fresh wt. (g)					
Control	1.20 e	3.14 a-c	2.90 a-d	3.44 ab	2.67 AB
1000	2.06 d	2.36 cd	2.90 a-d	2.55 b-d	2.35 B
2000	2.34 cd	2.67 a-d	2.46 cd	3.23 a-c	2.79 A
4000	2.95 a-d	3.49 a	2.43 cd	3.12 a-c	3.00 A
Mean	2.13 C	2.91 AB	2.67 B	3.09 A	

*Means in each row or column with the same letter(s) are not significantly different at 5% level using Duncan's test.

use of putrescine improved the roots and led to more acceptable roots. It may be concluded that the use of putrescine with auxin can improve the quality of roots.

Many studies have hypothesized a role for polyamines in the rooting process, and their relationship with auxins and peroxidases. According to Gaspar *et al*. (8) IAA and Putrescine, an important polyamine, might be required to initiate cell division at the end of the root induction phase. Polyamines induced rooting in olive (Rugini *et al*., 17), possibly at the very early stages of rooting. It has also been suggested that polyamines might be considered precocious markers of rooting.

The results also showed that putrescine can be a useful substance in increasing *Ficus elastica* qualitity of roots. In fact, it has been demonstrated that polyamines played an important role in primary, lateral, and adventitious root development (Pastur *et al*., 16; Naija *et al*., 15). The results obtained in this study are in agreement with those obtained by Rugini *et al*. (17) in olive, Cristofori *et al.* (4) in hazelnut, Wu *et al*. (2010) in trifoliate orange and Zikah *et al*. (22) in the cuttings of GF-677 (a hybrid of peach and almond). Cristofori *et al*. (4) found that, young cuttings collected from hazelnut 'Tonda Gentile Romana' in early September rooted poorly when treated with IBA alone, but showed the best rooting (80%) after the application of a combination of 1000 ppm IBA and 1600 ppm putrescine. Friedman *et al*. (7) suggested a possible regulatory role for PAs in combination with auxins in the early phase of adventitious root formation.

The highest lateral bud growth percentage (82%) was obtained on cuttings treated with 250 ppm BA, that was significantly different from other treatments and the lowest (74%) observed at control (Table 2). The highest shoot length (30.5 cm) was obtained on cuttings treated with IBA 4000 + Put 4000 + BA 1000 ppm and the lowest shoot length (14 cm) was observed at BA 250 ppm treatment that was not significantly different compared to control (Table 3).

Means showed that BA had a positive effect on the number of leaves, but showed no significant effect of IBA and Put (data not shown). The highest number of leaves (6.6) was obtained with IBA 1000 + BA 4000

Table 2. Effect of BA on bud growth of *Ficus* cuttings.

BA (ppm)	Bud growth (%)
Control	0.74 d*
250	0.82 a
500	0.76 c
1000	0.79 b

*Means in each row or column with the same letter(s) are not significantly different at 5% level using Duncan's test.

Table 3. Effect of IBA, putresine and BA concentrations on shoot length (cm) of *Ficus* cuttings.

IBA (ppm)	Put (ppm)	BA (ppm)				Mean	Put Mean
		Control	250	500	1000		
Control	Control	16.5 eg*	14.0 g	18.0 d-g	21.3 a-f	24.66 A	21.07 C
	1000	24.8 a-e	23.3 a-g	24.1 a-f	24.7 a-e		24.43 B
	2000	26.3 a-e	25.2 a-e	25.2 a-e	28.2 a-c		25.89 B
	4000	27.8 a-d	27.9 a-d	28.2 a-c	29.1 a		28.14 A
1000	Control	14.5 fg	16.5 e-g	18.3 b-g	24.9 a-e	24.59 A	
	1000	24.5 a-e	22.3 a-g	24.0 a-f	29.2 a		
	2000	27.2 a-d	27.0 a-d	25.3 a-e	26.8 a-d		
	4000	26.8 a-d	27.8 a-d	28.0 a-d	27.8 a-d		
2000	Control	18.2 c-f	21.8 a-g	23.2 a-g	26.6 a-d	25.25 A	
	1000	23.5 a-g	24.5 a-e	25.6 a-e	25.1 a-e		
	2000	23.0 a-g	22.5 a-g	23.3 a-g	29.1 a		
	4000	27.2 a-d	28.8 a	29.3 a	29.0 a		
4000	Control	23.2 a-f	25.6 a-e	26.3 a-e	27.4 a-d	26.19 A	
	1000	23.0 a-g	23.3 a-g	23.8 a-f	23.8 a-f		
	2000	23.9 a-f	25.2 a-e	28.4 ab	26.8 a-d		
	4000	27.8 a-d	26.2 a-e	28.2 a-c	30.5 a		
BA Mean		21.01 B	24.35 B	25.19 B	27.19 A		

*Means in each row or column with the same letter(s) are not significantly different at 5% level using Duncan's test.

ppm which was significantly different compared to the other treatments. The lowest number of leaves (3.0) obtained with control and BA 250 ppm (Table 4). Overall, means showed that BA positively affected the number of leaves but IBA and Put had no significant effects on this parameter. Interaction of different treatments had no significant effect on internode length (data not shown).

In general, all three growth regulators had positive effect on aerial plant growth. Putrescine and BA had a positive effect on shoots length and BA significantly increased number of leaves. The increase of vegetative growth due to IBA treatments is in agreement with the findings of Singh (20) on *Bougainvillea peruviana*, Sharma *et al*. (19) on *Gardenia lucida* and Hussein (11) on *Thunbergia grandiflora*. They reported that IBA improved the plant vegetative growth. The promotive effect of IBA on the vegetative growth may be due to the enhancement of rooting percentage and root growth on the treated cuttings, which leads to more uptake of water and nutrients from the growing medium, resulting in an increase in vegetative growth.

Although auxins are required for axillary meristem initiation (McSteen, 14). Outgrowth of axillary buds is well correlated with the cytokinin level in the buds (Bangerth, 1). This increment in plant height in our study may be due to the role of cytokinin (BA) in increasing cell division in apical meristems and cambium. Our results are comparable with those obtained by Mazrou *et al*. (13) on sage plant and Eraki (5) on *Hibiscus sabdartffa* L. Significant effects of putrescine on shoot growth in our study are in agreement with the findings of Rugini *et al.* (18). They showed that putrescine increase the number of stem and leaf blade expansion in the pear. Further, application time of auxin and putrescine on cuttings, their effects on shoot growth is more related to the rooting of cuttings so that cuttings with stronger root system have better absorbance of water and nutrients and consequently higher cytokinin production, that increases shoot growth.

ACKNOWLEDGMENTS

This work was supported by the Eram Botanic Garden of Shiraz (Iran). We also thank Mr. Sattari for supplying the plant material.

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Table 4. Effect IBA, Put and BA concentrations on No. of leaves per cutting in *Ficus*.

IBA (ppm)	Put (ppm)	BA (ppm)				Mean	Put Mean
		Control	250	500	1000		
Control	Control	3.0 d*	3.0 d	4.0 b-d	5.3 a-d	4.8 A	4.86 A
	1000	4.5 a-d	4.5 a-d	4.8 a-d	5.0 a-d		4.71 A
	2000	4.4 a-d	5.0 a-d	5.0 a-d	5.8 ab		5.06 A
	4000	5.0 a-d	5.4 a-d	5.2 a-d	5.5 a-c		5.18 A
1000	Control	3.3 cd	5.0 a-d	4.8 a-d	6.6 a	5.05 A	
	1000	4.5 a-d	4.5 a-d	4.8 a-d	4.8 a-d		
	2000	5.0 a-d	5.2 a-d	5.0 a-d	5.8 ab		
	4000	5.2 a-d	5.3 a-d	5.2 a-d	6.0 ab		
2000	Control	4.2 a-d	5.8 ab	6.0 ab	6.3 ab	5.09 A	
	1000	4.5 a-d	4.5 a-d	5.0 a-d	5.3 a-d		
	2000	4.3 a-d	4.5 a-d	4.4 a-d	5.3 a-d		
	4000	5.0 a-d	5.8 ab	5.0 a-d	5.0 a-d		
4000	Control	4.3 a-d	4.4 a-d	4.5 a-d	4.8 a-d	4.91 A	
	1000	4.0 b-d	5.0 a-d	5.0 a-d	5.0 a-d		
	2000	4.5 a-d	5.5 a-c	5.4 a-d	6.0 ab		
	4000	4.3 a-d	4.8 a-d	5.2 a-d	5.2 a-d		
Mean		4.39 C	4.97 B	4.98 B	5.47 A		

*Means in each row or column with the same letter(s) are not significantly different at 5% level using Duncan's test.

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Received : July, 2014; Revised : November, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00014.1

Indian J. Hort. 73(1), March 2016: 30-35

Effect of drip irrigation scheduling on yield and quality of Nagpur mandarin (*Citrus reticulata* Blanco) fruits

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ABSTRACT

A experiment was conducted on 7-9 year-old bearing Nagpur mandarin (*Citrus reticulata* Blanco) based on evaporation replenishment (ER) irrigation scheduling to identify the irrigation water requirement through drip irrigation system during 2009-2012 at different stages. The fruit and quality was found significantly influenced under various evaporation replenishment (ER) based drip irrigation scheduling treatments. The highest fruit yield (21.48 tonnes/ha) was observed under irrigation at 80% ER in stages I-V and 30% ER in stage VI. Among the fruit quality irrigation scheduled at 80% ER in stages I-V and 30% ER in stage VI produced higher TSS, juice content and lower acidity. The highest TSS: acid ratio (12.7) was found in the irrigation scheduled with 30% ER in stage VI and 80% ER in stages I-V followed by the drip irrigation scheduled with 80% ER in all I-VI stages (12.2).

Key words: Citrus, critical growth stage**,** irrigation scheduling, Nagpur mandarin, yield.

INTRODUCTION

Nagpur mandarin (*Citrus reticulata* Blanco) is one of the commercial citrus fruit crop grown in 1.48 lakh ha area (fruit bearing area is 86,200 ha) with production of 8.75 lakh tonnes (Shirgure, 16). The average productivity is 10-11 t/ha, which is very low as compared to other citrus cultivars grown in India. Besides other factors, it may be due to faulty irrigation. Due to increasing scarcity of water, the mandarin orchards are being covered under drip irrigation systems. Many times the drip irrigation system is not scheduled regularly and maintaining correct irrigation intervals is not taken care of properly. The fruit yield of mandarin can be increased from 10-11 t /ha and the productivity from 16-18 t/ ha with proper adoption of drip irrigation (Shirgure *et al.*, 13).

The irrigation water requirement of Nagpur mandarin and other citrus cultivars vary with season and age under different climatic conditions. The growth of plant gets retarded below certain critical level of available moisture depending upon soil type, climatic factors and plant genetic make up. Irrigation scheduling based on depletion of available water content as 65 and 85% (Peres, 6) in Valencia orange, 40-100% (Moreshet *et al.*, 5) in 'Shamouti' orange, 80% (Shirgure *et al.*, 12) in Nagpur mandarin and 70% (Shirgure *et al.*, 14) in acid lime have been suggested. Field experiment with a mature 'Valencia' orange trees showed that the water use pattern over the entire season reached a maximum of 87 l /day in January. The highest yield (190 kg/tree) and the largest average fruit size with irrigation at a crop factor of 0.9 on a 3 day cycle was obtained (Plessis, 7). In comparison to five flood irrigation treatments in Verna lemon with daily drip irrigation at 0.475 Epan, it was concluded that the drip irrigation produced higher yield as compared to flood irrigation (Sanehez *et al.*, 9). The mature 'Satstuma' trees grafted on sour orange rootstocks showed a good response in yield and quality when irrigated with 60% of the estimated ET losses from a class 'A' pan and 80% of the control throughout the year (Castel and Buj, 2). With such a view the present investigation was carried out to identify the critical growth stages of water requirement under pan evaporation based drip irrigation scheduling in bearing Nagpur mandarin.

MATERIALS AND METHODS

To identify the critical stages of water requirement based on open pan evaporation a field experiment on scheduling drip irrigation was conducted in the block of 0.5 ha m with 6 m spacing on 7-9 year-old Nagpur mandarin orchard at ICAR-National Research Centre for Citrus, Nagpur during 2009-2012. The irrigations were scheduled on percent of pan evaporation replenishment (ER) at various stages of growth and fruit development. The different stages considered for study were stage-I (Jan-Feb), stage-II (Mar-Apr), stage-III (May-Jun), stage-IV (Jul-Aug), stage-V (Sep-Oct) and stage-IV (Nov-Dec). The treatments were drip irrigation scheduled with 30% ER in stage-I

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and 80% ER in stages II to VI (I_1), drip irrigation scheduled with 30% ER in stage-II and 80% ER in stage I and stages III to VI (I_2), drip irrigation scheduled with 30% ER in stage-III and 80% ER in stage I, stages II and IV to VI (I_3), drip irrigation scheduled with 30% ER in stage-IV and 80% ER in stages I-III, V and Stage VI (I_4), drip irrigation scheduled with 30% ER in stage-V and 80% ER in stages I-IV and stage VI (I_5), drip irrigation scheduled with 30% ER in stage-VI and 80% ER in stages I-V (I_6), and drip irrigation scheduled with 80% ER in all stages I-VI (I_7) with three replications in Randomized Block Design. The texture of the soil was clay loam and depth of the soil is 40 cm. The composite soil samples were collected for determination of field capacity and permanent wilting point. Volumetric soil moisture content at field capacity (FC) and the permanent wilting point (PWP) soil moisture content was determined using pressure plate method. The FC and PWP of the field under study was 28.2 and 18.14%, respectively. The available water content of the soil was 10.06%. The bulk density of the soil in field was determined using core sampler having 100 cm^3 volume and oven drying. The bulk density of the field was 1.47 g/ cc. The water holding capacity of the soil was 14.78 cm/ m depth of soil. Based on the average weekly open pan evaporation, the irrigation quantities were calculated taking into account pan factor (0.7), canopy factor (0.8) and crop factor (0.6). Monthly quantity of irrigation scheduled and depth and quantity of irrigation was recorded from October to December *vis-a-vis* January to June. Soil-moisture status was recorded periodically during April, 2009 to March, 2012 with the help of a neutron moisture probe. Aluminum access tubes were installed to the depth of 70 cm within the tree basin and 70 cm apart from the trunk in between the two drippers. The biometric parameters of Nagpur mandarin plants (plant height and tree spread) were recorded during October, 2009, 2010 and 2011. The plant stock girth was taken 15 cm above the ground surface. The canopy volume of the mandarin tree was calculated according to formula as suggested by Castle (1). Fruit yield and quality analyses were made as per procedures described by Ranganna (8). Leaf samples were collected and analyzed as per procedures suggested by Srivastava *et al.* (17). The leaf N was determined using alkaline permanganate steam distillation method, P by vanadomolybdophosphoric acid method and K by flame photo-metric method (Chapman and Pratt, 3). The data on fruit yield and quality attributing to the different irrigation schedules for two years were analyzed by following analysis of variance method (Gomez and Gomez, 4).

RESULTS AND DISCUSSION

The requirement of irrigation water varied as per pan evaporation and growth stage of fruits. The daily weather data recorded from NRCC Observatory, Nagpur was used for irrigation scheduling based on evaporation. The average irrigation requirement of Nagpur mandarin per plant varied from 26.5, 52.8, 59.4 and 21.3 l / plant with irrigation scheduling with 30% ER in Stage I, II, III and VI during 2009-10. The same was 70.8, 143, 158.5 and 56.8 l/ plant with the irrigation scheduled at 80% ER in all the stages during 2009-10. The average irrigation water requirement of mandarin per plant varied from 16.5, 27.8, 57.5 and 17.5 l/ plant with irrigation scheduling with 30% ER in stage I, II, III and VI during 2010-2011. The same was 44.1, 74, 153.4 and 46.8 l/ plant with the irrigation scheduled at 80 % ER in all the stages during 2010-2011. Similarly, the average irrigation water requirement of mandarin per plant varied from 19.4, 30.1, 61.7 and 20.9 l/ plant with irrigation scheduling with 30% ER in stage I, II, III and VI during 2011-2012. The same was 51.6, 80.2, 164.5 and 55.7 l/ plant with the irrigation scheduled at 80% ER during all the stages during 2011-2012 (Table 1). The irrigation water requirement of mandarin was found lower during the year 2010-2011 and higher during 2009-2010 and 2011-2012. It may be due to the variation in evaporation rates during the various growth stages. The irrigation was not scheduled during the stages IV and V as these stages coincided with the rainy season.

The effect of different drip irrigation scheduling based on percent evaporation replenishment influenced the biometric growth of Nagpur mandarin. The data on biometric growth parameters revealed that out of various growth parameters, only canopy volume produced a significant response in relation to irrigation treatments (Table 2). The plant height, stock girth and scion girth is not significant. The canopy volume was found significant during the third year of the study. The average plant height ranged from 4.41-4.71 m and stock girth from 50-55.57 cm during 2009-2010. The same was 4.61-4.84 m and 52.04-56.52 cm during 2010-2011. Similarly, the average height of the mandarin plant ranged from 4.72-4.94 m and stock girth from 52.3-56.8 cm during 2011-2012. The significant difference was observed in canopy volume ranging from 61.69 to 68.34 m^3, 62.06 to 70.22 m^3 and 62.41 to 74.21 m^3 during the three years, respectively (Table 2). The average plant height (4.83 m) was higher in the irrigation schedule having 80% ER during all the six stages. The average stock girth (56.5 cm) was higher in the irrigation scheduled with 30% ER in stage-V and 80% ER in

Table 1. Weekly mean irrigation water applied (l/ plant) under various treatments.

Treatment*	Stage I (Jan.-Feb.)	Stage II (Mar.-Apr.)	Stage III (May-June)	Stage IV (July-Aug.)	Stage V (Sept.-Oct.)	Stage VI (Nov.-Dec.)
			2009-2010			
I_1	26.5	143.0	158.5	Rain	Rain	56.8
I_2	70.8	52.8	158.5	Rain	Rain	56.8
I_3	70.8	143.0	59.4	Rain	Rain	56.8
I_4	70.8	143.0	158.5	Rain	Rain	56.8
I_5	70.8	143.0	158.5	Rain	Rain	56.8
I_6	70.8	143.0	158.5	Rain	Rain	21.3
I_7	70.8	143.0	158.5	Rain	Rain	56.8
			2010-2011			
I_1	16.5	74.0	153.4	Rain	Rain	46.8
I_2	44.1	27.8	153.4	Rain	Rain	46.8
I_3	44.1	74.0	57.5	Rain	Rain	46.8
I_4	44.1	74.0	153.4	Rain	Rain	46.8
I_5	44.1	74.0	153.4	Rain	Rain	46.8
I_6	44.1	74.0	153.4	Rain	Rain	17.5
I_7	44.1	74.0	153.4	Rain	Rain	46.8
			2011-2012			
I_1	19.4	80.2	164.5	Rain	Rain	55.7
I_2	51.6	30.1	164.5	Rain	Rain	55.7
I_3	51.6	80.2	61.7	Rain	Rain	55.7
I_4	51.6	80.2	164.5	Rain	Rain	55.7
I_5	51.6	80.2	164.5	Rain	Rain	55.7
I_6	51.6	80.2	164.5	Rain	Rain	20.9
I_7	51.6	80.2	164.5	Rain	Rain	55.7

* I_1 = irrigation schedule with 30% ER in stage-I and 80% ER in stages II to VI; I_2 = irrigation schedule with 30% ER in stage-II and 80% ER in stage I and stages III to VI; I_3 = irrigation schedule with 30% ER in stage-III and 80% ER in stage I, II and stage IV to VI; I_4 = irrigation schedule with 30% ER in stage-IV and 80% ER in stage I-III, V and stage VI; I_5 = irrigation schedule with 30% ER in stage-V and 80 % ER in stages I-IV and stage VI; I_6 = irrigation schedule with 30% ER in stage-VI and 80% ER in stages I-V; I_7 = irrigation schedule with and 80% ER in all stages I-VI.

stages I-IV and stage VI during 2009-2012. This may be mainly due to the rains and high humid conditions favouring stock and scion development. Various drip irrigation schedules in six stages influenced the canopy volume significantly. Average canopy volume observed was 69.08, 70.22 and 74.21 m^3 and in the irrigation scheduled with 30% ER in stage-VI and 80% ER in stages I-V during 2009-2010, 2010-2011 and 2011-2012, respectively. The canopy volume was moderate in the irrigation schedule 80% ER in all the stages (68.34, 68.65 and 71.18 m^3) during 2009-2012. It was lowest in the irrigation schedule of 30% ER in stages III, II and I in three years of the study. This may be mainly due to availability of constant and continuous soil moisture in plant root zone. Similar observations were also recorded in the earlier studies on irrigation scheduling in Nagpur mandarin (Shirgure *et al.*, 11) and in acid lime (Shirgure *et al.*, 10) under central Indian citrus growing conditions.

Drip irrigation scheduled based on pan evaporation replenishment in six different stages had profound effect on the yield and quality of fruits during 2009-2012. The yield and fruit quality were significantly influenced by the different drip irrigation schedules during the six stages. The number of fruits per plant, fruit yield, average fruit weight, TSS and juice content was found significant during 2010-2012. The fruit acidity was not found significant. It may be due to internal maturity condition and internal fruit quality (Table 3).

Table 2. Growth in biometric parameters and leaf nutrient content of Nagpur mandarin as affected by drip irrigation schedule.

Treatment*	Year			
	2009-2010	2010-2011	2011-2012	Mean
	Plant height (m)			
I_1	4.41	4.61	4.72	4.58
I_2	4.52	4.72	4.81	4.68
I_3	4.48	4.68	4.78	4.65
I_4	4.33	4.63	4.74	4.57
I_5	4.66	4.79	4.80	4.75
I_6	4.57	4.82	4.93	4.77
I_7	4.71	4.84	4.94	4.83
CD *(P = 0.05)*	NS	NS	NS	NS
	Stock girth (cm)			
I_1	50.0	52.0	52.3	51.5
I_2	53.7	54.7	55.0	54.5
I_3	55.4	56.4	56.7	56.2
I_4	54.0	55.0	55.4	54.9
I_5	55.5	56.5	56.8	56.3
I_6	53.4	54.4	55.1	54.3
I_7	53.4	54.4	54.6	54.2
CD *(P = 0.05)*	NS	NS	NS	NS
	Canopy volume (m^3)			
I_1	61.69	62.06	63.41	62.4
I_2	64.02	67.28	67.14	66.1
I_3	64.08	68.01	64.05	65.4
I_4	65.27	70.05	69.93	68.4
I_5	66.05	69.86	71.32	69.1
I_6	69.08	70.22	74.21	71.2
I_7	68.34	68.65	71.18	69.4
CD *(P = 0.05)*	1.04	2.4	2.2	2.27

*The treatments are as highlighted in context to Table 1.

The average number of fruits per plant varied from 348, 332 and 311 in the irrigation schedule having 80% ER in stage I and II and 30% ER in stage III, in the irrigation schedule having 80% ER in stage I and III and 30% ER in stage II followed by the irrigation schedule having 30% ER in stage I and 80% ER II and III; respectively. The number of fruits per plant was highest (628 and 631) in the irrigation schedule with 30% ER in stage VI and 80% ER in stages I-V during 2010-2011 and 2011-2012. From this it is evident that the stages III, II and I are critical and the stages IV, VI and V are less critical from the point of irrigation water requirement of Nagpur mandarin. Various drip irrigation scheduling treatments significantly influenced the yield of the mandarin. The highest fruit yield was recorded in the drip irrigation schedule with 30% ER in stage VI and 80% ER in stages I-V (17.25 and 21.48 t/ ha) followed by irrigation schedule with and 80% ER in all stages (16.09 and 19.66 t/ ha) and irrigation schedule with 30% ER in stage-V and 80% ER in stages I-IV and stage VI (16.04 and 18.94 t/ ha) in 2010-2012 (Table 3). Moderately higher yield was observed in the drip irrigation schedule with 30% ER in stage I and 80% ER in stage II and III (8.85 and 10.7 tonnes/ha) followed by the irrigation schedule with 30% ER in stage II and 80% ER in stage I and III (8.54 and 9.84 t/ ha) and the irrigation schedule with 30% ER in stage III and 80% ER in stage I and II (8.15 and 8.76 t/ ha). This clearly indicates that the stage-III (May-June), stage-II (March-April) and stage-I (January-February) are critical for water requirement in the order of III, II and I due to increase in summer months and rise in evapo-transpiration demand of the plants. It may be due to the fact that drip irrigation schedules based on ER maintained higher as well as continuous soil moisture *vis-a-vis* nutrient uptake resulting in enhanced yield. The highest average fruit weight (121.1 and 122.4 g) and lowest acidity (0.81 and 0.82%) was observed in the drip irrigation scheduled with 30% ER in stage VI and 80% ER in stages I-V. The TSS (10.2 to 10.3°Brix) and juice percent (39.1 to 39.3%) was more in irrigation scheduled with 30% ER in stage VI and 80% ER in stages I-V. The high TSS: acid ratio is indicator of sweetness of the fruit of *Ambia* flush during October-November. If the TSS to acid ratio is high, it means that the fruits have more total soluble solids and less acidity (Table 3). The highest TSS: acid ratio (12.7) was found in the irrigation scheduled with 30% ER in stage VI and 80% ER in stages I-V followed by the drip irrigation schedule with 80% ER in all I-VI stages (12.2). The lowest TSS; acid ratio (10.7) was observed the drip irrigation scheduled with 30% ER in stage III and 80% ER in stages I-II and stages IV-VI. This clearly indicates that water supply in stage III (May-June) is very essential to get good quality fruits. The similar fruit yield and quality results were observed in mandarin (Shirgure *et al.*, 12) and acid lime (Shirgure *et al.*, 15).

ACKNOWLEDGEMENT

Authors are thankful to Project Coordinator, All India Coordinated Research Project on Tropical Fruits, ICAR-IIHR, Bengaluru for providing technical and financial supports.

Table 3. Effect of irrigation schedules on the Nagpur mandarin yield and fruit quality parameters during 2010-2011 and 2011-2012.

Treatment*	Yield parameter			Quality parameter			TSS: acid ratio
	No. of fruits/ plant	Fruit wt. (g)	Yield (t/ ha)	Juice (%)	Acidity (%)	TSS (°Brix)	
			2010-2011				
I_1	348	110.2	8.85	37.4	0.84	9.11	10.8
I_2	332	103.4	8.54	37.2	0.85	9.13	10.7
I_3	311	102.8	8.15	37.3	0.85	9.09	10.7
I_4	571	105.2	15.28	38.4	0.83	9.69	11.6
I_5	576	116.3	16.04	38.7	0.83	10.02	12.0
I_6	628	121.1	17.25	39.3	0.81	10.3	12.7
I_7	581	119.3	16.09	38.9	0.82	10.0	12.2
CD(*P = 0.05*)	102	8.1	0.71	0.54	NS	0.32	--
			2011-2012				
I_1	354	109.3	10.71	37.2	0.85	9.10	10.7
I_2	340	104.5	9.84	37.1	0.85	9.11	10.7
I_3	314	103.1	8.96	37.3	0.84	9.07	10.8
I_4	582	105.8	17.05	38.1	0.84	9.63	11.4
I_5	591	115.7	18.94	38.3	0.85	10.0	11.7
I_6	631	122.9	21.48	39.1	0.82	10.2	12.4
I_7	597	118.9	19.66	38.7	0.83	9.9	11.9
CD(*P = 0.05*)	92	7.9	0.81	0.45	NS	0.18	--

*The treatments as shown in Table 1.

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Received : December, 2012; Revised : September, 2014; Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00015.3

Indian J. Hort. 73(1), March 2016: 36-41

Combining fertigation and consortium of bio-fertilizers for enhancing growth and yield of banana cv. Robusta (AAA)

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ABSTRACT

The present investigation was carried out to study the effects of combination of fertigation and consortium of bio-fertilizers in enhancing the production of banana cv. Robusta (AAA) and improving the soil biological properties. The results shown that, fertigation with 100% recommended dose of fertilizers (RDF) and 300 g of consortium of bio-fertilizers (CBF) produced significantly higher yield (115 MT ha^{-1}) as compared to other treatments in the main crop. The yield increase was nearly 32 per cent as compared to soil application of fertilizers (78 MT ha^{-1}). However, the yield difference between 100 and 75% RDF with CBF was not significant. Moreover, there was no significant yield difference between 75 and 50% RDF. In the ratoon crop, fertigation with 100% RDF and 100 g of CBF produced significantly higher yield (109 MT ha^{-1}), which was 30 per cent higher as compared to soil application of fertilizers (76 MT ha^{-1}) and 42 per cent higher than the treatment comprising of farm yard manure (FYM) + 300 g of CBF. In the ratoon crop, the yield difference between 100 and 75% RDF with CBF was not significant. Similarly, yield difference between 75 and 50% RDF was not significant. In both the cropping seasons, the soil biological activity in terms microbial population of was enhanced at higher level of consortium of bio-fertilizers.

Key words: Banana, bio-fertilizers, drip irrigation, fertigation.

INTRODUCTION

Banana is one of the important fruit crops in the tropics and India is the largest producer of banana in the world. Banana being a nutrient and water loving crop, the demand for water and nutrient is high (Srinivas *et al*.,14; Robinson and Alberts, 12), thus, it is imperative to maintain high degree of soil fertility to ensure higher productivity. This can be managed effectively by drip-fertigation as this technology has revolutionized the commercial cultivation of banana in recent years. On the other hand, the increase in demand for inorganic fertilizers and their anticipated short supply will be a major threat in the production of horticultural crops in the near future. Therefore, there is a need to reduce the dependency on the usage of organic and inorganic fertilizer by supplementing the nutrients through microbial inoculants. These microbial inoculants are known to increase the soil aggregate formation and improve the soil health. Earlier studies have proved the efficacy of fertigation (Srinivas *et al*., 14) and bio-fertilizers (Jeeva *et al*., 8; Tiwary *et al*., 15) as two different production methodologies, whereas the synergetic effects of these two techniques for realizing maximum benefits have not been worked out for sustainable production of banana. In this back drop, the present study was attempted for maximizing the production of banana cv. Robusta (AAA) through combined application of fertigation and consortium of bio-fertilizers (CBF).

MATERIALS AND METHODS

Present study was conducted at the ICAR-IIHR, Hessarghatta, Bengaluru, situated at 13º 58' N latitude, 78º E longitude and at an altitude of 890 m during 2010-12. The climate of Hessaraghatta is moderately warm with mild summer months. The maximum temperature ranges from 27º to 35ºC with a mean of 29ºC, while the minimum temperature ranges from 10.9º to 21.5ºC with a mean of 17.5ºC. The mean relative humidity is 63.5 per cent and the average rainfall is around 850 mm annum^{-1}. Three levels of fertigation, *i.e.*, 100% recommended dose of fertilizer (RDF) @ 200 g N, 110 g P, 200 K g pl^{-1} crop^{-1} (Anon, 1), 75 and 50% RDF and three levels (100, 200, 300 g pl^{-1}crop^{-1}) of CBF (*Azospirillum*, phosphate solubilizing bacteria and AM fungi mixed in equal proportion) along with soil application of recommended dose of fertilizers were used. Another treatment was comprising of FYM and 300 g of CBF. These 12 treatment combinations were replicated thrice in a randomized block design using the cv. Robusta (AAA) and a total of 9 plants were used in each treatment. The planting material of banana (*Musa* AAA), Cavendish sub-group cv. Robusta consisted of healthy sword suckers weighing around

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0.80 to 1.0 kg each were planted during the first week of Januray 2010 at a spacing of 1.5 m × 1.5 m (4,444 plants ha^{-1}). The entire quantity of phosphorous as single super phosphate was applied in the pit before planting and 15 days after the CBF was incorporated before the fertigation was started. Nitrogen was applied in the form of calcium ammonium nitrate (CAN) and potassium as muriate of potash (MOP). The fertigation was started from 60th day of planting and continued upto 320 days. For the ratoon crop, it was started after the harvest of the main crop and continued up to 270 days at weekly interval. The fertigation was given at weekly intervals and irrigation was given on daily basis, replenishing 80% of evaporation losses. Any rain, which fell, was deducted from the evaporation but rain in excess of evaporation was disregarded (Hegde and Srinivas, 7). Two emitters were placed for each plant at equal distance of 30 cm from the pseudostem with a discharge rate of 4 l of water/ h. All the suckers were removed periodically until flowering and later one sword sucker per plant was retained for the ratoon crop.

The plant height was measured from ground level to the top of the curve of the bunch stalk. Pseudostem girth was measured at 0.3 m above ground level after flowering. The bunch was weighed and number of hands and fruits were recorded individually. Ten fruits were selected randomly from the middle portion of the bunch and the total soluble solid was recorded using a hand refractometer (ERMA, Japan). The data were analyzed using Web Agri Stat Package version WASP 1.0 developed by the Indian Council of Agricultural Research Complex, Goa. The data were subjected to one way analysis of variance (ANOVA). Treatment difference was evaluated using least significant difference (LSD) at p≥0.05. The data pertaining to population of *Azospirillum* and phosphobacteria were transformed through logarithmic transformation, while the AM fungi spore load and root colonization were transformed through square root transformation and Arc sine transformation respectively for realistic interpretation of the data.

RESULTS AND DISCUSSION

The plant height at harvest did not show any marked variations due to fertigation and consortium of bio-fertilizers (CBF) both in main and ratoon crops, whereas the pseudostem girth and the number of leaves significantly varied (Fig. 1-3). The plants treated with 100% recommended dose of fertilizers (RDF) + 300 g consortium of fertilizers (CBF) recorded maximum pseudostem girth at maturity in both main and ratoon crops and plants received farm yard manure (FYM) with 300 g CBF recorded the lowest

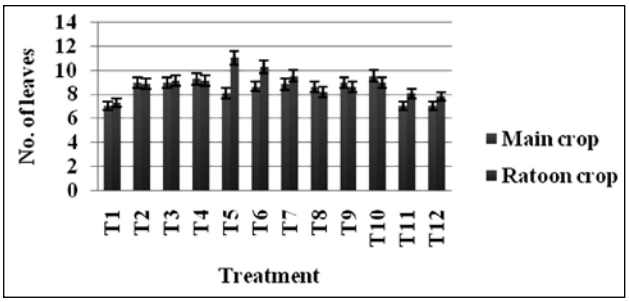

Fig. 1. Effect of fertigation and consortium of biofertilizers on No. of leaves at harvest.

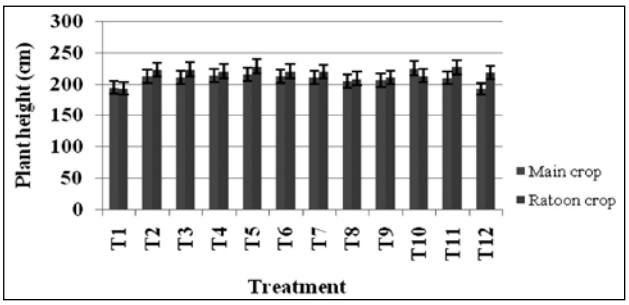

Fig. 2. Effect of fertigation and consortium of biofertilizers on plant height at harvest.

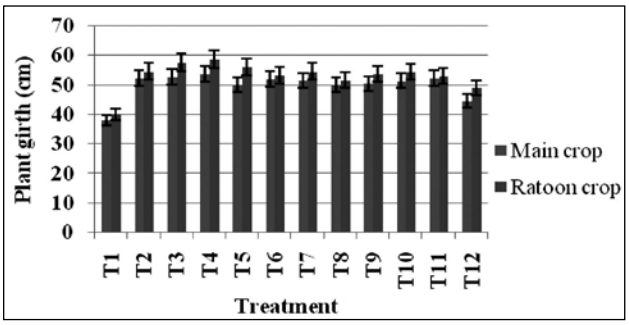

Fig. 3. Effect of fertigation and consortium of biofertilizers on plant girth at harvest.

followed by 100% RDF treatment. The combination of fertigation and CBF resulted in retention of more number of leaves till harvest. In the main crop, application of 50% RDF through fertigation with 300 g of CBF resulted in higher number of leaves per plant (9.5). Whereas, 75% RDF with 100 g of CBF retained more number of leaves in the ratoon crop. It indicates the role of CBF in slow release and mobility of nutrients to the plants even when the fertigation level was reduced to 50 and 75% of recommended dose. Present results are in conformity with the results reported by Niteen *et al*. (11) in banana.

Effect of the plants applied with the combination of fertigation and CBF recorded significantly higher yield

over control. The fruit number was highest with 100% RDF and 100 g CBF in main as well as ratoon crops (98 and 106). However, it was lower in treatment of FYM + 300 g CBF (62 and 77) and 100% RDF given through soil (79 and 87). It was also observed that the treatment of 100% RDF + 300 CBF recorded 33.65 and 26.76% higher fruit weight in the main and ratoon crops as compared to FYM + 300 g CBF treatment. It was evident that banana yield increased significantly through combined application of fertigation and CBF. In the main crop, application of 100% RDF with 300 g of CBF produced higher yield (115 MT ha^{-1}), which was 32% higher than the treatment 100% RDF applied through soil. However in the ratoon crop, 100% RDF with 100 g of CBF resulted in higher yield (109 MT ha^{-1}), which was 30 and 43% higher than the treatment of 100% RDF applied through soil and FYM + 300 g CBF (Table 1). The better growth and yield components might be attributed to reduced nutrient losses by deep percolation and leaching and also timely application of nutrients directly to the root zone of plants improving fertilizer use efficiency (Srinivas *et al*., 14). It was also observed that highest levels of fertigation resulted in heaviest bunches in both main and ratoon crops. Similar results were obtained by Mahalakshmi *et al*. (9) in banana.

In both main and ratoon crops, the yield difference between 100 and 75% RDF combined with CBF was not statistically significant. Likewise, the yield difference between 75% and 50% RDF was also not significant. The non-significant difference in yield observed with the 75 and 50% RDF might be attributed to the nutrient supplementation among the inoculated organisms, which might have mutually enhanced their efficiencies of N_2 fixation by *Azospirillum* and phosphorus solubilization by PSB (Rudresh *et al*., 13). Further, *Azospirillum* is known to produce bioactive substances having similar effect as that of the growth regulators besides N_2 fixation. Therefore, the enhanced uptake of nutrients such as N and auxins due to *Azospirillum*, could have diverted the photo-assimilates to the developing flower buds and helped in conversion of flowers to more femaleness to produce higher number of fingers which in turn increased the bunch yield (Dhanapal *et al*., 5). This improved growth parameters in turn resulted in higher bunch weight, number of fingers per hand. Besides, the increase in the growth parameters due to microorganisms may also be due to the direct role of *Azospirillum* spp. in nitrogen fixation (Jeeva *et al*., 8), phosphorus solubilization by PSB and production of plant growth substances by AM, which are known to mobilize more nutrients and make them available to the plants (Eswarappa *et al*., 6). Furthermore, the yield increase in treatments having combination of recommended dose of fertilizers and consortium of bio-fertilizers might be due to improvement in yield contributing attributes like increased number of fingers, fruit and bunch weight (Meena and Somasundram, 10).

The yield difference between 50% RDF + CBF and 75% RDF + CBF was not marked. This was largely due to the beneficial synthesis of hormones by the AM in increasing the cell division and cell

Table 1. Effect of fertigation and consortium of bio-fertilizers on the yield characters and TSS of banana.

Treatment	No. of fruits		Fruit wt. (g)		Bunch yield (kg)		Yield/ ha (MT)		TSS (°Brix)	
	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop	Main crop	Ratoon crop
1. FYM + 300 g CBF	62.00	76.80	195.16	196.74	12.10	15.71	53.77	62.79	18.25	18.50
2. 100% RDF + 100 g CBF	98.69	106.23	249.06	228.94	24.58	24.64	109.23	109.50	19.07	20.00
3. 100% RDF + 200 g CBF	97.54	98.13	250.89	243.59	24.93	24.10	110.92	107.10	19.68	20.50
4. 100% RDF + 300 g CBF	97.54	95.85	260.84	249.39	25.93	24.00	115.23	106.66	20.85	21.00
5. 75% RDF + 100 g CBF	95.92	96.67	249.00	238.85	23.87	23.09	106.08	102.61	20.35	20.75
6. 75% RDF + 200 g CBF	93.36	92.63	249.90	241.15	24.25	22.43	107.77	99.68	21.00	21.00
7. 75% RDF + 300 g CBF	94.63	94.78	257.75	232.12	24.86	22.00	111.89	97.77	21.55	21.55
8. 50% RDF + 100 g CBF	90.04	83.20	222.12	240.00	20.72	20.10	92.07	89.32	19.03	19.25
9. 50% RDF + 200 g CBF	91.40	88.00	227.27	232.00	21.15	20.50	93.99	91.10	19.07	19.50
10. 50% RDF + 300 g CBF	91.82	85.60	230.13	241.50	21.45	20.80	94.54	92.44	19.75	19.00
11. 100% RDF (fertigation)	93.60	90.50	246.00	209.25	23.00	19.00	101.06	84.44	20.06	21.00
12. 100% RDF (soil application)	78.90	86.83	230.64	198.00	17.50	17.17	77.77	76.30	18.85	18.85
CD at 5%	12.56	12.95	33.94	31.98	3.10	2.97	13.84	13.10	NS	NS

multiplication (Azcon and Bago, 2) at reduced levels of inorganic fertilizers. Tiwary *et al*. (15) also reported that inoculation with bio-fertilizers in various combinations increased the yield of banana by 18-84% over the control and the response was more pronounced when the N dose was reduced to half.

In ratoon crop, there was a decrease in the bunch weight in all the treatments except 100% RDF with 100 g CBF and FYM with CBF. The yield reduction in the ratoon crop might be basically due to reduced fruit weight as compared to the main crop. Though there was an increase in the fruit weight at 50% RDF with all the three levels of CBF. The bunch weight was also less as compared to main crop due to reduction in the number of fruits in the ratoon crop. Whereas, plants applied with FYM + 300 g of CBF resulted in higher number of fruits, fruit weight and bunch yield in the ratoon crop. This increase of yield might be due to enhanced root growth, which absorbed more nutrients and consequently accumulated higher nutrients (Baset Mia *et al*., 3).

The highest total soluble solids (TSS) was observed at the treatment combination of 100% RDF through fertigation with CBF. The TSS marginally reduced at lower doses (50% RDF fertigation + CBF), but the differences were not marked among the treatments in both main and ratoon crops. The increase in TSS at lower level of fertigation might be due to steady accumulation of nutrients especially K, all through the cropping season which resulted in higher level of sugars in the pulp. This finding corroborates with the report of Baset Mia *et al*. (3), which reveals that plant growth promoting rhizobacteria (PGPR) might improve the efficiency of absorbing applied mineral nutrients by helping the plant, scavenge limiting nutrients.

The initial population of *Azospirillum*, PSB and AM did not differ significantly in the samples, which indicated the overall uniformity of the microbial load that existed prior to planting. However, the application of CBF considerably increased the microbial population in the rhizosphere soil (Table 2). Twelve months after the application, the microbial population was significantly higher in the rhizosphere soil applied with CBF. The rhizosphere colonization of *Azospirillum* was higher (4.48×10^4 cfu g^{-1} soil) at 75% RDF + 300 g CBF in the main crop and 4.61×10^4 cfu g^{-1} soil at 75% RDF + 200 g CBF in the ratoon crop. The bacterial population was higher at 75% RDF + 300 g CBF in both main and ratoon crops with 4.76×10^4 $cfug^{-1}$ soil 4.77×10^4 cfu g^{-1} soil, respectively. Among the treatments 75% RDF + 300 g CBF recorded significantly higher microbial load in terms of AM spore density in plant (3.14 g^{-1} of dry soil) in the main and ratoon crops. AM root colonization was also higher at 75% RDF through fertigation with 300 g CBF in main and ratoon crops with a value of 56.06 and 59.03 per cent, respectively. Higher level of fertigation (100% RDF) with the combination of CBF could result in lesser microbial population compared to the 75 and 50% levels of fertigation in both the crop cycles. This indicated that the microbes would have a better association and colonization when the inorganic fertilizer level was reduced. In a similar experiment, plant growth promoting strains inoculated with minimal N fertilizer supply were found to be more effective as a bio-enhancer and bio-fertilizer to fix N_2 and increase plant growth, nutrient uptake, yield and fruit quality of banana (Baset Mia *et al*., 4). In both the crop cycles, the treatments without the combination of CBF recorded less microbial population.

In conclusion, it may be stated that growth and yield of banana can be significantly enhanced with 100% RDF, followed by 75% RDF through fertigation with a combination of a consortium of bio-fertilizers. It was noted that even a dosage of 50% RDF when combined with the consortium of bio-fertilizers could result in 18 to 23 and 17 to 21% higher yield in the main and the ratoon crop, respectively when compared to 100% RDF applied to soil. It is also an indication that, through combined application of fertigation and consortium of bio-fertilizers, the inorganic fertilizers can be saved from 25-50%. The microbial population being the biological indicators of the soil health found higher at 75% recommended dose of fertilizer through fertigation combined with consortium of bio-fertilizers.

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Table 2. Effect of fertigation and consortium of bio-fertilizers on the microbial population.

Treatment	*Azospirillum* (10^4cfu g^{-1} dry soil)			Phospobacteria (10^4cfu g^{-1} dry soil)			AM spore load (No. g^{-1} dry soil)			AM fungal root colonization (%)	
	Main crop		Ratoon crop	Main crop		Ratoon crop	Main crop		Ratoon crop	Main crop	Ratoon crop
	Initial	12th month	12th month	Initial	12th month	12th month	Initial	12th month	12th month	12th month	12th month
1. FYM + 300 g CBF	0.198 (3.29)	1.16 (4.06)	1.13 (4.05)	0.243 (3.38)	2.72 (4.43)	2.77 (4.41)	4.74 (2.17)	6.56 (2.56)	6.62 (2.57)	45.11 (42.17)	52.46 (46.42)
2. 100% RDF + 100 g CBF	0.211 (3.32)	1.21 (4.08)	2.01 (4.30)	0.251 (3.40)	2.36 (4.37)	2.14 (4.32)	4.30 (2.07)	6.65 (2.58)	7.13 (2.67)	49.32 (44.61)	58.77 (50.06)
3. 100% RDF + 200 g CBF	0.223 (3.35)	2.03 (4.31)	2.51 (4.40)	0.273 (3.43)	3.62 (4.55)	3.27 (4.51)	4.48 (2.11)	7.88 (2.80)	8.00 (2.82)	50.83 (45.60)	60.06 (50.87)
4. 100% RDF + 300 g CBF	0.193 (3.29)	1.24 (4.09)	2.38 (4.38)	0.227 (3.36)	2.84 (4.45)	2.91 (4.46)	5.01 (2.24)	6.91 (2.63)	6.89 (2.62)	57.01 (49.03)	62.16 (52.04)
5. 75% RDF + 100 g CBF	0.199 (3.30)	2.31 (4.36)	2.81 (4.45)	0.230 (3.36)	3.72 (4.57)	3.90 (4.59)	5.12 (2.26)	8.14 (2.85)	8.28 (2.88)	56.28 (48.61)	66.89 (54.88)
6. 75% RDF + 200 g CBF	0.212 (3.33)	2.77 (4.44)	4.03 (4.61)	0.263 (3.41)	4.98 (4.70)	5.05 (4.70)	4.97 (2.23)	8.83 (2.97)	8.92 (2.99)	58.15 (49.70)	68.05 (55.61)
7. 75% RDF + 300 g CBF	0.182 (3.25)	3.01 (4.48)	3.75 (4.57)	0.247 (3.39)	5.83 (4.76)	5.96 (4.77)	4.88 (2.21)	9.92 (3.14)	9.86 (3.14)	68.57 (56.06)	73.11 (59.03)
8. 50% RDF + 100 g CBF	0.204 (3.32)	1.19 (4.08)	1.64 (4.21)	0.271 (3.43)	2.49 (4.40)	2.62 (4.42)	4.77 (2.18)	7.40 (2.72)	7.52 (2.74)	51.42 (45.81)	54.48 (47.57)
9. 50% RDF + 200 g CBF	0.192 (3.29)	1.32 (4.12)	1.59 (4.20)	0.282 (3.45)	2.76 (4.44)	2.80 (4.45)	5.21 (2.28)	7.61 (2.76)	7.73 (2.78)	52.62 (46.50)	55.35 (48.07)
10. 50% RDF + 300 g CBF	0.201 (3.30)	1.57 (4.20)	1.89 (4.28)	0.264 (3.43)	3.59 (4.56)	3.73 (4.57)	5.18 (2.28)	8.60 (2.93)	8.71 (2.95)	58.34 (46.91)	58.66 (49.99)
11. 100% RDF (fertigation)	0.213 (3.33)	0.89 (3.95)	0.68 (3.83)	0.252 (3.40)	1.59 (4.20)	1.31 (4.12)	5.00 (2.24)	5.23 (2.29)	5.37 (2.32)	38.83 (38.54)	42.61 (40.75)
12. 100% RDF (soil application)	0.188 (3.29)	0.57 (3.75)	0.52 (3.71)	0.239 (3.38)	1.41 (4.14)	1.69 (4.23)	4.81 (2.19)	5.43 (2.33)	5.42 (2.33)	32.16 (34.52)	38.29 (38.21)
CD at 5%	NA	0.06	0.06	NA	0.06	0.06	NA	0.20	0.20	4.45	5.20

Figures given in the parenthesis are transformed values

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Received : June, 2013; Revised : January, 2016;
Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00016.5

Indian J. Hort. 73(1), March 2016: 42-47

Seasonal variation in leaf nutrient concentration of grapefruit

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ABSTRACT

Leaf samples of 'Star Ruby' grapefruit (*Citrus paradisi* Macf.) were collected from fruiting terminals (FT) and non-fruiting terminals (NFT) at monthly interval during the growing period to study the nutrient dynamics. The mean nutrient concentrations for N, P, K, Zn, Cu and Mn were found higher in the leaves from non-fruiting terminals (2.21%, 0.13%, 2.07%, 20.81 ppm, 10.68 ppm, 17.06 ppm) as compared to fruiting terminals (1.60%, 0.12%, 2.01%, 18.87 ppm, 9.92 ppm, 15.88 ppm). However, the mean nutrient concentrations for Ca, Mg and Fe were recorded higher in the leaves from fruiting terminals (4.10%, 0.412%, 145.50 ppm) as compared to non-fruiting terminals (3.94%, 0.367, 141.51 ppm). Similarly, K, Mg, Zn and Cu contents were higher in younger leaves, while, Ca, Fe and Mn contents were more in older leaves. There were non-significant variations in the content of N, P, K, Ca, Mg, Fe, Zn, Cu and Mn in the leaves from FT and NFT during September-October. The dynamics of leaves micronutrient content showed higher intensity of seasonal variations than the macronutrient content. The regression analysis explained the correlation between type of terminals and the linear regression in leaves accounted 86, 75 and 79% variability in data for the N, K and Mn, respectively.

Key words: Grapefruit, seasonal variations, leaf nutrients content, fruiting terminals, non-fruiting terminals.

INTRODUCTION

Grapefruit is a sub-tropical citrus plant known for its sour fruit. The fruit is valued for its pharmaceutical properties. Grapefruit juice combines the sweet and tangy flavour of the orange and shaddock and also provides up to 69% of the RDA for vitamin C along with as many as 250 mg of potassium and antioxidants. It has been reported that grapefruit juice reduces atherosclerotic plaque formation and inhibits breast cancer cell proliferation and mammary cell tumorigenesis (So *et al*., 12; Guthrie *et al*., 6).

Optimal nutrition of fruit plants is a key factor for determining growth and development of plants. Leaf mineral analysis is the best diagnostic tool for determining nutritional status of plants and represents an efficient guide for fertilization (Chatzissavvidis *et al*., 4). The position of leaf and time of sampling are quite essential to assess the nutritional status of fruit trees. The past studies have shown large variation in mineral composition of leaves due to difference in age of leaves, position of leaf on a shoot, leaf sample size and time of sampling (Srivastava and Singh, 13). Earlier studies were conducted to pinpoint a stable period and type of leaf for standardizing leaf sampling procedures in Kinnow mandarin under semi-arid conditions of north-west India. Leaf N contents increased and those of P, K and S decreased with progressive sampling beginning 3-month-old leaves on fruiting and non-fruiting terminals (Chahil *et al*., 3). Similarly, the data were collected from 30 orchards of grapefruit cv. Star Ruby showed that the optimal leaf nutrient concentrations for the trees are 1.7 to 2.1% dry weight for N, 0.08 to 0.010% for P, 0.37 to 0.48% for K, and 0.33 to 0.45% for Mg. Maintaining leaf nutrient concentrations within these ranges will support maximal yields of 110 to 120 t ha^{-1} for grapefruit (Raveh, 10). However, no information is available about the changes in mineral nutrients during the growth and development of grapefruit under arid irrigated conditions. The objective of the present investigations was to study the seasonal accumulation of the nutrient contents of grapefruit leaves.

MATERIALS AND METHODS

The trial was conducted during the year 2012 at PAU Regional Station, Abohar (Punjab) located at 30°12' N and 74°21' E with an altitude of 190 m above mean sea level. The soil was sandy loam having 8.7 pH, 0.19 dSm^{-1} EC, 0.29% organic carbon, 4.5% calcium carbonate, 0.26% N, 1.95 kg ha^{-1} P, 71.40 kg ha^{-1} K, 1.06 ppm Fe, 0.13 ppm Zn, 0.22 ppm Cu and 2.12 ppm Mn. The grapefruit cv. Star Ruby plants, budded on rough lemon were spaced at 6 m × 6 m in a square system of planting. The full bearing stage plants were maintained under standard orchard management programme. For estimation of mineral elements, spring flush leaves from the fruiting terminals (behind the fruits) and non-fruiting terminals (middle of shoots) of the plants were taken at monthly intervals in May, June, July, August, September, October, November and

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December. Each replication comprises three plants and 100 leaves per replication were collected. Leaves were collected from all the directions of plants in paper bags and stored in portable ice box before taken to Leaf Analysis Laboratory of PAU, Ludhiana for elemental analysis. Leaves were carefully rinsed with distilled water to remove surface residue and were kept at 65°C in oven until they reached stable weight. Subsequently, leaves grounded for further nutrient analysis. Nitrogen was analyzed by Kjeldahl's method using Kelplus semi-auto analyzer nitrogen estimation system (M/s Pelican Equipment, Chennai). For other elements, 0.50 g of samples was wet digested using concentrated nitric acid and perchloric acid (4:1 v/v). Phosphorus content of samples were determined by vandate-molybdate colorimetric method. Leaf K, Ca, Mg, Fe, Zn, Cu and Mn concentrations were determined using atomic absorption spectrophotometer (AAnalyst 200, Perkin Elmer, Shelton, CT, USA). All the nutrient contents were expressed on dry matter basis.

Data were analyzed using ANOVA and differences among the means were determined for significance by LSD test using the statistical analysis system software version 9.3 (SAS Institute Inc., Cary, NC, USA) at 5% level of probability. Mean and standard errors of each sample were calculated for statistical comparison. Regression analysis was undertaken to find the relative correlation for seasonal variation of nutrient between fruiting and non-fruiting terminals.

RESULTS AND DISCUSSION

The higher N content in the spring flush leaves from fruiting terminals (FT) and non-fruiting terminals (NFT) was recorded during mid growing season (Fig. 1a). In leaves from FT, the N content significantly ($p<0.05$) increased in June (1.62%) and remained statistical *at par* from June to November followed by a decline in December (1.55%). Similarly in leaves from NFT, the N content registered an increase from May (2.02%) to June (2.28%) and remained statistical *at par* from June to November followed by a decline in December (2.07%). However, the mean N content in leaves from NFT (2.21%) was higher as compared to FT (1.60%). This difference may be due to nitrogen consumption by fruits for developmental processes. Results indicate that spring season leaves tended to accumulate N during the mid-season of growth. Similar results reported by the Chahil *et al*. (3) during foliar analysis in Kinnow.

In the leaves from both FT and NFT, the P content was higher in younger leaves in May (0.123% FT, 0.130% NFT) followed by steady decline in mid-season (Fig. 1b). However, significant ($p < 0.05$) highest concentration of leaf P was noted in December. In fruiting terminals, leaf P content was lowest in August (0.115%), while in NFT it was lowest in July (0.118%). Throughout the sampling period, the mean P concentration was recorded higher in leaves from NFT (0.128%) as compared to FT (0.121%). The accumulation of P during end of season may indicate the storage of this element in leaves. Present findings are in agreement with those of Acharaya *et al*. (1).

The K content in leaves from both FT and NFT increased from May (1.87% FT, 1.96% NFT) to the significant highest level in July (2.47% FT, 2.47% NFT) and thereafter declined steadily until the end of the season (Fig. 1c). Potassium content remained steady from August to October in leaves from both type of terminals. The mean K content did not varied significantly from August to October and September to December in leaves from FT and NFT, respectively. The present findings are in congruence with the observation of Sharma and Rehman, (11).

In general, Ca content increased progressively with age of leaves although fluctuations were detected (Fig. 1d). In leaves from fruiting terminals, Ca content was highest in November (4.56%) while in NFT, the highest value was recorded in December (5.05%). Similarly, seasonal variation about the Ca content was recorded higher in the leaves from FT (4.10%) and NFT (3.94%) than the dynamic intensity of other macronutrients. At all sampling dates fruiting terminals recorded higher leaf Mg content as compared to non-fruiting terminals (Fig. 1e). In leaves from fruiting terminals, the Mg content was the highest in June (0.467%) followed by a decrease until December (0.384%). Similarly, the leaf Mg content of NFT was the maximum in July (0.408%) and decreased as in FT. The Mg content in leaves showed non-significant variations from August to October in both types of terminals. Similar results were reported by Khan *et al*. (7) on fruiting and non-fruiting terminals in oranges.

The leaf N content of FT was highly significant ($p<0.05$) and positively correlated with NFT. The common regression in leaves accounted 86% variability in data (Fig. 2a). The determination ratio $R^2 = 0.375$ for the P content, which showed 37% variability in the data within the FT and NFT (Fig. 2b). However, the F-test's p-value 0.04 is below 0.05; therefore the weak correlation about the P content within the FT and NFT. Changes within FT and NFT in leaf K and Ca content are described by the linear regression curve and the determination ratio are $R^2 = 0.748$ and 0.592, which accounted 75 and 59% variability in data, respectively (Fig. 2c & 2d). The correlation between FT and NFT macronutrient concentrations were relatively linear and in the case of N and K the ratio was essentially 1:1. But the correlation between FT and NFT for Ca concentrations was curvilinear. F-test's p-value 0.02 is below 0.05; therefore the regression equity is a

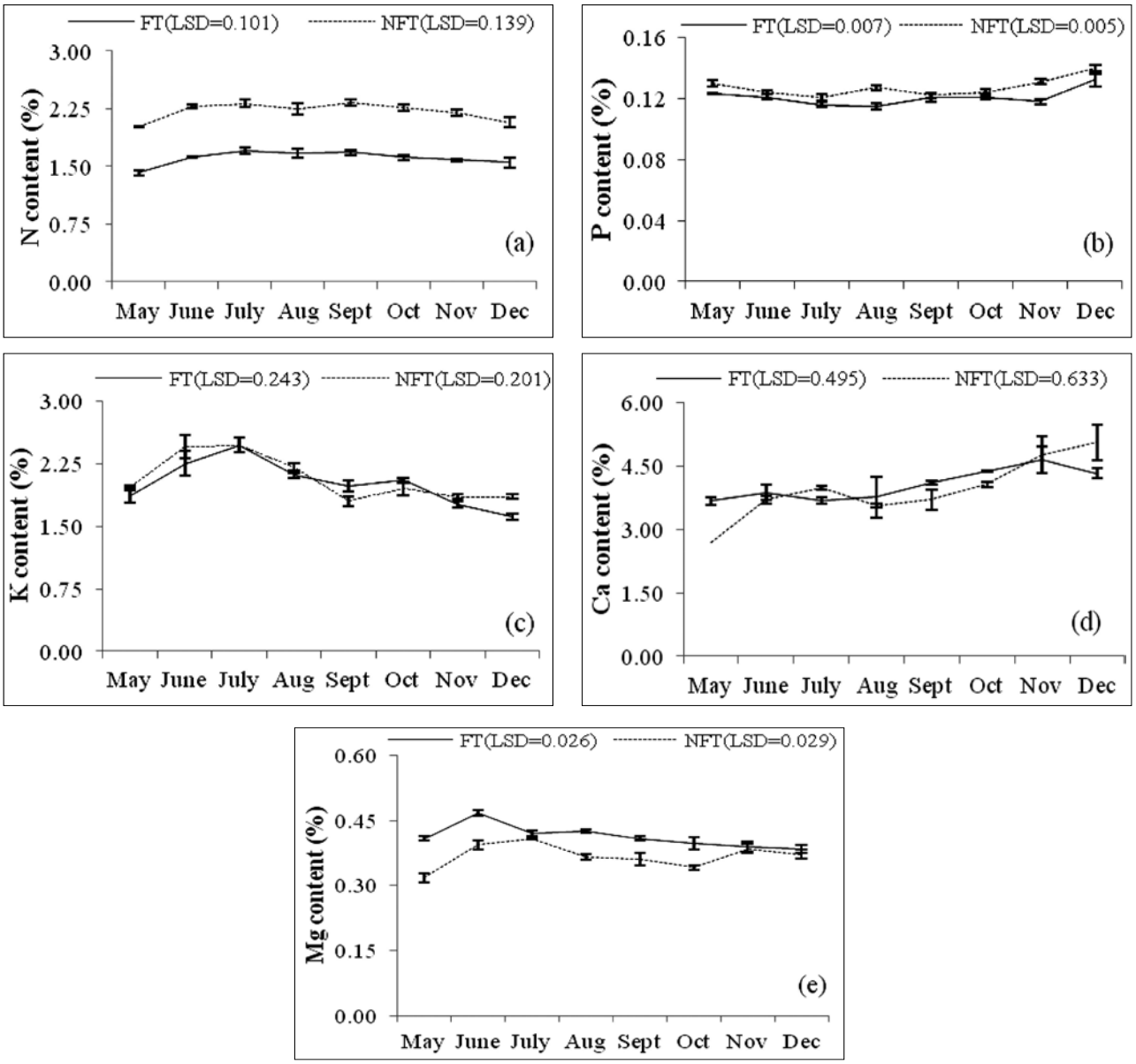

Fig. 1 (a-e). Seasonal variation of macronutrient contents in grapefruit leaves developed on fruiting and non-fruiting terminals. Vertical bar represents ± SE. LSD (0.05) indicates the least significant difference test at P<0.05.

statistically important explanation of the changes within the FT and NFT. F-test's p-value 0.410 was more than 0.05 for the leaf Mg content values within FT and NFT; therefore the regression analysis was not explained the changes. FT and NFT for Mg concentrations were not correlated (Fig. 2e). In general macronutrients (N, P and K) showed similar seasonal variations in the FT and NFT except Ca, which showed higher seasonal variation in the NFT. Earlier studies also reported significantly larger amounts of N, P and K in leaves from non-fruiting terminals, while Ca and Mg from fruiting terminals in oranges (Khan *et al*., 7). Similarly, Khokhar *et al*. (9) reported positive relationship within foliar nutrient contents in Kinnow.

The Fe content in the leaves from fruiting and non-fruiting terminals were significantly ($p < 0.05$) higher at the end of the season in December (Fig. 3a). In leaves from fruiting terminals Fe content was increased in June (161.10 ppm) sampling followed by a sharp decline in mid-season, while, non-fruiting terminals showed steady decline in Fe content up to August followed by sharp rise. The Fe content was recorded higher in the leaves from FT at all sampling dates, except for December sampling. There was more dispersion of Fe content from the mean

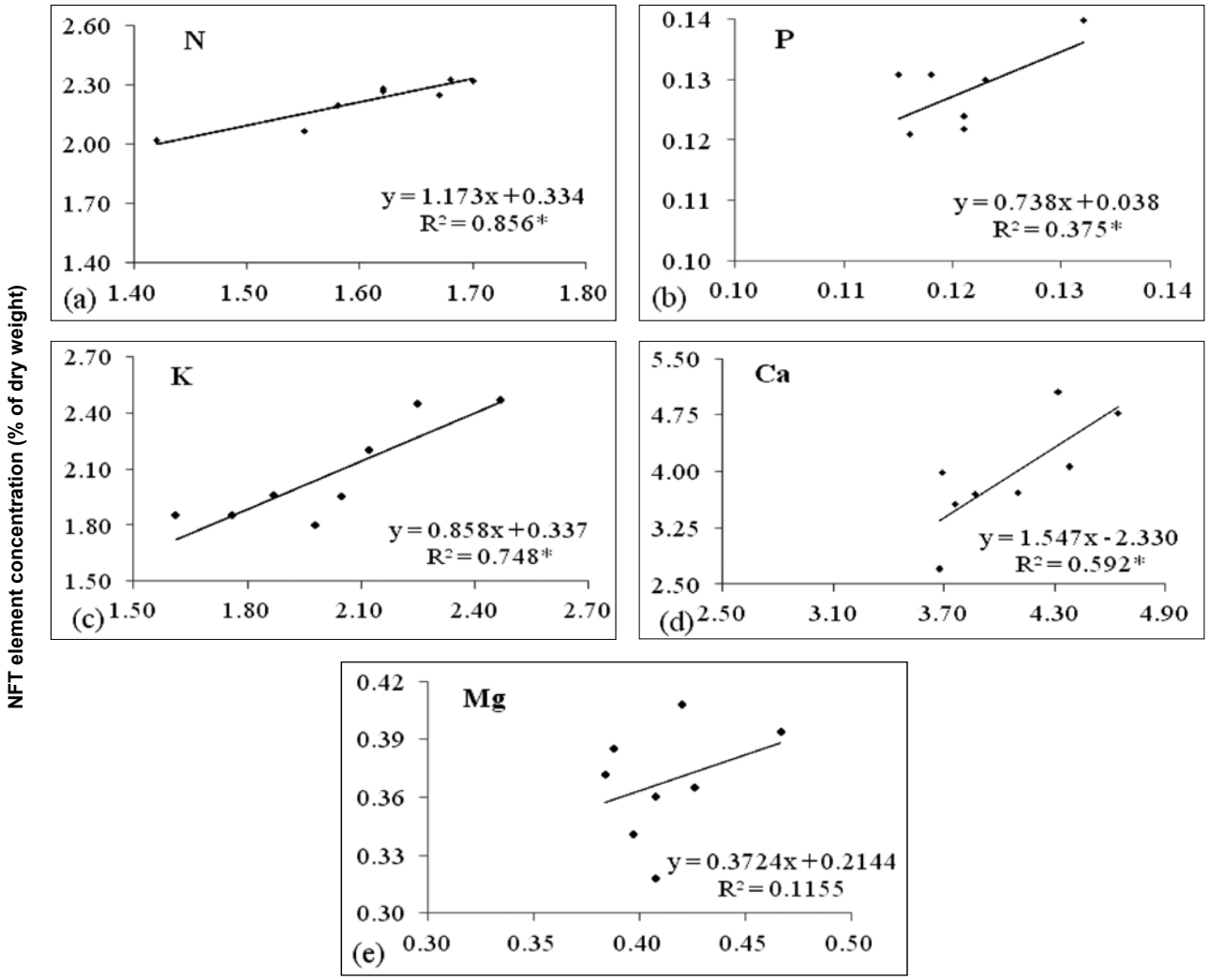

Fig. 2 (a-e). Mean leaf macronutrient concentration and correlation between fruiting and non-fruiting terminals. Asterisks indicate significance of R^2.

values in the FT (145.50 ppm) and NFT (141.51 ppm) than the other micronutrients. A similar pattern has been observed in other tree species (Brown, 2).

The higher mean Zn content in leaves from non-fruiting terminals (20.81 ppm) over fruiting terminals (18.87 ppm) was noted (Fig. 3b). The Zn content in leaves from FT was the maximum in June (23.20 ppm), then it declined up to September and subsequently an increase in Zn level was observed towards end of growth period in December (21.20 ppm). Similarly, The Zn content in leaves from NFT was the maximum in May (26.20 ppm), then it declined up to October and subsequently an increase in Zn level has been observed towards end of growth period in December (20.90 ppm). In general, higher levels of Zn in the FT and NFT were recorded during the beginning and end of the season and lowest during the mid growing season.

Large fluctuations in the content of Cu was observed in leaves from fruiting terminals and non-fruiting terminals but no categorical seasonal trend was noted (Fig. 3c). Leaves from NFT showed higher Cu content as compared to FT except for May and December sampling. The Cu content in leaves from FT was the significantly higher in May (15.88 ppm), while in NFT Cu content was recorded maximum in July (15.06 ppm). The Mn content was recorded significantly higher in older leaves in both types of terminals (Fig. 3d). The Mn content in leaves from FT and NFT was recorded minimum in May (9.00 ppm FT, 9.60 ppm NFT), while it was stabilized in middle of season followed by increase reaching maximum in December (26.50 ppm FT, 27.30 ppm NFT). Little differences were observed between leaves from FT and NFT for Mn content. Earlier workers also reported an increase in leaf Mn content with

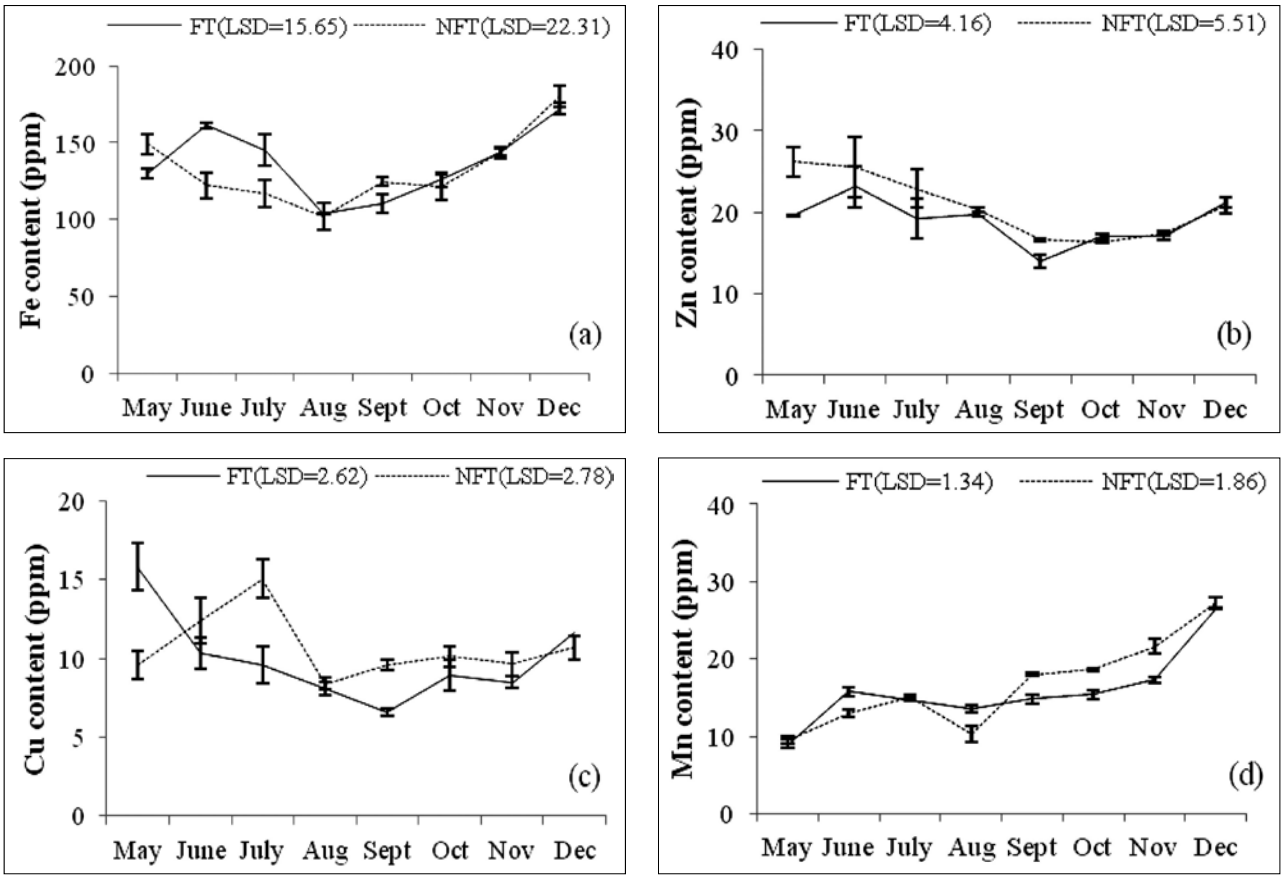

Fig. 3 (a-d). Seasonal variation in leaf micronutrient contents in grapefruit on fruiting and non-fruiting terminals. Vertical bar represents ± SE. LSD (0.05) indicates the least significant difference test at $p < 0.05$.

season. The results are in conformity with findings of Fernandez-Escobar *et al*. (5).

Leaf Zn and Mn concentrations were significant ($p < 0.05$) and positively correlated within FT and NFT. The common regression in leaves accounted 60 and 79% variability in data, respectively (Fig. 4b & d). The relationship between FT and NFT for Mn concentration was essentially 1:1 and the correlation between FT and NFT for Zn concentration was clearly curvilinear. F-test's p-value is 0.888 more than 0.05 for the leaf Cu concentrations within FT and NFT; therefore the changes were highly non-significant (Fig. 4c). Similarly, the changes for the leaf Fe concentrations within the FT and NFT were non-significant ($p > 0.05$) and the 42% variability in the data (Fig. 4a). Micronutrients (Zn, Fe, Cu, Mn) showed different seasonal variations in the FT and NFT and showed much higher intensity of seasonal variation than macronutrients. Similarly, Mirsoleimani *et al*. (9) examined seasonal variations of micronutrient contents were neither uniform nor affected by the fruiting state of trees. Khan *et al.* (7) also observed significantly higher concentration of Fe, Mn, and Cu in non-fruiting terminal leaves of Washington Naval sweet orange.

The mean values of nutrient concentrations showed non-significant variations from September to October (6-7 month-old leaves) from fruiting and non-fruiting terminals suggesting ideal period for estimating the macro- and micro-nutrient status of grapefruit plants.

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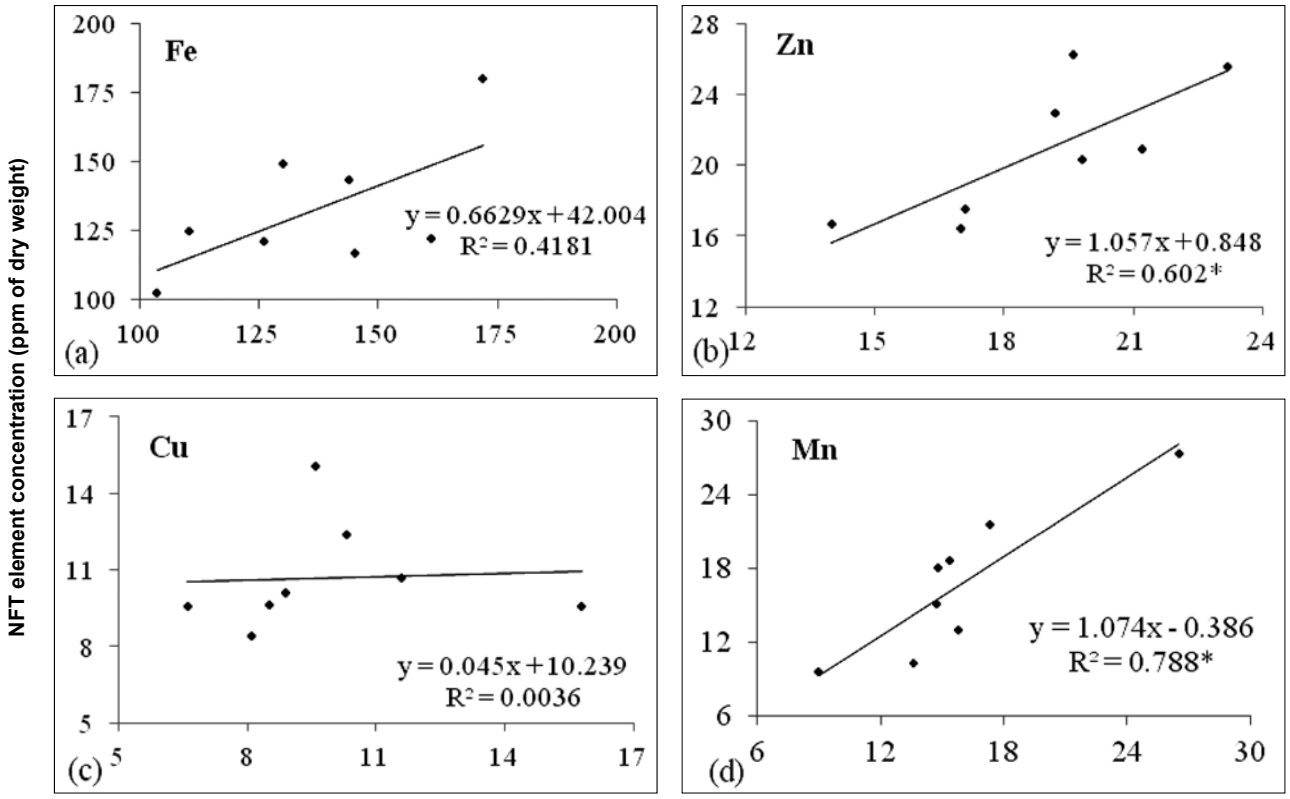

Fig. 4 (a-d). Mean micronutrient concentration in leaves and correlation between fruiting and non-fruiting terminals. Asterisks indicate significance of R^2.

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Received : July, 2014; Revised : December, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00017.7

Indian J. Hort. 73(1), March 2016: 48-53

Effect of salinity on gas exchange parameters and ionic relations in *bael* (*Aegle marmelos* Correa)

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ABSTRACT

Salt stressed *bael* cultivars showed marginal scorch, necrosis and abscission of leaves under both moderate (6.5 dS m^{-1}) and high (10.7 dS m^{-1}) salinity but control plants (1.3 dS m^{-1}) did not exhibit these injury symptoms. While cvs NB-5 and CB-1 showed delayed onset and gradual progression of the stress symptoms, while NB-9 and CB-2 were worst affected and exhibited severe marginal scorch and necrosis in over 70% of the leaves in saline soils. At high salinity, NB-9 and CB-1 plants did not survive. Salt stress significantly ($p \leq 0.05$) reduced gas exchange and 6.5 dS m^{-1} salinity caused 28-32% decline in net photosynthesis and 29-39% reduction in transpiration rate in all cultivars relative to control. Although Na^+ accumulation significantly increased in salt treated plants, cultivar NB-5 exhibited relatively similar distribution of Na^+ ions in different plant parts and also maintained higher K^+ concentrations in aerial parts. In spite of significantly high leaf Na^+ (0.29%) at 6.5 dS m^{-1} salinity, cultivar NB-5 did not exhibit severe injury symptoms. Although CB-1 cultivar showed the tendency to retain Na^+ ions in stem and root tissues, it failed to avoid the injury symptoms. Calcium was acquired in high amounts by salinized NB-5 plants as compared to others. Restricted Na^+ uptake and preferential K^+ accumulation seemed to contribute to alleviate the salt stress in cultivar NB-5.

Key words: *Aegle marmelos* Correa, gas exchange, salt stress, sodium uptake.

INTRODUCTION

Soil salinity refers to the build-up of soluble salts and/or exchangeable sodium in soil profile in excess amounts resulting in severe limitations for agricultural production. It is estimated that about 6.73 m ha lands in India are affected by salinity and sodicity stresses. In saline soils, initial osmotic stress due to low water potential in root zone followed by nutrient imbalances and toxicities caused by the excessive uptake of Na^+ and Cl^- ions impair the plant growth (Sharma and Singh, 7). Although majority of fruit crops are categorized as sensitive to salinity, wide genetic differences exist and some of the scion and rootstock cultivars may exhibit higher salt tolerance as compared to others. While considerable work has been done to identify salt tolerant types in crops such as citrus (Awasthi *et al*., 2), some of the underutilized fruits of Indian origin remain under-researched. *Bael* (*Aegle marmelos* Correa; Rutaceae) is such a fruit which is widely distributed throughout the Indian subcontinent, particularly in arid and semi-arid tracts where salinization is a major problem. Different parts of *bael* tree, rich in bioactive compounds such as marmelosin, have long been used as ingredients in traditional Indian medicine. While mature and half-ripe fruits are used to cure stomach ailments, ripe fruits have restorative and laxative properties (Singh *et al*., 9).

In recent past, commercial cultivation of *bael* has steadily increased in many arid and semi-arid regions of India, which suffer from constraints such as secondary salinity and increasing scarcity of good quality irrigation water. Previous salinity studies in *bael* have mostly been conducted with seedling plants (Singh *et al*., 9) and detailed information on commercial cultivars is lacking. Given the growing interest in improved cultivars, their salinity tolerance needs to be worked out to arrive at viable recommendations for commercial cultivation in saline environments. The identification of salt tolerant cultivars may also facilitate their use in future improvement programmes. In this backdrop, observations on growth, gas exchange parameters and mineral nutrition were recorded to evaluate the threshold of salt tolerance and to understand the physiological basis of salt stress alleviation in *bael* cultivars.

MATERIALS AND METHODS

This experiment was carried out during 2012-2013 at ICAR-Central Soil Salinity Research Institute (CSSRI), Karnal, India. One-year-old, grafted plants of four *bael* cultivars, namely, Narendra Bael-5 (NB-5), Narendra Bael-9 (NB-9), CISH Bael-1 (CB-1) and

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CISH Bael-2 (CB-2) procured from the ICAR-Central Institute of Subtropical Horticulture, Lucknow, India were used. The saline soils (6.5 and 10.7 dS m^{-1}) used in this experiment were obtained from the salt-affected CSSRI-Nain Experimental Farm, Panipat, India, while control soil (1.3 dS m^{-1}) was obtained from the crop fields. The soil was filled in large, metallic experimental columns of approximately 74 cm length, 44 cm width and 166 cm circumference (each column containing approx. 76 kg soil). After transplanting, the plants were irrigated with normal water (EC_{IW} 0.5 dS m^{-1}) at weekly intervals till the time of data recording (75 days after planting). Salinity in saturation extract (EC_e) of the experimental soil was evaluated at fortnightly intervals and the values presented here (control- 1.3 dS m^{-1}, moderate salinity- 6.5 dS m^{-1} and high salinity- 10.7 dS m^{-1}) represent the means of three replicates of the five consecutive measurements over the experimental period (data not presented).

The visible symptoms of salt stress were recorded at fortnightly interval during the course of investigation. The leaves showing stress symptoms were counted at each stage and the extent of damage (%) was expressed as percentage of total leaves per plant. The relative chlorophyll concentration of the fully expanded, attached leaves from middle layer of plants was obtained using a leaf chlorophyll meter (SPAD-502, Minolta, Japan). Chlorophyll readings were taken from the centre of leaves (excluding mid-rib) between 9 and 11.30 AM. To ensure consistency in results, the same leaves were used for gas exchange measurements. Next day, net photosynthesis (P_N), stomatal conductance (g_s) and transpiration rate (E) were measured during the same hours using the portable photosynthetic system (Li-Cor Biosciences, Nebraska, USA). Water use efficiency was obtained as the ratio of P_N and E. After 75 days of salt treatment, the plants were harvested and washed with distil water to remove the dust and salt particles. After drying by wrapping in paper sheets, the whole plant was divided into different parts (leaves, upper stem, basal stem, primary roots and root hairs) for mineral analyses. These samples were subsequently dried in a forced-draft oven at 60ºC for 48 h, weighed and crushed in a hammer mill. Approximately 50 mg of dried and powdered leaf material was extracted with 1 M HNO_3 at 100ºC. Na^+ and K^+ contents were determined by using the flame photometer (Systronics, India), while Ca^{2+} concentration was determined through atomic absorption spectrometry (Analytik Jena, Germany). The experiment was laid out in a randomized complete block design with three replications. The data were analysed for analysis of variance using the SPSS 11.0.1 for Windows statistical package (SPSS, Chicago, IL, USA). For comparison of the means, the Duncan's multiple range test ($P \leq 0.05$) was used.

RESULTS AND DISCUSSION

Data on salt stress symptoms recorded at fortnightly interval (Table 1) revealed significant differences ($p \leq 0.05$) in the appearance and progression of injury symptoms among the tested cultivars. NB-9 and CB-2 were the most affected cultivars as even moderate salinity caused severe marginal scorch and necrosis in over 70% of the leaves in these cultivars after 75 days of salt treatment. Contrary to NB-5 and CB-1, which showed delayed appearance and gradual development of injury symptoms, NB-9 and CB-2 cultivars exhibited severe injury within a few days of salinity exposure. Between 60th and 75th days, all the leaves became necrotic in NB-9, CB-1 and CB-2 but remained intact. At 10.7 dS m^{-1} salinity, salt injury appeared as early as 15 days after planting. Initially, the leaves turned chlorotic followed by necrosis and eventual abscission in all the cultivars. However, the pattern and extent of necrosis and abscission varied among the cultivars. The upper leaves were

Table 1. Percentage of leaves with salt stress symptoms after 75 days of salinity exposure.

Cultivar	Salinity (dS m^{-1})	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP
NB-5	6.5	1.3e	1.3f	8.4e	16.5g	33f
	10.7	4.2de	11.6e	22d	38.3e	67d
NB-9	6.5	13.7bc	20.3bc	34c	43.2d	70.5cd
	10.7	17b	23b	40b	62b	91.6a
CB-1	6.5	5.4d	15.7d	22d	31.5f	46.3e
	10.7	11.6c	18.3cd	32.3c	52c	80.7b
CB-2	6.5	15.7b	20.8bc	37bc	47.7cd	73.3c
	10.7	26.3a	34.7a	46.7a	70.3a	94.4a

Means with at least one letter common in a column are not statistically significant using Duncan's Test at 5% level of significance. The control plants did not show any injury symptoms and were thus excluded from statistical analysis, DAP = Days after planting.

first to abscise followed by middle and lower leaves. In NB-5 plants, however, some of the lower and middle leaves remained attached to plants till the end of experiment. In citrus, absence of such injury symptoms is frequently used as a criterion for salt tolerance (Lopez-Climent *et al*., 6). Adverse effects of salinity on plant growth seem to be mainly due to excessive Na^+ and Cl^- accumulation in vegetative tissues as reported in citrus (Lopez-Climent *et al*., 6) and *bael* (Singh *et al*., 9). Although all the tested cultivars showed significant increase in toxicity symptoms with increasing salinity, these effects were less pronounced in cv. NB-5 indicating its relatively higher salt tolerance.

All the cultivars exhibited significant decrease ($P \leq 0.05$) in relative chlorophyll (SPAD) values (Table 2) with increasing salinity. The maximum (50.5%) and the minimum (36.7%) reductions in SPAD at 6.5 dS m^{-1} salinity occurred in CB-2 and NB-5, respectively, as compared to control. High salinity (10.7 dS m^{-1}) caused drastic reductions in SPAD with cultivar CB-2 exhibiting as high as 73% decrease relative to control. The observation that SPAD only marginally decreased in NB-5, while other cultivars exhibited moderate to high reductions is supported by previous findings on *bael* (Singh *et al*., 9) and citrus (Anjum, 1). Leaf chlorophyll relations in salt stressed plants vary with the magnitude of salinity, genotype and growth stage and often depend on the integrity of cell membranes and the activities of key enzymes (Singh *et al*., 18; Anjum, 2). As salt tolerant types often exhibit significantly lower membrane damage, they succeed in maintaining favourable chlorophyll levels under salt stress (Singh *et al*., 8; Singh *et al*., 9). Similar to SPAD, P_N of attached leaves significantly decreased ($P \leq 0.05$) in salt treated plants (Table 2). At moderate salinity, P_N decreased by about 28 to 32% in different cultivars. At high salinity, the maximum (67.11%) reduction in P_N was noted in CB-2 and the minimum (59.29%) in NB-5 plants. Salinity induced photosynthetic decline in studied cultivars coincided with a significant decrease ($p \leq 0.05$) in stomatal conductance (g_s) and transpiration rate (*E*, Table 2). At moderate salinity, *E* decreased by 39% in NB-5, 33% in CB-1 and 29% each in NB-9 and CB-2 plants as compared to control. Such genotypic variations in photosynthetic relations have also been reported in fruit crops such as citrus (López-Climent *et al*., 6) and olive (Chartzoulakis, 3). Salt tolerant types in citrus either maintain equilibrium or alternatively exhibit substantial declines in gas exchange and CO_2 assimilation under salinity (López-Climent *et al*., 6). A decrease in stomatal conductance and the concurrent lowering of transpiration rate was noted in all the cultivars but this effect was more pronounced in NB-5. It probably implied a strategy by NB-5 plants to improve water use efficiency under salt stress. The high water use efficiency of NB-5 plants at moderate salinity (about 13% high as compared to control) explains their relative salt tolerance as decline in stomatal conductance may allow the plants to economize water use to partially alleviate the effects of salinity (Chaves *et al.*, 4).

Salt treated plants recorded significantly ($p \leq 0.05$) higher Na^+ ions in all plant parts as compared to control (Table 3). Nevertheless, data on Na^+

Table 2. Salinity induced changes in gas exchange parameters in *bael* cultivars.

Cultivar	Soil salinity (dS m^{-1})	SPAD	P_N	g_s	*E*	WUE
NB-5	1.3	44.1b	10.12ab	215.33a	2.34a	4.32b
	6.5	27.91c	7.07c	78.33d	1.42d	4.97a
	10.7	19.61f	4.12d	55.33e	0.86e	4.81a
NB-9	1.3	45.4a	9.89b	218.33a	2.39a	4.15bc
	6.5	25.65d	6.73c	186b	1.7c	3.96c
	10.7	14.62h	3.28e	106cd	1.33d	2.47d
CB-1	1.3	46.21a	10.44a	212.33a	2.18b	4.8a
	6.5	25.67d	7.06c	94bc	1.46d	4.86a
	10.7	16.26g	3.81d	82.33cd	0.9e	4.23bc
CB-2	1.3	45.95a	9.82b	209a	2.4a	4.09bc
	6.5	22.74e	7.05c	105.67b	1.71c	4.13bc
	10.7	12.44i	3.23e	91cd	1.38d	2.34d

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance. Note: SPAD= relative chlorophyll, P_N=net photosynthesis (µmol $CO_2/m^2/s$), g_s = stomatal conductance (mol $H_2O/m^2/s$), *E* = transpiration (mmol $H_2O/m^2/s$), WUE= water use efficiency ($P_{N/} g_s$).

Table 3. Effect of salinity on Na^+ (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m^{-1})	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	0.17de	0.08h	0.08e	0.08g	0.18ef
	6.5	0.29b	0.49c	0.23c	0.15f	0.17fg
	10.7	0.16de	0.21g	0.2cd	0.15f	0.15g
NB-9	1.3	0.06g	0.07hi	0.05ef	0.08g	0.11h
	6.5	0.21c	0.29f	0.17d	0.22d	0.44b
	10.7	0.21c	0.39d	0.28b	0.29c	0.33c
CB-1	1.3	0.05g	0.05ij	0.05ef	0.09g	0.2e
	6.5	0.14ef	0.34e	0.2cd	0.2de	0.24d
	10.7	0.12f	0.48c	0.36a	0.34b	0.49a
CB-2	1.3	0.12f	0.04j	0.03f	0.03h	0.08h
	6.5	0.36a	0.6b	0.2cd	0.19e	0.44b
	10.7	0.18d	0.88a	0.32b	0.57a	0.49a

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

partitioning indicated a strong tendency for its restricted uptake with increasing salinity in NB-5 (Table 3). While, leaf Na^+ concentrations were statistically similar at both moderate and high salinities in NB-9 and CB-1, NB-5 and CB-2 exhibited significantly lower ($p \leq 0.05$) leaf Na^+ concentrations (about 50% in each case) at high salinity as compared to moderate salinity. In upper stem (primary and secondary branches; excluding the leaves) tissues, cultivar NB-5 exhibited significantly lower Na^+ (0.21% DW) at high salinity as compared to moderate salinity (0.49% DW). In contrast, other cultivars exhibited significantly higher ($p \leq 0.05$) Na^+ concentrations in upper stems at high salinity. In basal stems (5-6 cm above the graft union; below the branching point), primary roots and root hairs, cultivar NB-5 exhibited non-significant differences for Na^+ accumulation at both moderate and high salinity treatments. Although leaf Na^+ concentration in NB-5 (0.29%) was almost two-fold high relative to CB-1 (0.14%) at 6.5 dS m^{-1} salinity, it did not exhibit severe injury symptoms while the latter showed severe chlorosis, marginal scorch and downward leaf curling. It appears, therefore, that besides restricted uptake and subsequent translocation to foliage, NB-5 cultivar had a higher threshold for Na^+ induced toxicity. Na^+ partitioning under salinity indicated distinct behaviour of the tested cultivars. Cultivar NB-5, in spite of higher leaf Na^+ concentration at moderate salinity, did not exhibit pronounced injury symptoms till the end of experiment as compared to other cultivars. It points to relative cellular tolerance for Na^+ as well as probable osmotic adjustment in NB-5 plants. At high salinity, NB-5 plants showed almost similar distribution of Na^+ ions in leaf, stem and root tissues and this could have prevented excessive Na^+ accumulation in aerial parts. Cultivar CB-1 showed partial retention of Na^+ ions in stem and root tissues, but failed to avoid the injury symptoms. Both NB-9 and CB-2 cultivars showed excessive Na^+ build up in leaves and stems. The different patterns of sodium uptake and accumulation observed in these cultivars highlight the existence of separate mechanisms, which operate to limit the transport of Na^+ from the roots to aerial parts. In a nutshell, cultivar NB-5 exhibited higher tolerance threshold for Na^+ at moderate salinity and restricted Na^+ uptake at high salinity.

The leaf, stem and root K^+ concentrations significantly decreased with increasing salinity in all the cultivars (Table 4). At both moderate and high salinities, cultivar NB-5 maintained significantly higher leaf K^+ as compared to others. Moreover, it also exhibited a tendency for higher K^+ accumulation at high salinity as compared to moderate salinity with significant differences for upper and basal stems and primary roots and non-significant differences for leaves and root hairs. At moderate salinity, 1.25 to two-fold more K^+ ions were retained in the root hairs of NB-9, CB-1 and CB-2 plants as compared to those of NB-5. At moderate salinity, the highest K^+ concentration in upper stems was noted in CB-1 (0.61% DW) and the lowest (0.49% DW) in NB-9. At high salinity, the highest K^+ concentration in upper stems was noted in NB-5 (0.68% DW) and the lowest (0.45% DW) in NB-9. The higher K^+ concentrations in leaf and stem tissues of NB-5 indicated an easier translocation of K^+ from root to aerial parts where it seems to have partially substituted for toxic Na^+ ions

Table 4. Effect of salinity on K^+ (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m^{-1})	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	0.95b	1.05b	0.73a	0.51b	1.01a
	6.5	0.34d	0.54g	0.34e	0.37ef	0.27g
	10.7	0.39d	0.68de	0.4d	0.51b	0.28g
NB-9	1.3	0.92b	0.73d	0.57b	0.45c	0.98a
	6.5	0.17f	0.49gh	0.34e	0.44cd	0.53d
	10.7	0.24e	0.45h	0.4d	0.4de	0.25g
CB-1	1.3	0.83c	0.88c	0.6b	0.73a	0.68c
	6.5	0.22ef	0.61f	0.32ef	0.34fg	0.34f
	10.7	0.11g	0.63ef	0.29f	0.36ef	0.47de
CB-2	1.3	1.31a	1.13a	0.47c	0.5b	0.75b
	6.5	0.26e	0.5gh	0.31ef	0.3h	0.42e
	10.7	0.18f	0.61f	0.43cd	0.31gh	0.34f

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

and thus created a favourable K^+/Na^+ ratio for plant growth under elevated salinity.

Data on Ca^{2+} partitioning in different plant parts (Table 5) indicated that moderate salinity induced slight decrease in NB-5 and CB-2 while marginal increase in leaf Ca^{2+} in NB-9 and CB-1 cultivars. In contrast, at high salinity, only NB-5 plants showed significantly higher Ca^{2+} in leaves while other cultivars exhibited significant decrease relative to control. In upper stems, Ca^{2+} concentration either showed significant increase (NB-5 and CB-2) or decrease (NB-9) or no change (CB-1). In basal stems, Ca^{2+} concentration significantly decreased in all the cultivars with increasing salinity except in NB-5 which showed higher Ca^{2+} as compared to control. Ca^{2+} concentration in primary roots increased in salinized plants of all cultivars except NB-9. Cultivar NB-5 almost invariably showed increase in Ca^{2+} concentrations with increasing salinity except the slight reduction in leaf as compared to control. Cultivar NB-5 either maintained Ca^{2+} concentrations in different plant parts at par with control or even showed significantly higher Ca^{2+} levels under elevated salinity. It pointed to preferential accumulation and easy translocation of Ca^{2+} to foliage tissues under saline conditions. On the contrary, salinity induced reduction in Ca^{2+} levels hampered the growth in other cultivars. Similar findings have earlier been reported in citrus (García-Sánchez *et al*., 5) where salt tolerant lines did not show impaired Ca^{2+} nutrition under salinity.

Table 5. Effect of salinity on Ca^{2+} (% DW) partitioning in different plant parts in *bael* cultivars after 75 days of salt treatment.

Cultivar	Soil salinity (dS m^{-1})	Leaf	Upper stem	Basal stem	Primary roots	Root hairs
NB-5	1.3	2.8efg	1.8f	3.88d	1.8fg	2.04h
	6.5	2.33h	2.36d	3.95d	3.55b	9.64a
	10.7	3.03cde	3.43b	4.5c	4.5a	9.61a
NB-9	1.3	3.89b	4.12a	5.45a	2.92c	2.54fg
	6.5	4.51a	3.33b	3.24e	1.67gh	3.29cd
	10.7	3.26c	1.88ef	3.23e	2.37d	6.29b
CB-1	1.3	2.94def	2.4d	4.97b	1.08i	2.23gh
	6.5	3.11cd	2.33d	2.32g	2.03ef	3.65c
	10.7	2.65g	2.4d	2.62f	1.56gh	2.87ef
CB-2	1.3	2.95def	1.98e	3.87d	1.08i	1.12i
	6.5	2.93def	2.73c	2.23g	1.45h	3.22de
	10.7	2.76fg (	2e	2.12g	2.25de	2.26gh

Means with at least one letter common in a column are not statistically significant using Duncan's test at 5% level of significance.

Based on the above results, it can be said that salt stressed *bael* cultivars tend to arrest gas exchange in leaves so as to economize water use as well as to restrict the uptake of toxic sodium ions through transpiration stream. Ionic partitioning in salt treated plants indicated restricted Na^+ uptake and preferential K^+ accumulation in NB-5 cultivar. Based on the extent of salt injury symptoms in leaves, gas exchange characteristics and ionic distribution in leaf, stem and root tissues, cultivar NB-5 outperformed other tested cultivars in moderately saline soils (EC_e ~6.5 dS m^{-1}).

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Received : January, 2014; Revised : December, 2015; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00018.9

Indian J. Hort. 73(1), March 2016: 54-59

Genetic diversity analysis of indigenous and exotic chilli genotypes

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ABSTRACT

Genetic diversity analysis of 64 chilli accessions of Indian and exotic origins was performed using 14 qualitative and quantitative traits. Variation was not observed for mature fruit colour, corolla colour and number of anthers. Based on 11 polymorphic traits, the analysis allowed grouping of the accessions into 9 clusters. Using the polymorphic traits, all but one pair of accessions, *viz*. Selection 40 and ELS 82 were differentiated from each other. Euclidean's inter-cluster distances varied from 12.68 between clusters 5 and 6 to 90.77 between clusters 4 and 5. Intra-cluster distance was maximum in cluster 1 (21.18) and minimum in cluster 5 (6.99). cluster 2 was represented by only one genotype 'Faslima'. Based on the diversity analysis, parental lines were identified for their utilization in hybrids development and genetic improvement of chilli.

Key words: Capsicum, genetic diversity, morphological descriptors.

INTRODUCTION

Chilli (*Capsicum* sp.), also known as hot pepper, belongs to the family Solanaceae and has a chromosome number 2n = 2x = 24. Chilli is indigenous to South America and was first introduced in India from Brazil by Portuguese towards the end of 15th century (Krishna De, 8). Chilli is an often cross-pollinated crop and, therefore, exhibits wide variability for different qualitative and quantitative traits (Tanksley, 12). There are five cultivated species of chilli including *Capsicum annuum* L, *C. frutescens*, *C. chinense*, *C. pubescens* and *C. baccatum* (Smith and Heiser Jr, 11). India is considered to be the secondary centre of diversity of chilli (IBPGR, 7), especially of *C. annuum*, the most important cultivated species. North-Eastern states are home to the genetic variability for *C. chinense* to which Naga King, one of the world's hottest chilli also belongs. Over the years, chilli has become an important commercial crop of India. India is currently the leading producer, consumer and exporter of chilli in the world. Total area under the crop is estimated to be 792 thousand hectare with the production of 1,260 thousand tonnes of dry chilli during 2011-2012 (Anon, 1). Although chilli is cultivated almost throughout the country, Andhra Pradesh alone accounts for 25% of the total area and 40-50% of the total national production. In the world trade, India contributes about 25% of the total global chilli exports (Anon, 2).

Genetic resources are the most valuable and essential basic raw material to meet the current and future needs for genetic improvement of any crop. Characterization of the germplasm is important for its identification and registration with the competent authority for plant variety protection. The uniqueness of a variety is established by tests for distinctiveness, uniformity, and stability (DUS) for which the International Union for the Protection of New Varieties of Plants has provided guidelines in the case of most economically useful plant species (UPOV, 13). Characterization of the germplasm is also important from the point of view of its utilization in crop breeding programmes. The present investigation was, therefore, undertaken to characterize the available germplasm of cultivated chilli, assess genetic diversity using qualitative and quantitative traits and identify genotypes for hybrid development and genetic enhancement of the crop.

MATERIALS AND METHODS

The experiment was conducted at Vegetable Research Farm of the Punjab Agricultural University, Ludhiana, India during the crop season 2011-2012. The plant material comprised of 64 chilli germplasm of which 49 accessions belong to the indigenous sources and the remaining 15 to the exotic sources (Table 1). Except 'Naga King', 'Tabasco' and 'Punjab Longi', all accessions belong to the cultivated species, *Capsicum annuum* L. 'Naga King' is believed to be a naturally occurring hybrid between *C. chinense* and *C. frutescens* and 'Tabasco' belongs to *C. frutescens*. Phylogeny of 'Punjab Longi' is not clear but resembles that of *C. frutescens*. For diversity analysis, the germplasm was screened for 14 descriptors. These included growth habit, stem colour, leaf colour, leaf size, corolla colour, number of anthers, anther colour, fruit position, fruit length, fruit tip, fruit colour, fruit shape, seediness and pungency. Growth habit, stem colour, leaf colour, leaf size, corolla colour,

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Table 1. List of chilli accessions used for diversity analysis.

Genotype	Source
Selection-11, Selection-15, Selection-40, Selection-8E, Selection-40-1, Selection-20-1, S-217621, PAU Selection Long, ELS-82, PG-1-1, SAS-39, PC-7-1, Acc-34-1, Punjab Surkh, Punjab Gucchedar, Selection-7-1, MS 12, Acc-33-1, C-31-1, S-2530, Acc-06-01, Acc-06-02, Dev Long, LLS	PAU, Ludhiana
VR-338, Perennial, EC-532386, Long thick USA, PC-1, Chilli Shining, Yellow Bird Dark, VR-36, Tabasco, EC-532390, Pubescent	USA
JCA-283, JCA-288, DCL-524, PC-2062, Acc-2-1, PC-6, Acc-34, ATG	AICRP, India
PP91-7195-1, PP-9852-173, PP-0237-7508, CCA-4261	AVRDC, Taiwan
VS-5, Kashi Anmol, VS-5	IIVR, Varanasi
PLS-3, PLS-2, PLS-5	Moga, Punjab
Pepsi-17-2, Pepsi-8-1	Pepsi Foods, India
SHHP-404, SHHP-4884	SKUAST, Srinagar
Local Line Ropar	Ropar, Punjab
Punjab Longi	Amritsar, Punjab
Faslima	Rajasthan, India
NSS-2	Namdhari Seeds, India
Utkal Yellow	Bhubaneswar, Odisha
Naga King	Nagaland, India
Mehma Sarja	Bathinda, Punjab

anther colour, fruit position, fruit tip, fruit colour and fruit shape were scored visually. Number of anthers was counted from five flowers per plant. Fruit length was measured with the help of scale. Seediness was scored by counting seeds from five red ripe fruits per plant; and pungency was assessed by organoleptic test. Clustering was done by UPGMA using SHAN module of Windostat version 8.6.

RESULTS AND DISCUSSION

Among 14 qualitative and quantitative traits scored, variation was not observed for mature fruit colour, corolla colour and number of anthers. These traits were, therefore, not considered for genetic diversity analysis. Earlier, Fonseca *et al.* (5) also reported that variation was not observed for corolla color, corolla shape, calyx pigmentation, days to fructification, duration of fructification and seed color. Bozokalfa *et al*. (4) studied patterns of phenotypic variation in a germplasm collection of chilli from Turkey and reported that all lines had green stem. Yumnam *et al*. (14) reported variation for fruit colour as the green colour shades. However, variation was reported for corolla colour and plant growth habit by Bozokalfa *et al*. (4); fruit shape by Fonseca *et al*. (5) and Yumnam *et al*. (14); leaf size by Yumnam *et al*. (14); pungency, fruit tip and leaf colour by Bozokalfa *et al*. (4); and fruit position by Fonseca *et al*. (5).

In the given set of collections, intermediate type of growth habit was found to be the most frequent (45.35%). This was closely followed by erect growth habit (43.75) and only few genotypes (4.6%) showed prostrate type of growth habit. Among the characters analyzed, pungency, fruit length, fruit shape and seediness are commercially important. With regard to pungency level, the germplasm was classified into three groups. These included mildly pungent (32.8%), pungent (64.1%) and highly pungent (3.1%). However, none was found to be non-pungent. Other than 'Naga King', the world known hot pepper, 'Punjab Longi' was regarded as highly pungent. Data regarding fruit length showed wide variation among the genotypes. Mean values for fruit length ranged from 1.46 cm in 'Punjab Longi' to 16.23 cm in 'Faslima'. Of the total germplasm analyzed, 2.6% have fruit length ≤ 1.0 cm, 15.8% between 1.1 and 2.0 cm, 39.5% between 2.1 and 4.0 cm, 39.5% between 4.1 and 8.0 cm and 12.6% have fruit length more than 8.0 cm. A wide variation among the test genotypes was also observed for seediness. Number of seeds per fruit ranged from 46.0 in 'EC 532386' to 273.0 in 'MS-12'. Other genotypes with higher seed content included 'ACC-34-1' (269.0) and 'ACC-34' (205.0). The detailed genotypic data are not presented here.

Based on similarity co-efficient values, genetic relationship among the test genotypes were presented

in the form of dendrogram (Fig. 1). The UPGMA cluster analysis showed that the 64 genotypes were divided into two main clusters at similarity coefficient of 0.85. The first major cluster consisted of 45 genotypes and the second cluster consisted of 19 genotypes. The major cluster was further divided into two sub-groups at similarity coefficient of 0.62. The first sub-group of major cluster consisted of 26 genotypes, while the second sub-group consisted of 19 genotypes. The smaller cluster was further divided into two sub-groups at similarity coefficient of 0.44. The first sub-group consisted of seven, while second sub-group consisted of twelve genotypes. 'Punjab Longi' and 'Naga King' were clustered together in one group. Both the lines belong to high pungency group. The sixty four genotypes were finally divided into nine clusters. However, there were three clusters (1, 2 and 3) in the smaller group and six clusters (4 to 9) in the major group. Using 11 morphological descriptors, it was possible to distinguish 62 of the 64 genotypes evaluated. However, the available set of descriptors could not distinguish 'Sel 40' from 'ELS 82' (Fig. 1). There is need to evaluate these genotypes for additional descriptors to differentiate them from each other.

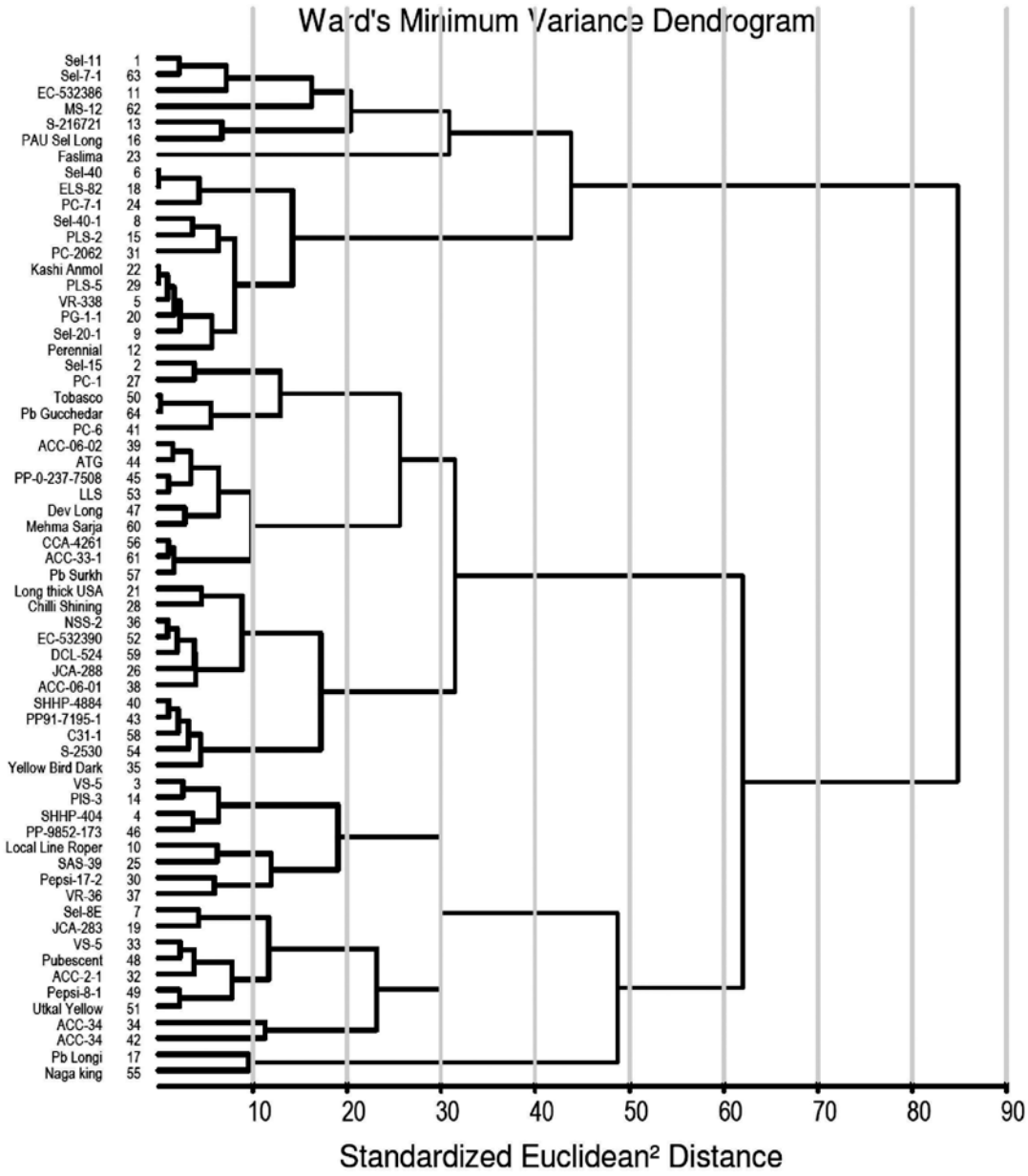

Fig. 1. Dendrogram depicting classification of 64 chilli genotypes based on 11 morphological descriptors.

Euclidean's inter- and intra-cluster distance matrices of 64 chilli genotypes based on 11 morphological descriptors are presented in Fig. 2 where each circle is represented by a cluster number. Numerical within the circle represent intra-cluster distance, whereas figures on the connecting lines denote the inter-cluster distances. The inter-cluster distance varied from 12.68 between Clusters 5 and 6 to 90.77 between Clusters 2 and 9. This indicated comparatively low to high level of genetic divergence between any two accessions of chilli. The genotypes, therefore, contained neither totally similar nor totally dissimilar accessions. The maximum intra-cluster distance matrix was observed in Cluster 1 (21.18) followed by Clusters 9 (19.15) and 8 (16.66). Cluster 5 exhibited the minimum intra-cluster variation (6.99). The Cluster 2 was represented by only one genotype 'Faslima'. Clustering patter of 64 chilli genotypes based on the Euclidean's analysis is given in Table 2.

The diversity analysis revealed that grouping of the genotypes was not based on their geographic distribution indicating that the geographic origins may not be true index for sampling genetic diversity in chilli. This is contrary to the earlier report of Loaiza Figueroa *et al.* (9) who found correlations of genetic differentiation with geographic isolation in chilli accessions from Mexico. Baral and Bosland (3) analyzed genetic diversity in *C. annuum* var. *annuum* landraces from Nepal and *C. annuum* var. *annuum* landraces from the centre of diversity, Mexico. All accessions from Nepal grouped into one cluster at a similarity index value of 0.80; whereas, accessions from Mexico grouped into 8 clusters at the same similarity level indicating greater genetic diversity in the accessions. This could be attributed to the fact that chilli has originated in Mexico and wide genetic diversity is still represented by different ecological regions of the country, whereas, in other geographic regions of the world only the improved genetic materials were introduced.

Based on the diversity analysis, genotypes clubbed in Cluster 9 (Punjab Longi and Naga King) and nuclear male sterile line MS 12 (Cluster 1), with inter-cluster distance of 44.99, were found to be the most divergent. Similarly, the cytoplasmic male sterile lines PP-0237-7508 and CCA 4261 represented in Cluster 5; PP-91-7195-1 in Cluster 6; and PP 9852-17 in Cluster 7 were found to be the most divergent from Faslima represented in Cluster 2; and Selection-8E, JCA 283, VS 2, Pubescent, Acc 2-1, Pepsi 8-1, Utkal Yellow, Acc 34 and Acc 34-1 represented in Cluster 9. Since heterosis has direct correlation with diversity between the parental lines (Geleta *et al.*, 6; Reif *et al.*, 10), crosses between the identified parental lines are likely to produce heterotic hybrids. For estimation of gene effects, mapping of useful genes and breeding

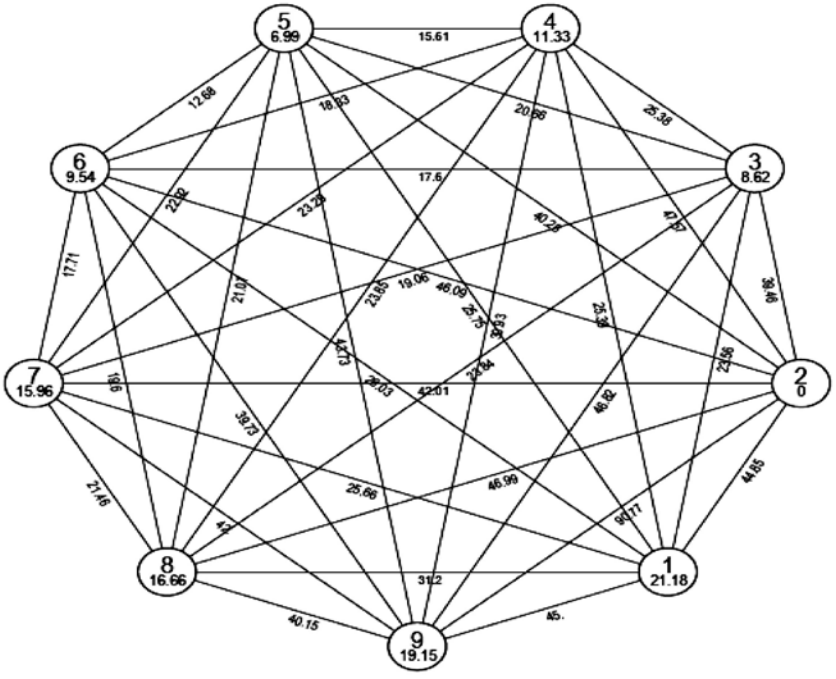

Fig. 2. Euclidean inter- and intra-cluster distance matrix of 64 chilli genotypes based on 11 morphological descriptors.

Table 2. Clustering pattern of 64 chilli genotype on the basis of Euclidean's analysis.

Cluster No.	No. of genotype(s)	Name of accession(s)
Cluster-1	6	Selection-11, Selection-7-1, EC-522386, MS-12, S217621, PAU Selection Long
Cluster-2	1	Faslima
Cluster-3	12	Selection-40, ELS-82, PC-7-1, Selection-40-1, PLS-2, PC-2062, Kashi Anmol, PLS-5, VR-338, PG1-1, Selection-20-1, Perennial
Cluster-4	5	Selection-15, PC-1, Tobasco, Punjab Gucchedar, PC-6
Cluster-5	9	ACC-06-02, ATG, PP-0-237-7508, LLS, Dev Long, Mehma Sarja, CCA-4261, ACC-33-1, Punjab Surkh
Cluster-6	12	Long Thick USA, Chilli Shining, NSS-2, EC-532390, DCL-524, JCA-288, ACC-06-01, SHHP-4884, PP-91-7195-1, C31-1, S-2530, Yellow Bird Dark
Cluster-7	8	VS-5, PLS-3, SHHP-404, PP-9852-173, Local Line Ropar, SAS-39, Pepsi-17-2, VR-36
Cluster-8	9	Selection-8E, JCA-283, VS-2, Pubescent, ACC-2-1, Pepsi-8-1, Utkal Yellow, ACC-34, ACC-34-1
Cluster-9	2	Punjab Longi, Naga King

of superior performing crop cultivars, parental lines from Clusters 2 and 9, with maximum inter-cluster distance, can be involved.

Our results vindicated that India is an important source of genetic variability of cultivated chilli. The relevance of morphological descriptors in genetic diversity analysis in chilli has been emphasized. This is evident from the fact that by using 11 polymorphic traits, the clustering analysis differentiated all 64 chilli genotypes from each other, except Selection 40 and ELS 82.

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Received : December, 2013; Revised : November, 2015; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00019.0

Indian J. Hort. 73(1), March 2016: 60-65

Response of some wild species of tomato against *Peanut bud necrosis virus* under open-field conditions

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ABSTRACT

Thrips-borne *Tospovirus* pathogens adversely affect many globally important crops. Among 16 distinct virus species in the *Tospovirus* genus, four species including *Peanut bud necrosis virus* (PBNV) causing necrosis disease in tomato have been reported in India. Identification of stable sources and further utilization of wild relatives as gene sources to increase levels and diversify the bases of resistance may offer good management for the disease. A total of 13 wild species of tomato (*Solanum peruvianum*), two *S. pimpinellifolium*, one *S. chilense*, one *S. pennellii* and three check cultivars (*S. lycopersicum*) along with two cultivars (*S. lycopersicum*) having the *Sw-5* and *Sw-7* genes were evaluated under field conditions during three consecutive seasons (June to October 2008, July to December 2009, August 2010 to February 2011). Among all, a high degree of field resistance (>80%) was detected in seven lines of *S. peruvianum* (L00735, L00671, L00887, L06138), *S. chilense* (TL02226) and *S. pimpinellifolium* (L03708, TL02213) lines. The field data was also supported by negative reaction against a polyclonal antiserum of the nucleocapsid protein (N) of PBNV in direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA). The cultivars with *Sw-5* and *Sw-7* genes were highly susceptible to PBNV.

Key words: *Solanum lycopersicum*, *S. peruvianum*, *peanut bud necrosis virus.*

INTRODUCTION

Thrips are extremely difficult to find on plants, making control problematic. Tomato necrosis disease caused by *Peanut bud necrosis virus* (PBNV) in India is a distinct *Tospovirus* belonging to serogroup IV (Jain *et al.*, 8; Soler *et al.*, 19; Akram *et al.*, 2). The disease is a serious constraint to production of several crops including tomato in various locations of the subcontinent. PBNV and *Tomato leaf curl virus* are considered to be among the most destructive diseases of tomato in India, causing yield losses ranging from 27 to 90% in summer (Singh and Tripathi, 15). PBNV not only reduces yield up to 90%, but also diminishes the quality of fruit harvested from infected plants (Sain and Chadha, 14). Integrated disease management (IDM) strategies like use of cultural practices, plastic mulches, fine-mesh netting at nursery stage, and resistant or tolerant varieties are effective and ecologically friendly method for reducing the impact in tomato, pepper and peanut (Greenough *et al.*, 7). Cultivar choice is an additional method available to control plant virus disease. Virus-resistant cultivars are one of the most cost-effective IDM components as the resistant cultivars have low environmental impact and have proven to be the most consistent way to minimize losses from *Tospovirus* (Soler *et al.*, 19; Zaccardelli *et al.*, 20). Finding the source of resistance genes and utilizing them in breeding for resistance is an important process for safe and effective *Tospovirus* control.

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MATERIALS AND METHODS

A germplasm collection of 13 wild species of tomato (*Solanum peruvianum* (L.) Mill), two *S. pimpinellifolium,* one *S. chilense*, one *S. pennellii*, two cultivated tomato (*S. lycopersicum*) BL1022 and CK 12 having *Sw-5* and *Sw-7* genes, and three susceptible cultivars TLB 182, K555, TLCV15 were chosen for evaluation for resistance to *Peanut bud necrosis* (PBNV) disease. These entries were evaluated under field conditions using the natural inoculum with infector row method in three consecutive years (2008, 2009, and August 2010-February 2011) (Table 1). Susceptible line *S. lycopersicum* (CNL-2498 E) was selected to be planted as the infector row. The germplasm/line material was procured from AVRDC-The World Vegetable Center, Taiwan.

The field experiments were laid out following a randomized complete block design (RCBD) with three replications for two consecutive years (June-November 2008, July-December 2009, and August 2010-February 2011) in Hyderabad, India to evaluate the resistance of wild tomato germplasm against PBNV infection.

Table 1. PBNV incidence in tomato entries evaluated during 2008, 2009 and 2010-11.

Entry	Species	Percent disease incidence[a]									
		Year 2008			Year 2009			Year 2010-11			Pooled mean
		49 DAT[b]	89 DAT	AUDPC[c]	50 DAT[b]	75 DAT	AUDPC[c]	90 DAT[b]	120 DAT	AUDPC[c]	
L 00671	*S. peruvianum*	04.2 (06.90)	17.5 (24.61)	16.26	13.9 (21.9)	17.8 (24.9)	18.39	2.38 (8.9)	14.29 (22.2)	16.67	16.53 (23.99)
L 00673	*S. peruvianum*	16.7 (19.93)	26.7 (30.29)	19.62	19.0 (25.9)	30.2 (33.3)	22.96	19.05 (25.9)	52.38 (46.4)	10.72	36.43 (37.13)
L 00678	*S. peruvianum*	06.7 (08.85)	30.0 (32.21)	21.04	-	-	-	-	-	-	30.0 (33.21)
L 00687	*S. peruvianum*	36.7 (36.14)	43.3 (41.15)	16.02	-	-	-	-	-	-	43.3 (41.15)
L 00688	*S. peruvianum*	21.4 (22.41)	40.5 (39.42)	25.62	-	-	-	-	-	-	40.5 (39.53)
L 00689	*S. peruvianum*	24.1 (24.36)	31.5 (33.74)	29.61	-	-	-	-	-	-	31.5 (34.14)
L 00737	*S. peruvianum*	20.7 (21.49)	47.8 (43.76)	33.12	-	-	-	-	-	-	47.8 (43.74)
L 00735	*S. peruvianum* var. *humifusum*	-	-		09.5 (18.0)	09.5 (18.0)	14.25	2.38 (8.9)	9.52 (18.0)	19.05	9.51 (17.96)
L 00738	*S. peruvianum*	20.0 (21.93)	33.3 (34.93)	25.51	-	-	-	-	-	-	33.3 (35.25)
L 00882	*S. peruvianum*	18.3 (21.07)	36.9 (37.15)	28.93	30.0 (33.2)	37.8 (37.9)	32.11	4.76 (12.6)	16.7 (24.1)	14.29	30.47 (30.50)
L 00887	*S. peruvianum*	04.2 (06.90)	35.6 (31.34)	31.41	05.6 (13.6)	05.6 (13.6)	10.09	4.76 (12.6)	16.7 (24.1)	14.29	16.53 (23.99)
L 00890	*S. peruvianum*	20.0 (21.93)	23.3 (28.07)	26.32	-	-	-	-	-	-	23.3 (28.86)
L 06138	*S. peruvianum*	10.7 (15.52)	10.7 (15.52)	39.15	16.7 (24.1)	22.2 (28.1)	20.88	14.29 (22.2)	28.57 (32.3)	3.57	20.49 (26.92)
BL 1022 (*Sw-5*)	*S. lycopersicum*	76.7 (66.15)	100 (90.00)	63.65	84.1 (66.5)	94.4 (76.4)	75.83	59.52 (50.5)	85.71 (67.8)	47.62	93.37 (75.08)
CK-12 (*Sw-7*)	*S. lycopersicum*	56.7 (48.93)	92.9 (77.37)	78.58	-	-	-	-	-	-	92.9 (74.55)
TLB-182 (SC)	*S. lycopersicum*	92.9 (77.37)	100 (90.00)	84.19	80.2 (63.6)	90.5 (72.0)	72.36	54.76 (47.7)	64.29 (53.3)	34.52	84.93 (67.16)
L05789	*S. pennellii*	-	-	-	-	-	-	47.62 (43.6)	47.62 (43.6)	22.62	47.62 (43.6)
L03708	*S. pimpinellifolium*	-	-	-	-	-	-	4.76 (12.6)	11.90 (20.2)	16.67	11.90 (20.2)
TL02213	*S. pimpinellifolium*	-	-	-	-	-	-	7.14 (15.5)	16.67 (24.1)	13.09	16.67 (24.1)
TL02226	*S. chilense*	-	-	-	-	-	-	21.43 (27.6)	21.43 (27.6)	3.57	21.43 (27.6)
TLCV15 (SC)	*S. lycopersicum*	-	-	-	-	-	-	33.33 (35.3)	88.09 (69.8)	35.71	88.09 (69.8)
K555 (SC)	*S. lycopersicum*	-	-	-	-	-	-	50 (45.0)	71.43 (57.7)	35.71	71.43 (57.7)
CD at ($p \leq 0.05$)		26.93	21.77		26.62	27.54		17.09	18.64		20.18

[a]Value in parentheses are Arc sine transformed values; [b]DAT = days after transplanting; [c]AUDPC = area under disease progress curve

Tomato seedlings were raised in trays using sand, soil, and compost mixture @ 1:1:2 under 60-mesh nylon net. Seed of *S. peruvianum* species were sown 10 days earlier (June 23, 2008) than the check lines (CK12, BL1022) and susceptible tomato line (TLB-182), as the wild tomato grows slower than improved varieties. Transplanting of the wild entries was done 10 days after transplanting (DAT) of (August 2) the improved cultivars. Ten seedlings of each entry were transplanted on 5 m long raised ridges in a double-row plot, at 90 cm × 90 cm spacing at M/s JK Seeds Research farm, Hyderabad. Cultural practices were followed according to the recommendation for irrigated tomato in Andhra Pradesh (Anon, 1). One PBNV 'infector' tomato (highly susceptible line CNL-2498 E) row in every fifth plot and susceptible check (TLB-182) in every replication were transplanted 15-20 days before transplanting of the screening material. The lines L00671, L00673, L00882, L00887 and L06138 observed with low disease incidence and negative reaction with DAS-ELISA in the 2008 experiment were selected for field confirmation trials during 2009 and 2010-11. These entries, along with *S. peruvianum* var. *humifusum* (L00735), check line BL1022 having *Sw-5* genes, and a susceptible control were evaluated (7 plants per replication). During the 2010-2011 trial, six *S. peruvianum*, one *S. chilense* and two *S. pimpinellifolium* lines along with BL1022, TLB 182 and two new lines K555 and TLCV15 as checks were evaluated using the infector row method. The nursery was raised on July 16 and transplanted on August 9, 2010 at the RCSA field (14 plants in each replication).

PBNV disease incidence was identified on the basis of field symptoms (Swift, 18). The plants were inspected at seven-day intervals to note the appearance and development of the symptoms of PBNV infection starting from transplantation to last harvest. The tomato plants that remained asymptomatic until last harvest was designated as healthy plants. On the basis of the symptoms caused by the virus, the data on the incidence of PBNV was collected at two stages of the plant growth: at 49 and 89 DAT in 2008. 50 and 75 DAT in 2009, and 80 and 120 DAT in 2010-11 using 0-1 scores, 0 = healthy plant, 1 = systemic symptoms without or with stunting. The percent incidence of PBNV was calculated by counting the plants showing PBNV infection by following the formula: Percent incidence $=(X_1/X) \times 100$, where X_1 = number of infected plants and X = total number of plants. Because the standard deviations for PBNV score were proportional to their corresponding means, disease incidence data were arcsine-transformed to stabilize error variance before analysis of variance (ANOVA) at P <0.05% and <0.01% levels of significance (Snedecor and Cochran, 16). The Arc sine formula gives the values being identical to the Arc sine tabular values and also facilitates the calculation in the Microsoft Excel program.

Area under disease progress curve (AUDPC) was also calculated (for assessment of disease incidence) for each genotype using disease incidence (transformed data), which was the proportion (0-1.0) of symptomatic plants in the plot, using the formula: $AUDPC = {}^{n-1}_{i=1}\Sigma ([Y_i + Y_i+1]/2) [T_i+1 - T_i]$ where: Y_i+1 = apparent incidence (0-1.0) at the i^{th} observation, T_i = time (days) at the i^{th} observation, n = total number of observations. Where y_i is the disease incidence in percent at i^{th} assessment, t_i is the time of the i^{th} assessment in days from the first assessment date, and is the total number of days the disease was assessed (Campbell and Madden, 3).

Enzyme-linked immunosorbent assay (ELISA) was carried out and PBNV infections were verified by serological identification. Infected *S. lycopersicum* leaves were used as a positive control. Two to 10 fresh leaves samples from symptomatic and non-symptomatic surviving plants of each entry were collected. The samples were subjected to a polyclonal antiserum against a nucleocapsid protein of PBNV in an alkaline phosphate-based direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA) (Cho *et al.*, 4; Jain *et al.*, 8). Results of finished ELISA plates were measured at 405 nm on Molecular Devices-E-Max plate reader. Samples were considered positive if the absorbance at 405 nm was more than the twice the average buffer of healthy tomato control reading, whichever was higher.

RESULTS AND DISCUSSION

Most of the tomato species evaluated (Table 1) showed the prevalence of PBNV incidence with the highest percent (85-100%) in susceptible checks (TLB-182- BL1022, TLCV15, CK-12). Symptom expression varied among the accessions ranging from sudden wilt to severe necrosis of leaves, stem, meristem, buds, pods, and fruits. Necrosis and wilting were the most common symptoms observed at 40 and 60 DAT. Leaves became necrotic but remained attached to the stem. Stems showed necrosis and irregular brownish-black patches resulting in death of the plants.

Percent disease incidence at 89 DAT during 2008 ranged between 17.5-47.8% in *S. peruvianum* species, and 90-100% *S. lycopersicum* species. In 2009 it was 9.5-94.4%, and during 2010-2011

the percentage of disease incidence ranged from 9.5 to 88.1% in *S. peruvianum*, *S. chilense*, *S. pennellii*, *S. pimpinellifolium* and *S. lycopersicum* (Table 1). Overall, *S. lycopersicum* lines were more susceptible than the other lines evaluated. However, the level of prevalence varied significantly among accessions. Based on the visual symptom scores and their pooled mean, the lowest PBNV incidence was recorded in L06138, L03708, L00887, L00671 followed by TL02213, TL02226 and L00890. Similar results on TSWV prevalence on different tomato lines/varieties in the field have been reported earlier by Greenough *et al.* (7) and Swift (18) and also in previous reports on resistance of tomato wild species against *Tospovirus* (Paterson *et al.*, 11; Krishna Kumar *et al.*, 9; Stevens *et al.*, 17). Of the 68 fresh leaf samples from symptomatic and nonsymptomatic plants of 15 lines, all samples from 7 tomato lines (L00735, L00887, L00882, L00889, L0673, L00671, L00688) I and 11 from 13 samples of L06138 showed negative reaction against a polyclonal antiserum of the nucleocapsid protein (N) of peanut bud necrosis in DAC-ELISA.

Thus results of our study are strongly in contrast to the earlier findings, suggesting that the wild tomato species, especially *S. peruvianum*, *S. pimpinellifolium* and *S. chilense*, are resistant or immune against TWSV with no systemic infection under field conditions (Paterson *et al.*, 11; Krishna Kumar *et al.*, 9; Stevens *et al.*, 17; Rosello *et al.*, 13) and laboratory conditions (Cho *et al.*, 4; Paterson *et al.*, 11). However, the resistance conferred by the *Sw-5* gene, which relies on the development of a hypersensitive response (Soler *et al.*, 19), no longer constitutes a durable resistance system against the disease. Cho *et al.* (4) has reported that certain TSWV isolates overcome this resistance. Our results support the finding of Gordillo *et al.* (6) who have reported a varied range of resistance against TSWV6 isolate from Hawaii and An_{wa}-1 from Western Australia in *S. lycopersicum* accessions ranging from 33-68%. They have reported the highest percentage of resistance against TWSV6 isolate in L00689, L00887 and L00673.

In our study, cultivated tomato lines CK-12 and BL01022 having genes *Sw-7* and *Sw-5*, respectively were found to be highly susceptible to PBNV. This data contrasts with the previous reports suggesting that *Sw-5* confirms dominant resistance against TSWV. In our study *Sw-5* and /or *Sw-7* did not impart PBNV resistance as previously reported, which suggests that *Sw-5* and *Sw-7* genes are isolate-dependent or that resistance breakdown, has occurred. Various researchers are of the opinion that TSWV is found in nature as a heterogenous population of isolates (de Avila *et al.*, 5) with genetic potential for adoption to a wide range of hosts (Qui *et al.*, 12). Resistance-breaking occurs when new, more virulent isolates arise through mutation, selection, or introduction from other countries (Qui and Moyer, 12). Furthermore, through multiplication or prolonged contact of TSWV isolates with the resistance gene, carrier plants can lead to the development of new, more virulent isolates that overcome the resistance (Qui and Moyer, 12).

In conclusion, our study shows that four out of 13 *S. peruvianum*, two *S. pimpinellifolium,* and one *S. chilense* showed high degree of resistance (>75%) and reacted negatively to PBNV antiserum. These accessions are likely to be useful in developing an IDM strategy to reducing the impact of PBNV. Cultivated tomato lines CK-12 and BL01022 with the genes *Sw-7* and *Sw-5,* respectively, were highly susceptible as well as positive to PBNV antiserum. The difference between previous reports and the susceptibility observed in the wild tomato accessions may be due to the natural isolate (PBNV) variation or due to the use of different accessions in previous screening programmes. The high selection pressure associated with this gene has probably contributed to the emergence of resistance-breaking isolates throughout the world. It is now essential to continue the search for new source of resistance, as well as to confirm the genetic control of the resistance already identified in several tomato accessions.

The highest level of field resistance was observed in four *S. peruvianum* (L00735, L00671, L00887 and L06138), two *S. pimpinellifolium* accessions (L03708, TL02213), and one *S. chilense* accession (TL02226) (Fig. 1) and the results suggest that these accessions may be used as a source of PBNV resistance in tomato breeding programmes for the region. However, the resistance also may be due to the lack of thrips transmission/ infestation on the accessions showing resistance (Krishna-Kumar *et al.*, 10), and some genotypes may be susceptible when the mechanical or viruliferous-thrips inoculation method is used (Rosello *et al.*, 13). Therefore, laboratory screening through mechanical inoculation and thrips transmission as well as molecular studies should be carried out to identify valuable accessions. In addition, to speed up conventional improvement programs, the results of both methods coupled with molecular marker techniques in screening germplasm for resistance to PBNV should be used to ascertain the resistance mechanism. With the availability of more durable resistance genes from wild sources, there is scope to develop PBNV-resistant elite lines and cultivars.

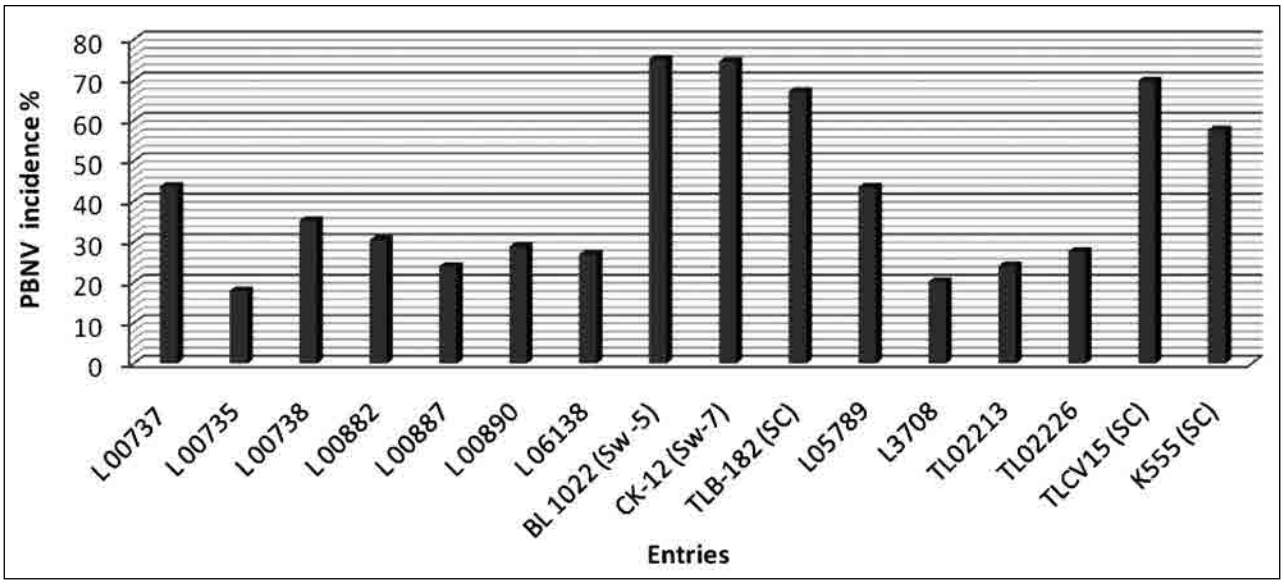

Fig. 1. Pooled mean for PBNV incidence (arcsine transformed) in different entries (CD = 20.18 at *P = 0.05*).

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Received : June, 2016; Revised : November, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00020.7

Indian J. Hort. 73(1), March 2016: 66-73

Growth, yield and important quality attributes chilli (*Capsicum* sp.) genotypes under the Sub Himalayan tracts of West Bengal

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ABSTRACT

An experiment was undertaken to study the performance of 65 chilli genotypes during the *rabi* (*i.e.* winter) season 2005-06 and 2006-07. Mean performance revealed significant variation in yield and quality characters among the different genotypes. Maximum green fruit yield was recorded in CA-29 (262.25 g/ plant), which was statistically *at par* with CA-47 (244.89 g/ plant). Higher yield was also recorded in CA-34 (233.38 g/ plant), CA-48 (232.62 g/ plant), CA-30 (230.27 g/ plant) and CA-40 (219.89 g/ plant). Ascorbic acid content in green fruit varied from 74.78 to 168.10 mg/100 g fresh. The highest capsaicin content in green fruit was recorded in CA-60 (2.08%) the lowest in CA-55 (0.19%). Highest amount of extractable colour in red ripe fruit was recorded in CA-55 (185.34 ASTA) followed by Utkal Abha (156.31 ASTA).

Key words: Chilli, capsaicin, genotypes, growth, quality, yield.

INTRODUCTION

Chilli (*Capsicum annuum* L.) is emerging as one of the commercial vegetable crops at the global level, and is probably most important vegetable after tomato (Grubeen, 6). It is also one of the most valuable and commercial vegetable and spice crops of West Bengal as well as in India. It is considered as one of the important cash crop in the northern parts of the West Bengal. Chillies are sold in local market or supplied to distant places as cash crop fetching a good return to the farmers. It is used for its pungency, colour and its spicy taste. Green chillies are also rich in vitamins A and C (Rahaman, *et al.*, 12). The average dry chilli yield of the country is low as compared to the progressive chilli producing countries like USA, Korea and Taiwan. Productivity of chilli in the Sub-Himalayan region is low (0.96 tonnes/ ha) as compared to national level (1.2 tonnes/ ha). Among several factors, lack of improved varieties is the main constraints for getting production. Studies on chilli genotypes revealed that great variation exists in ability to flowering, fruit set, yield and other qualitative attributes under different agro-climates (Wien *et al.*, 17; Rani, 14; Gupta, 7). Identification of a variety better suited for a particular region and its improvement is of immediate task to exploit its potential (Tembhurne *et al.*, 16).

Though a large number of varieties have developed from different research station but very few information is available for this agro-climatic region. Therefore, area based screening of superior genotypes is an important task for promoting its production, productivity and quality of the produce for improving the productivity of this crop is an important step to increase the production. Considering these points, the present investigation was undertaken to find out the suitable chilli genotypes for West Bengal.

MATERIALS AND METHODS

An experiment was conducted to study the performance of the 65 green chilli genotypes (among them 61 belongs under *Capsicum annuum* L. and rest 4 genotypes under *Capsicum frutescens* group) at the Experimental Farm (26°19'86" N latitude and 89^{0}23'53" E longitude) of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal during the winter season of two years. The experimental soil was sandy clay loam having pH 5.5, 0.91% organic carbon, 133.81 kg/ha available nitrogen, 45.62 kg/ha available phosphorus and 59.43 kg/ha potash. The climatic condition of this region is sub-tropical humid in nature. The experiment was laid out in randomized block design with three replications. Among the 65 genotypes, 58 (CA-1 to CA- 55 and CA-58 to CA-60) collected from different parts of the country and 7 were varieties collected from different research stations (Table 1). Healthy and uniform seedlings were transplanted in plots of 3.60 m × 3.0 m size with a spacing of 30 cm × 45 cm during Middle of November. The crops were grown with standard package of practices as suggested by Anon (1). Observations on yield attributing characters were recorded from

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Table 1. Collection area/ source of the different chilli genotypes.

Sl. No.	Variety/ cultivar	Collection area/ Source	Sl. No.	Variety/ cultivar	Collection area/ Source
1	CA-1	Raijanj, Uttar Dinajpur, W.B.	34	CA-34	Madhupur, Cooch Behar, W.B
2	CA-2*	Kaminighat, Coochbehar, W.B.	35	CA- 35	Madhupur, Cooch Behar, W.B
3	CA-3	Tufanjang, Coochbehar , W.B.	36	CA-36	Madhupur, Cooch Behar, W.B
4	CA- 4	Sealdah market, Kolkata, W.B.	37	CA-37	Madhupur, Cooch Behar, W.B
5	CA-5	Suri, Birbhum, W.B.	38	CA- 38	Madhupur, Cooch Behar, W.B
6	CA-6	Malda, W.B.	39	CA-39	Bhubaneswar, Orissa
7	CA-7	Haldi bari, Cooch Behar, W.B.	40	CA-40	Madhupur, Cooch Behar, W.B
8	CA-8	Balurghat, Dahshin Dinajpur, W.B.	41	CA- 41	Cooch Behar market
9	CA-9	Jalpaiguri, W.B.	42	CA-42	Cooch Behar market
10	CA-10	Jalpaiguri, W.B.	43	CA-43	Chilapata,
11	CA-11	Haldibari, Cooch Behar, W.B.	44	CA-44	Chilapata,
12	CA-12	Haldibari, Cooch Behar, W.B.	45	CA-45	Rajabhat Khawa, jalpaiguri,W.B.
13	CA-13	Ghugumari, Cooch Behar, W.B.	46	CA-46	Madhupur, Cooch Behar, W.B
14	CA-14	Pundibari, Cooch Behar, W.B.	47	CA-47	Madhupur, Cooch Behar, W.B
15	CA-15	Pundibari, Cooch Behar, W.B.	48	CA-48	Madhupur, Cooch Behar, W.B
16	CA-16	Balurghat, Dahshin dinajpur, W.B.	49	CA-49	Tufanjanj, Cooch Behar, W.B.
17	CA-17	Raiganj , Uttar dinajpur, W.B	50	CA-50	Chilapata,
18	CA-18	Balurghat, Dahshin Dinajpur, W.B.	51	CA-51	Tufanjanj, Cooch Behar, W.B
19	CA-19	Balurghat, Dahshin Dinajpur, W.B.	52	CA-52	Tufanjanj, Cooch Behar, W.B
20	CA-20	Murshidabad, W.B.	53	CA-53	Varanasi, UP.
21	CA-21	Murshidabad, W.B.	54	CA-54	Tufanjanj, Cooch Behar, W.B.
22	CA-22	Burdwan, W.B.	55	CA-55	Udaipur, Rajasthan
23	CA-23	Katwa, Burdwan, W.B.	56	C. Philhal	PAU, Ludhiana, Punjab
24	CA-24	Bolpur, Birbhum, W.B.	57	DKC-8	YSPUHF, Solan
25	CA-25	Beldanga, Murshidabad, W.B.	58	Utkal Abha	OUAT, Bhubaneswar, Odisha
26	CA-26	Delhi	59	Pusa Sadabahar	NSC, Delhi
27	CA-27	Delhi	60	G-4	Sungro Seeds Limited, Delhi
28	CA-28	Delhi	61	Pusa Jwala	NSC, Delhi
29	CA-29	Sajerpar, Cooch Behar, W.B.	62	PC-1	Lead Better Seeds Pvt. Ltd., Hyderabad.
30	CA-30	Dinhata, Cooch Behar, W.B.	63	CA-58*	Kalimpong, W.B
31	CA-31	Madhupur, Cooch Behar, W.B	64	CA-59*	Hashimara, Jalpaiguri
32	CA-32	Madhupur, Cooch Behar, W.B	65	CA-60*	Gunjabari, Cooch Behar
33	CA-33	Madhupur, Cooch Behar, W.B			

*Indicates that genotype belongs under *Capsicum frutescens* group (and rest under *C. annuum* L.).

ten randomly selected plants for each replications. Ascorbic acid in chilli fruit was determined by colorimetric method based on the reduction of 2,6-dichlorophenol indophenol by ascorbic acid and was expressed in milligram of ascorbic acid per 100 g of sample (Ranganna, 13). Capsaicin content (%) of green fruits is measured by spectrophotometer method as described by Sadasivam and Manickam (15). In this method capsaicin is extracted with ethyl acetate and then made to react with ethyl acetate

solution of vanadium oxychloride. Then it is read at 720 nm. Total extractable colour (in ASTA unit) of red fruits was measured by using American Spice Trade Association techniques as suggested by Pruthi (11). Statistical analysis were done as per method suggested by Gomez and Gomez (4).

RESULTS AND DISCUSSION

Perusal of the data presented in Tables 2 & 3 revealed that there was a significant variation with respect to yield and quality characters among the different genotypes. Significantly, the highest (468.54) number of fruits per plant was recorded in CA-60

Table 2. Number of fruits and yield of different chilli genotypes.

Genotype	No. of fruits per plant			Individual plant yield (g/ plant)			Fresh green fruit yield (tonnes/ ha)		
	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
CA-1	66.73	72.33	69.53	95.38	97.38	96.38	6.67	6.93	6.80
CA-2	49.30	89.93	69.62	81.96	116.31	99.14	5.31	8.59	6.95
CA-3	129.40	114.00	121.70	155.42	147.86	141.64	10.71	10.43	10.57
CA-4	146.20	126.13	136.17	175.31	143.21	159.26	12.24	10.63	11.44
CA-5	139.80	167.27	153.54	185.31	183.21	184.26	10.71	14.94	12.83
CA-6	122.53	162.00	142.27	112.66	172.25	142.46	8.82	13.19	11.01
CA-7	102.67	137.80	120.24	81.96	155.24	138.60	7.05	12.37	9.71
CA-8	114.07	128.27	121.17	132.19	165.84	149.02	10.53	10.87	10.70
CA-9	122.80	103.40	113.10	159.00	124.79	141.90	10.92	9.32	10.12
CA-10	93.00	153.93	123.47	154.33	197.05	175.69	8.92	14.67	11.80
CA-11	86.13	136.40	111.27	138.00	192.52	165.26	7.97	12.53	10.25
CA-12	69.53	103.93	86.73	104.08	104.62	104.35	6.01	9.13	7.57
CA-13	68.17	61.73	64.95	93.77	79.17	86.47	6.57	5.72	6.15
CA-14	68.27	75.20	71.74	88.06	97.04	92.55	6.24	6.84	6.54
CA-15	62.73	54.20	58.47	88.44	79.28	83.86	5.97	5.40	5.69
CA-16	125.60	151.73	138.67	151.13	221.04	186.09	11.04	14.41	12.73
CA-17	79.33	73.20	76.27	84.86	82.69	83.78	6.64	6.35	6.50
CA-18	97.13	85.40	91.27	143.17	124.79	133.98	8.85	7.38	8.12
CA-19	88.00	134.13	111.07	120.31	184.67	152.49	8.11	11.87	9.99
CA-20	80.13	60.33	70.23	134.16	76.73	115.45	8.91	6.27	7.59
CA-21	123.67	117.27	120.47	172.12	161.08	151.60	11.97	10.69	11.33
CA-22	117.13	110.73	113.93	159.75	152.73	156.24	10.96	10.28	10.62
CA-23	68.87	64.20	66.54	121.38	108.75	115.07	7.01	6.64	6.83
CA-24	89.33	117.33	103.33	112.92	149.06	130.99	7.85	10.71	9.28
CA-25	53.87	86.27	70.07	95.42	127.11	111.27	5.51	8.12	6.82
CA-26	116.27	125.93	121.10	154.50	164.10	159.30	10.66	10.85	10.76
CA-27	102.67	137.27	119.97	127.23	186.98	157.11	8.51	12.40	10.46
CA-28	163.73	146.53	155.13	206.59	197.63	207.11	13.76	11.77	12.77
CA-29	167.20	181.67	174.44	247.50	277.00	262.25	14.68	15.96	15.32
CA-30	158.60	147.73	153.17	223.15	217.38	220.27	14.37	13.10	13.74
CA-31	119.27	85.40	102.34	139.52	99.04	119.28	10.53	7.72	9.13
CA-32	100.33	86.47	93.40	182.31	185.27	183.79	8.11	7.53	7.82
CA-33	108.60	152.40	130.50	140.42	135.65	138.04	9.91	13.92	11.92

Contd...

Table 2 Contd...

Genotype	No. of fruits per plant			Individual plant yield (g/ plant)			Fresh green fruit yield (tonnes/ ha)		
	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
CA-34	123.77	190.53	157.15	205.38	261.38	233.38	11.57	16.42	14.00
CA-35	108.47	143.47	125.97	147.25	187.34	167.30	9.17	12.97	11.07
CA-36	102.13	161.07	131.60	158.77	241.33	200.05	9.57	15.38	12.48
CA-37	93.20	142.80	118.00	135.64	182.29	158.97	8.75	13.26	11.01
CA-38	89.33	162.60	125.97	151.38	228.65	190.02	7.84	15.60	11.72
CA-39	124.93	155.00	139.97	175.75	216.98	196.37	10.74	13.55	12.15
CA-40	119.33	187.27	153.30	175.94	263.83	219.89	10.42	16.39	13.41
CA-41	108.87	140.13	124.50	140.40	204.23	172.32	9.11	13.12	11.12
CA-42	90.13	139.53	114.83	137.62	198.52	168.07	8.62	12.51	10.57
CA-43	45.53	71.33	58.43	60.05	91.00	75.53	3.58	6.10	4.84
CA-44	97.73	140.87	119.30	119.19	181.94	150.57	8.62	12.76	10.69
CA-45	121.27	150.13	135.70	109.14	189.75	149.45	9.80	13.77	11.79
CA-46	113.60	182.40	148.00	149.67	245.94	197.81	9.22	15.95	12.59
CA-47	147.07	176.27	161.67	240.71	249.06	244.89	13.95	15.24	14.60
CA-48	128.67	170.20	149.44	221.38	243.86	232.62	12.57	15.20	13.89
CA-49	125.27	151.60	138.44	187.56	242.75	220.16	10.24	14.01	12.13
CA-50	124.20	102.67	113.44	157.29	135.92	146.61	11.53	9.75	10.64
CA-51	98.80	182.67	140.74	139.50	232.25	185.88	8.87	14.13	11.50
CA-52	124.53	166.20	145.37	163.46	231.83	197.65	10.92	14.56	12.74
CA-53	100.27	131.00	115.64	129.54	169.92	149.73	8.65	11.34	10.00
CA-54	112.20	151.77	131.99	149.65	200.79	175.22	9.72	12.66	11.19
CA-55	124.00	158.27	141.14	169.34	215.25	192.30	10.94	12.86	11.90
DKC-8	122.07	145.73	133.90	146.96	173.08	160.02	9.65	11.55	10.60
Pusa Sadabahar	121.47	126.87	124.17	112.89	176.83	154.86	8.83	11.70	10.27
Chilli Philhal	85.97	141.07	113.52	110.52	144.71	127.62	6.96	10.47	8.72
Pusa Jwala	95.87	83.73	89.80	119.33	97.93	108.63	7.63	6.90	7.27
Utkal Abha	109.47	180.47	144.97	110.71	96.06	103.39	9.86	15.37	12.62
G-4	104.60	75.87	90.24	145.13	125.67	135.40	8.96	7.08	8.02
PC-1	121.87	137.30	129.59	158.17	191.04	174.61	10.17	11.24	10.70
CA-58	78.20	81.87	80.04	105.96	113.92	109.94	7.20	7.42	7.31
CA-59	283.60	302.00	292.80	120.62	126.06	123.34	8.51	8.80	8.65
CA-60	460.87	476.20	468.54	97.29	106.60	101.95	6.96	7.12	7.04
Range	45.53-460.87	54.20-476.20	58.47-468.54	60.50-247.50	82.69–277.00	75.53-262.25	3.58-14.68	5.40-16.42	4.84-15.32
CD (P = 0.05)	8.24	7.79	7.98	9.02	8.52	8.65	1.17	1.44	1.30

followed by CA-59 (292.80) and CA-29 (174.44. But it was significantly less in CA-43 (58.43), which was statistically *at par* with CA-15 (58.47). Finding regarding the fruit number of Pusa Jwala is also corroborate with findings of Phulari (10). It was noted from the different chilli genotypes showed a variation among themselves and year to year with respect fresh green fruit yield. Among the different genotypes maximum fresh fruit yield was recorded in CA-29 (262.25 g/ plant, 15.32 tonnes/ha, respectively), which was statistically *at par* with CA-47 (244.89 g/ plant, 14.60 tonnes/ha, respectively). The higher

Table 3. Ascorbic acid content, capsaicin content and extractable colour content of different chilli genotypes.

Genotype	Ascorbic acid (mg per 100 g FW)			Capsaicin content in green fruit (%)			Extractable colour (ASTA)		
	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
CA-1	135.57	140.57	138.07	0.52	0.53	0.53	77.74	86.86	82.30
CA-2	102.96	104.39	103.68	0.84	0.83	0.83	81.51	78.57	80.04
CA-3	124.75	118.01	121.38	0.64	0.63	0.63	119.21	110.86	115.03
CA-4	111.53	114.15	112.84	0.23	0.24	0.24	126.42	129.26	127.84
CA-5	116.59	121.26	118.93	0.51	0.50	0.50	116.21	103.15	109.68
CA-6	121.01	124.25	122.63	0.37	0.39	0.38	91.12	102.24	96.68
CA-7	136.96	139.69	138.33	0.31	0.30	0.31	99.84	87.77	93.80
CA-8	74.78	75.94	75.36	0.48	0.48	0.48	75.43	82.90	79.17
CA-9	132.04	138.41	135.23	0.55	0.54	0.54	121.60	117.11	119.35
CA-10	140.27	132.86	136.57	0.74	0.75	0.75	119.39	123.05	121.22
CA-11	92.68	95.06	93.87	0.66	0.65	0.66	77.13	83.56	80.34
CA-12	128.02	120.70	124.36	0.78	0.78	0.78	106.19	111.73	108.96
CA-13	109.3	113.84	111.57	0.65	0.67	0.66	105.38	95.60	100.49
CA-14	158.24	154.34	156.29	0.57	0.59	0.58	101.89	97.92	99.91
CA-15	72.18	77.37	74.78	0.68	0.71	0.70	110.76	119.23	114.99
CA-16	137.93	135.67	136.80	0.30	0.30	0.30	76.88	71.27	74.07
CA-17	129.76	135.64	132.70	1.11	1.03	1.07	46.32	43.77	45.05
CA-18	102.94	98.54	100.74	0.60	0.63	0.62	107.93	111.89	109.91
CA-19	152.03	157.77	154.90	0.54	0.51	0.53	95.51	87.93	91.72
CA-20	168.69	161.33	165.01	0.26	0.27	0.26	56.66	50.57	53.62
CA-21	111.47	108.65	110.06	0.61	0.63	0.62	78.41	72.61	75.51
CA-22	119.90	124.33	122.12	0.52	0.50	0.51	70.75	68.04	69.40
CA-23	124.71	116.35	120.53	0.79	0.78	0.79	65.82	61.49	63.65
CA-24	131.46	125.57	128.52	0.49	0.48	0.48	92.82	97.44	95.13
CA-25	127.94	134.72	131.33	0.68	0.69	0.69	95.77	89.81	92.79
CA-26	104.53	111.77	108.15	0.41	0.42	0.42	81.87	77.52	79.70
CA-27	172.07	164.12	168.10	0.62	0.63	0.63	125.05	116.07	120.56
CA-28	113.27	109.12	111.20	0.65	0.66	0.65	115.75	109.17	112.46
CA-29	143.82	145.31	144.57	0.51	0.52	0.51	116.55	111.97	114.26
CA-30	131.19	131.97	131.58	0.36	0.36	0.36	79.44	84.18	81.81
CA-31	120.83	111.04	115.94	0.64	0.65	0.64	131.50	125.50	128.50
CA-32	109.40	107.12	108.26	0.55	0.55	0.55	98.16	93.41	95.79
CA-33	125.02	120.05	122.54	0.51	0.53	0.52	116.87	122.13	119.50
CA-34	112.31	115.62	113.97	0.34	0.32	0.33	92.17	88.17	90.17
CA-35	134.58	124.33	129.46	0.62	0.60	0.61	113.32	115.67	114.49
CA-36	139.78	130.80	135.29	0.36	0.35	0.36	104.27	111.99	108.13
CA-37	113.40	117.53	115.47	0.30	0.28	0.29	138.92	143.83	141.38

Contd...

Table 3 Contd...

Genotype	Ascorbic acid (mg per 100 g FW)			Capsaicin content in green fruit (%)			Extractable colour (ASTA)		
	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
CA-38	140.68	136.58	138.63	0.98	0.97	0.98	134.81	140.75	137.78
CA-39	140.93	134.66	137.80	0.45	0.47	0.46	101.95	96.62	99.29
CA-40	98.15	102.84	100.50	0.35	0.34	0.35	145.80	143.22	144.51
CA-41	117.54	111.96	114.75	0.31	0.31	0.31	77.05	81.89	79.47
CA-42	145.64	147.74	146.69	0.47	0.69	0.58	123.81	114.18	119.00
CA-43	107.1	111.95	109.53	0.44	0.67	0.55	83.74	89.69	86.71
CA-44	103.05	107.97	105.51	0.29	0.43	0.36	106.56	98.75	102.66
CA-45	120.6	123.71	122.16	0.66	0.67	0.66	43.43	36.15	39.79
CA-46	115.53	122.21	118.87	0.64	0.65	0.64	121.14	126.65	123.89
CA-47	126.36	123.11	124.74	0.37	0.36	0.36	60.81	65.78	63.29
CA-48	119.15	114.61	116.88	0.54	0.56	0.55	88.28	92.89	90.58
CA-49	113.23	113.23	113.23	0.64	0.63	0.64	149.91	154.72	152.32
CA-50	123.92	122.70	123.31	0.40	0.60	0.50	106.24	110.70	108.47
CA-51	135.77	128.02	131.90	0.44	0.42	0.43	97.42	93.45	95.44
CA-52	103.03	104.22	103.63	0.96	0.98	0.97	103.88	98.77	101.33
CA-53	124.56	125.93	125.25	0.46	0.69	0.58	129.48	123.22	126.35
CA-54	126.29	128.17	127.23	0.52	0.55	0.54	107.50	107.09	107.30
CA-55	152.48	156.88	154.68	0.19	0.19	0.19	190.95	179.73	185.34
DKC-8	128.15	123.79	125.97	0.36	0.36	0.36	152.36	133.57	142.97
Pusa Sadabahar	147.88	140.42	144.15	0.49	0.48	0.49	107.71	101.95	104.83
Chilli Philhal	122.35	127.04	124.70	0.56	0.56	0.56	138.56	145.24	141.90
Pusa Jwala	134.43	139.50	136.97	0.51	0.54	0.52	127.11	120.74	123.93
Utkal Abha	146.56	141.13	143.85	0.84	0.83	0.83	151.79	160.84	156.31
G-4	142.8	147.49	145.15	0.79	0.82	0.81	112.30	125.49	118.90
PC-1	107.60	104.16	105.88	1.16	1.21	1.19	144.36	140.62	142.49
CA-58	114.11	110.55	112.33	1.51	1.52	1.52	124.87	128.18	126.53
CA-59	102.95	106.59	104.77	1.68	1.69	1.69	76.54	74.66	75.60
CA-60	87.30	81.70	84.50	2.10	2.06	2.08	93.53	86.21	89.87
Range	72.18-172.07	75.94-164.12	74.78-168.10	0.19-210	0.19-2.06	0.19-2.08	46.32-190.95	43.77-179.73	45.05-185.34
CD (P = 0.05)	7.54	5.74	7.52	0.03	0.03	0.03	7.35	6.54	6.92

fresh fruit yield per hectare was also recorded in CA-34 (233.38 g/ plant, 14.00 tonnes/ha, respectively), which was also statistically *at par* with CA-48 (232.62 g/plant, 13.89 tonnes/ha, respectively), CA-30 (230.27 g/plant, 13.74 tonnes/ha, respectively), CA-40 (219.89 g/plant, 13.41 tonnes/ha, respectively). The lowest yield was recorded in CA-43 (75.53 g/ plant, 4.84 tonnes/ha, respectively), which was also statistically *at par* with CA-15 (83.86 g/plant, 5.69 tonnes/ha, respectively). The higher yield CA-29, CA-47, CA-34 and other genotypes might be due higher number of fruits per plant and moderate individual fruit weight. The genotypes CA-60 produced the highest number of fruits and CA-59 produced the second highest number of fruits but produced lower yield than the high yield genotypes could be due to

very low individual fruit weight. Hundal and Khurana (8) reported that fruit yield in chilli varied from 0.23 to 33.52 tonnes per ha. This finding support the observation on fresh yield of the present experiment.

Maximum ascorbic acid content (168.10 mg/100 g fresh) was recorded by CA-27, which was statistically *at par* with CA-20 (165. mg/100 g fresh). Significantly lowest ascorbic acid content (74.78 mg/100 g fresh) was observed in CA-15, which was also statistically *at par* with CA-8 (75.36 mg/100 g fresh). In an experiment Chaudhary and Samadia (2) reported ascorbic acid content of chilli was ranged from 70.83 to 237.30 mg/100 g fresh with mean value 151.38 mg/100 g fresh. Besides Deshpande and Anand (3) estimated ascorbic acid content ranged from 58.7 to 192.1 mg/100 g fresh. The significantly highest capsaicin in green fruit was recorded in CA-60 (2.08%) followed by CA-59 (1.69%) and CA-58 (1.52%). More than 1% capsaicin content was recorded in PC-1 (1.19%), CA-17 (1.07%). The significantly lowest capsaicin content in green fruit was recorded in CA-55 (0.19). Mathur *et al.* (9) reported that Tezpur variety of Indian chilli contains maximum capsaicin (4.27%) and it seems to be the hottest chilli in the world. Whereas, Govinda Reddy *et al.* (5) recorded the maximum capsaicin upto 0.52% in Variety LCA-235. Hence, the finding indicated that variation in capsaicin content might be due to different genotypes used for different experiment.

The significantly maximum extractable fruit colour was recorded in CA-55 (185.34 ASTA). The higher extractable fruit colour was also recorded in Utkal Abha (156.31 ASTA) and CA-49 (152.32 ASTA). The significantly lowest fruit colour was observed in CA-45 (39.79 ASTA), which was statistically *at par* with CA-17 (45.05 ASTA). From the above discussion it may be concluded that the genotype CA-29, CA-47 and CA-48 may be selected in further crop improvement programme under *terai* zone of West Bengal for their higher yield and good quality characters.

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Received : July, 2013; Revised : January, 2016;
Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00021.9

Indian J. Hort. 73(1), March 2016: 74-77

Biological control of *Fusarium* wilt of chillies using *Trichoderma* spp.

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ABSTRACT

Chilli wilt caused by *Fusarium solani* is a serious menace in black cotton soils of Karnataka. Wilt incidence of 19.8 to 31.5 and 7.5 to 12.3% was recorded in major chilli growing regions of Karnataka during 2011-12 and 2012-13 respectively. *Trichoderma harzianum* isolates 1, 2 and 5 and *T. viride* isolate 2 completely overgrew *F. solani* and inhibited mycelia growth by 40-50% *in vitro*. *T. viride* gave moderate effect against wilt and increased yield upto 30% in Haveri district, while at Bellary, *T. harzianum* isolate 1 reduced wilt incidence by 39.7%.

Key words: Bio-control, *Capsicum annuum*, *Fusarium* wilt, Karnataka, *Pseudomonas* fluorescens, Trichoderma.

INTRODUCTION

Chilli (*Capsicum annum*) is a major commercial crop grown as vegetable and spice having value addition in pharmaceuticals, cosmetics and beverages. India is a major producer, exporter and consumer of chilli with a cultivated area of 7.94 lakh ha and annual production of 13.04 lakh tonne (Anon, 2). It is grown primarily in Andhra Pradesh, Gujarat, Karnataka, Odisha and Maharashtra. In the recent decades, fungal wilts and dry root rots caused by *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotium rolfsii* have caused severe crop losses in chilli (Devika Rani *et al*., 5; Singh, 15). Area under chilli is dwindling due to *Fusarium* wilt in the intermediate hill zone of Jammu and Kashmir (Nayeem *et al*., 10). Yield loss to the tune of 50-80% was reported under heavy incidence (Madhavi *et al*., 7). In Karnataka, incidence is particularly high in the irrigated tracts of black cotton soil (Devika Rani *et al*., 5). At experimental level, chemicals gave promising results against chilli wilt (Singh, 15; Singh *et al*., 14). However, application of fungicides under field conditions results in high cost, environment pollution and inconsistency in efficacy. Chilli varieties / hybrids that are popular are highly susceptible to wilt and resistant accessions fail to make any dent with available strategies having little impact against chilli wilt. Bio-control agents also have been tested and successfully employed against soil borne pathogens including *Fusarium* (Mukhopadhyay, 8). Present study was undertaken to identify effective bio-agents against chilli wilt for irrigated (Haveri) and rain-fed/ irrigated (Bellary) chilli growing tracts in Karnataka during 2011-12 and 2012-13.

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MATERIALS AND METHODS

A roving survey for the incidence of chilli wilt was undertaken during January, 2012 and 2013 in the major chilli growing regions of Haveri (Haveri, Hangal, Hirekerur, Byadgi and Ranebennur), Bellary (Hospet, Sirugoppa and Bellary), Raichur (Raichur and Deodurga), Koppal (Gangavathi) and Yadgir (Shahapur) districts of Karnataka. In each location, 10 villages with ten fields/ village were surveyed. Occurrence of wilt in 15 plants in each of 10 spots per field was recorded and incidence per cent was calculated. Average incidence for the locations was calculated and means incidence for each district was also obtained. Samples of affected plants were brought to the laboratory and isolated on potato dextrose agar (Aneja, 3). Based on morphological and microscopic observations, identification of the fungus was made and pure culture of the pathogen deposited in ITCC, New Delhi after proof of pathogenicity (Fiume and Fiume, 6).

Twelve *Trichoderma* isolates isolated by serial dilution (Aneja, 3) from the rhizosphere of potato, chilli and bell pepper and five isolates of *Pseudomonas fluorescens* (Division of Plant Pathology, IARI, New Delhi) were evaluated for their efficacy against *Fusarium solani* by dual culture technique. Five mm discs from seven-day-old cultures of *F. solani* (slow growing) were placed at one end of the petridishes and exactly after 86 h, five mm discs of *Trichoderma* (fast growing) was placed at the other end of petridish to compensate for their growth behavior. In all, three replications were maintained for each treatment with suitable control for *Trichoderma* and *Fusarium.* Petridishes were incubated at 27°C and observations were made at regular intervals for mycelia inhibition by each of the *Fusarium* and *Trichoderma* isolates on one another (Sharma, 13) and overgrowth if any

was recorded. Five mm plug from the leading edge of freshly growing culture of *F. solani* was placed at the centre of the plate and after 86 h, 48-h-old culture of *P. fluorescens* was streaked at both ends equidistant point along the perimeter of the plate. In all, three replications were maintained for each treatment and plates without bacteria served as control. Plates were incubated at 27°C till 12 days and per cent inhibition was calculated based on the radial growth of *F. solani* (Dennis and Webster, 4).

Formulations of selected *Trichoderma* and *P. fluorescens* were made by following the methods of Ramanujam *et al.* (12) and Nandakumar *et al.* (9). *Trichoderma* was multiplied in pre-soaked sterilized sorghum seeds for 10 days at 29°C in a BOD incubator. Sorghum seeds covered with dark green *Trichoderma* mass were shade dried and ground to a fine powder using a blender. Entire content was mixed with sterile talcum powder and calcium carbonate (10 g/kg) to get the desired population of 2-5 × 10^8 cfu/g powder. *P. fluorescens* was cultured in Kings B broth for 48 h in an orbital shaker at 150 rpm. One kg sterile talcum powder, 15 g calcium carbonate and 10 g carboxy methyl cellulose were added to the 400 ml bacterial broth, mixed and dried overnight. Formulation was packed in polypropylene bag and sealed till further use in fridge. Bacterial population at the time of application was 2.5 × 10^8 cfu/g.

Field testing of bio-agents: Based on *in vitro* studies, three isolates of *T. harzianum*, one each of *T. viride* and *P. fluorescens* were tested against *F. solani* under field conditions at two locations in Haveri and one at Bellary district, Karnataka. At Haveri, experiment was taken up during the crop season of October to May (irrigated/ rainfed) during 2011-2012 / 2012-13 using the local popular and highly susceptible variety BSS 414. At Bellary, local popular variety and highly susceptible Byadgi Kaddi was used and experiment was carried out during July to February 2011-12/ 2012-2013 under irrigated / rainfed conditions. In Haveri, chilli seeds were treated with the formulations @ 10 g/kg seeds and raised in nursery during October-November and transplanted in November, while in Bellary, treated seeds were directly sown in the main field. At the time of transplanting, seedlings in bundles were dipped in water containing talc based formulations (20 g/l) for 2 h. Carbendazim 12% + mancozeb 63% (std. check) seed treatment was given @ 2 g/kg seed. *Trichoderma* and *P. fluorescens* were applied to soil at 5 kg/ha twice, one at the time of sowing / transplanting and the other at the time of flowering mixed with well rotten FYM. Carbendazim 12% + mancozeb 63% WP was applied @ 2 g/l water through drenching at the time of transplanting and flowering. Crop was raised by following the standard package of practices. In all, there were seven treatments including five bio-control agents with untreated check and standard control (carbendazim + mancozeb). Each treatment was replicated thrice in plot of size 10 m × 10 m with spacing of 75 cm × 60 cm in RBD design. At Bellary, spacing was 60 cm × 60 cm. Observation on *Fusarium* wilt was made at the final stage of the crop and incidence (%) was calculated. Harvest of each green chilli picking at Haveri was weighed and expressed as yield / plot of 100 sq. m was calculated. At Bellary, only disease incidence was recorded. Treatment means were compared by Duncan's Multiple Range test (DMRT).

RESULTS AND DISCUSSION

Wilt incidence was high during 2011-12 compared to 2012-13 in all the regions of survey. Haveri recorded the highest mean incidence of 31.5%, while Koppal recorded the least 19.8%. During 2012-13, wilt incidence was low (1-18%) in the surveyed districts (Fig. 1). Bellary recorded the highest (12.3%) closely followed by Bellary and Raichur (upto 8.9%) while Yadgir recorded the least (5.1%). Repeated isolation yielded the fungus *Fusarium solani*, which was confirmed by ITCC Delhi (ID No. 8760.12). Pathogenicity was proven using 25% culture filtrate wherein treated seedling collapsed within 36 h. In all the twelve dual culture sets, initial counter inhibition was observed between *Trichoderma* and *Fusarium*, which posed varying degree of inhibition on each other. Inhibition of *Trichoderma* by *Fusarium* ranged from 38.9 to 66.7% by three days (data not shown). During the same period, inhibition of *Fusarium* by *Trichoderma* ranged from 0 to 28.6%. *T. harzianum* 1, 2 and 5 and *T. viride*, 2 isolates completely overgrew *Fusarium*, while *T. hamatum and Trichoderma* sp. 2 failed to overcome inhibition posed by *Fusarium* (Fig. 2). In a similar type of study, Sharma (13), reported pre-contact inhibition followed by chemo attractive and parasitic phase between *F. oxypsorum* f.sp. *pisi* and *Trichoderma* interaction. Among *P. fluorescens* isolates, pf3 isolate

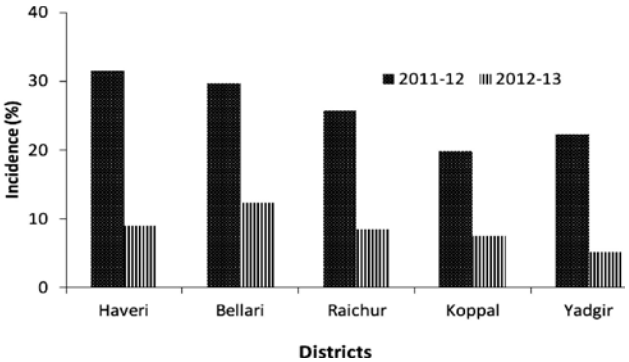

Fig. 1. *Fusarium* wilt incidence (%) in chilli in Karnataka.

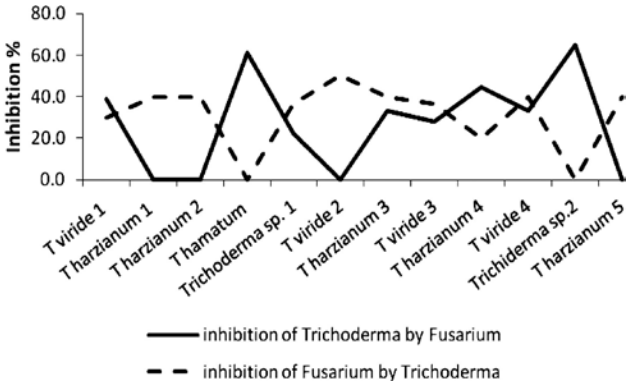

Fig. 2. Counter inhibition of *Trichoderma* and *Fusarium*.

gave the maximum inhibition (39.2%), which was at par with pf6 and pf80 isolates.

At Tavaragopppa, Haveri, *T. viride* was superior to other bio-agents in managing *Fusarium* wilt (Table 1). Incidence of 15.4 and 6.2% was recorded in *T. viride* treatment in two years as against 31.4 and 15.4% respectively in untreated control. Singh (15) reported moderate effect of *T. viride* and *T. harzianum* against chilli wilt at Himachal Pradesh. Lowest incidence (12.6 and 4.1%) was recorded in carbendazim + mancozeb treatment. At Hosalli, incidence of wilt was very high in all the treatments and none found effective including the chemical. The failure of various treatments could be due to very high load of the inoculun and favourable conditions for the pathogen. In general, *Fusarium* wilt was low during 2012-13 possibly because of dry weather. During 2011-12, highest yield (125.7 kg/ 100 sq.m) was recorded by *T. viride* treatment closely followed by carbendazim + mancozeb, which were at par. Almost a similar trend was noticed during 2012-13. *P. fluorescens* though failed to give protection, yielded 14 and 11% higher yield compared to untreated control. *Pseudomonads* are reported to be involved in growth promotion by the production of phytohormones, nitrogen fixation, phosphate solubilization (Park *et al*., 11). At Hosalli, crop yield was adversely affected due to failure of the crop. However, *T. viride* and carbendazim + mancozeb treated plots yielded almost twice the yield of untreated control. At Bellary, during 2011-12, *T. viride* and two isolates of *T. harzianum* gave moderate effect against chilli wilt with 27-35% protection over control (Table 2). During 2012-13, all treatments except control recorded < 10% incidence.

At low to moderate level of incidence, *T. viride* and *T. harzianum* gave encouraging results and be part of management strategy. However, at high level of incidence, resistance source, bio-agents and modified cultural practices (drip irrigation wherever feasible, planting in raised ridges, level land) should be part of the management strategies.

Table 1. Effect of bio-agents against chilli wilt at Haveri, Karnataka.

Treatment	Tavaragoppa						Hosalli					
	Incidence (%)			Yield (kg/100 sq.mt)			Incidence (%)			Yield (kg/100 sq.mt)		
	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean	2011-12	2012-13	Mean
T. viride 2	15.4 (3.9)^{cd}	6.2 (2.49)^{d}	10.8 (3.2)^{d}	125.7^{a}	131.3^{a}	128.5^{a}	70.1 (56.9)^{cd}	7.6 (2.74)^{d}	38.8 (38.5)^{c}	40.0^{a}	128.3^{a}	84.2^{a}
T. harzianum 1	17.6 (4.2)^{cd}	9.9 (3.15)^{c}	13.8 (3.7)^{c}	114.3^{bc}	125.7^{ab}	120.0^{bc}	77.0 (61.5)^{bc}	8.0 (2.83)^{d}	42.5 (40.6)^{b}	29.0^{b}	130.3^{a}	79.7^{b}
T. harzianum 2	16.6 (4.1)^{cd}	10.4 (3.22)^{bc}	13.5 (3.6)^{c}	113.3^{bc}	120.0^{bc}	116.7^{c}	83.0 (65.6)^{b}	9.3 (3.05)^{cd}	46.1 (42.8)^{b}	26.0^{bc}	125.3^{a}	75.7^{b}
T. harzianum 5	22.4 (4.7)^{bc}	11.1 (3.33)^{bc}	16.7 (4.0)^{b}	110.7^{c}	125.3^{ab}	118.0^{c}	79.3 (63.0)^{b}	10.8 (3.29)^{bc}	45.3 (42.3)^{b}	23.3^{c}	119.7^{b}	71.5^{d}
P. fluorescence pf 3	26.7 (5.2)^{ab}	12.4 (3.52)^{b}	19.6 (4.3)^{b}	107.7^{c}	118.3^{c}	113.0^{d}	77.0 (61.5)^{bc}	11.7 (3.42)^{b}	44.4 (41.8)^{b}	29.0^{b}	128.0^{a}	78.5^{bc}
Carbendazim + mancozeb	12.6 (3.5)^{d}	4.1 (2.03)^{e}	8.4 (2.8)^{e}	120.0^{ab}	126.3^{ab}	123.2^{b}	62.1 (52.0)^{d}	4.6 (2.14)^{e}	33.3 (34.6)^{d}	38.3^{a}	129.3^{a}	83.8^{a}
Control	31.4 (5.6)^{a}	15.4 (3.92)^{a}	23.4 (4.8)^{a}	91.7d	106.0^{d}	98.8^{e}	89.6 (71.4)^{a}	17.5 (4.18)^{a}	53.6 (47.1)^{a}	22.0^{c}	110.0^{c}	66.0^{e}

Data followed by the same letter(s) in column are not significantly different at 5% level

Table 2. Effect of bio-agents against chilli wilt at Bellary, Karnataka.

Treatment	2011-12	2012-13	Mean
T. viride 2	44.3 (41.7)^{bc}	9.3 (3.1)^{b}	26.8 (31.1)^{cd}
T. harzianum 1	39.5 (38.9)^{c}	7.1 (2.7)^{c}	23.3 (28.8)^{d}
T. harzianum 2	41.2 (39.9)^{c}	8.4 (2.9)^{b}	24.8 (29.9)^{d}
T. harzianum 5	52.1 (46.2)^{ab}	9.0 (3.0)^{b}	30.5 (33.3)^{bc}
P. fluorescence pf3	55.8 (48.3)^{a}	8.8 (3.0)^{b}	32.3(34.6)^{b}
Carbendazim + mancozeb	37.1 (37.4)^{c}	6.6 (2.6)^{c}	21.8(27.9)^{d}
Control	61.3 (51.4)^{a}	16.1 (4.0)^{a}	38.7(38.4)^{a}

Data followed by the same letter(s) in column are not significantly different at 5% level

ACKNOWLEDGEMENTS

Senior author is grateful to Dr O.M. Bambawale, Ex Director, NCIPM, Delhi for encouragement and Mrs. Neelam Mehta, T-5 (TO) for technical support.

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Received : October, 2014; Revised : January, 2016; Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00022.0

Indian J. Hort. 73(1), March 2016: 78-81

A simple DSS for potato crop scheduling in Nilgiri hills of Western Ghats

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ABSTRACT

A decision support tool has been developed for providing information on the optimum time of planting and the likely consequences of early or late planting of potato in about 173 locations of Nilgiris region of Tamil Nadu state in India. This DSS was developed for the most popular variety of the region *i.e.*, Kufri Jyoti, and the choice to select right time of harvest is also incorporated in this DSS by simulating the yields at 100 and 120 days. The tool consists of a database of simulated yield at 100 and 120 days after planting derived through InfoCrop-potato model. This DSS is developed with the database generated using the daily weather data developed through weather generators and the potential yields estimated with the help of InfoCrop-Potato model. These data were generated by running the model under rainfed conditions for five dates of planting using the weather data for each location generated through weather generators. A user interface was developed in Visual Basic to access this database. The DSS developed for the purpose of potato crop scheduling is of great significance, which enables the farmers as well as extension functionaries for taking right decisions on timing the planting and harvesting of potato crop on which the major fraction of Nilgiri's economy depends. The simulated results when compared with the actual data and a good degree of correlation was observed between two.

Key words: Decision support system, Infocrop-potato model, planting date, potential yield.

INTRODUCTION

Potato is an important crop in India which occupies 4th position in area and 3rd in production after China and Russia in the list of global potato producers. Potato is grown under different production situations in Nilgiris. The altitude ranges between 400 to 2,600 m above mean sea level. The soil types of the region also vary widely between clay loam to sandy loam in different locations. The mild climatic condition prevalent throughout the year makes it possible to grow three crops in a year and out of them, summer and autumn crops are grown entirely under rainfed conditions. Crop scheduling, being a non monetary input, under such widely varying production situations is much more challenging as all the locations are not equally suitable for growing potato crop under different seasons. The decisions of scheduling planting and also selecting a suitable hybrid are among the most important decisions in agriculture (Nelson, 5). Generating recommendations for crop scheduling through field experimentation is an impossible task under such delicate situations and using simulation modeling technique for deriving results seems to be a better alternative. A crop model InfoCrop-Potato has been developed and calibrated for simulating the growth and development of Indian potato varieties under the subtropical conditions (Singh *et al.*, 7). In the case of crop scheduling, not many tools for determining the optimum time of planting have been developed. A DSS which can give information on optimum time of planting in every 15 km^2 grid will be of immense use especially under Nilgiri conditions. A Decision Support System for potato crop scheduling was developed by Central Potato Research Institute, Shimla but, it can be used only for winter potato crop that too under potential situations. Proper crop scheduling is required to extend the potato cultivation even to even non-traditional areas (Shashi Rawat *et al*., 6). Hence, the present investigation was undertaken during the years 2009, 2010 and 2011 to develop a DSS for potato crop scheduling in Nilgiris under two different seasons, *i.e.*, summer and autumn under rainfed conditions.

MATERIALS AND METHODS

In general any DSS consists of three important components, *viz.*, 1. Data base 2. Model (Decision context and user criteria) and 3. User interface. This DSS consists of a database (back end in MS Access) and a user interface (front end in Visual Basic). The database consists spatial data, *viz.* location names and attribute data, *viz.* InfoCrop-potato model derived yield outputs for two different seasons, *i.e.*, summer and autumn in which potato is grown under rainfed conditions with the variety Kufri Jyoti. In total 173 locations were surveyed and the co-ordinates of each location were collected with the help of GPS instrument. The attribute data was developed like Weather database (daily weather data

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for running InfoCrop model) was generated for each location with the help of NewLoc_clim and Global Rain weather generators.

In Nilgiris, more than 60 per cent of total potato crop is cultivated during summer season. During summer season, planting is taken up during the month of April with the help of pre-monsoon showers and the south-west monsoon sets on in the first week of June. Hence, five dates were selected starting from first of April spaced at 10 days interval till 10th of May (1st, 10th, 20th and 30th of April and 10th of May) for running the Infocrop-potato model under rainfed conditions under existing major soil types (loam, sandy clay loam and sandy loam) for each of the locations to generate the simulated results of yield under two different harvesting times (100 and 120 days). For each date of planting, the model was run for the most popular variety Kufri Jyoti for which genetic coefficients are available. Similarly, for autumn season crop, which is entirely dependent upon North-East monsoon the attainable yield was calculated under five different dates, *i.e*., 1st, 10th, 20th and 30th of August and 10th of September as it is usually planted during mid of August. Yield output at 100 days or the latest date at which crop matures earlier to 100 and at 120 days after planting or the latest date at which crop matures after 100 days and at more or less than 120 days at each scenario were extracted and linked to corresponding spatial attributes, *viz*., location names in MS Access.

The decision context is defined as the optimum time of planting and harvesting of potato based on the potential yield data obtained from InfoCrop potato model for different sites in Nilgirs. This was planned based on the existing conditions, *i.e*., for summer and autumn seasons each five different planting dates around the general recommended planting time were taken and for each planting date two harvesting periods were considered. In user interface, the user can extract the desired information through a series of selections in the order of season (*i.e*., summer or autumn), view details - location (173 locations) and also date of planting. The information pertaining to a particular query is filtered out through these series of selections. Finally the model output in tabular format containing the attainable yield data of the variety Kufri Jyoti at two durations of harvest, corresponding to 100 and 120 days after start of the planting can be derived. In some locations, due to unsuitability of weather, the crop may not stand up to 100 or 120 days in the field and in such conditions the yield at maturity is recorded.

The use of simulation models requires a comparison between estimated and measured data to assess model reliability (Thomas, 11). For the evaluation of prediction efficiency of the DSS and InfoCrop-potato model for Nilgiri conditions, certain deviance measures, modelling efficiency and coefficient of residual mass and Peason's correlation coefficient were estimated.

RESULTS AND DISCUSSION

The InfoCrop model used radiation use efficiency (RUE) for predicting potential yields in different production environments. The yield predictions are made based on the varietal genetic coefficients developed for Kufri Jyoti, soil characters and weather parameters on daily step basis of each particular location. The entire data set is presented in the DSS un MS-Access. For comparison sake, the predicted data was generated with four levels of nitrogen doses (0, 50, 100 and 150 kg per hectare) for potato (for which observed data was available) for two seasons, *viz*., summer and autumn (Table 1). The

Table 1. Simulated and observed yields of potato (q/ha) under varying nitrogen levels at CPRS, Ooty.

N level (kg ha^{-1})	Summer season					
	2009		2010		2011	
	Observed	Simulated	Observed	Simulated	Observed	Simulated
0	102.6	121.5	99.7	115.3	111.5	124.3
50	149.7	172.3	138.5	147.2	162.3	186.1
100	273.6	302.4	224.1	234.6	301.4	322.4
150	281.4	311.6	230.2	245.2	312.6	330.4
	Autumn season					
	2009		2010		2011	
	Observed	Simulated	Observed	Simulated	Observed	Simulated
0	124.3	132.6	108.2	124.1	128.2	132.2
50	162.7	182.9	142.6	150.6	158.6	174.9
100	294.1	304.6	276.3	280.5	300.5	310.6
150	302.6	312.7	282.9	291.5	304.3	314.2

results were compared with actual observed yields and found that the difference in predicted and observed yields for nitrogen trial had been between 5 to 10 per cent more than the actual. The variation was more in summer season than in winter. This difference was mainly because of change in severity of potato late blight disease, which is more severe in summer and this information is not incorporated in the model. The deviation of model predictions from the actual shows the efficiency of the model in predicting under conditions of the defined domain (Singh *et al*., 8). The InfoCrop-potato has been used successfully for pre-harvest yield forecasting (Govindakrishnan *et al*., 1; Singh *et al*., 10) and for optimizing the date of planting (Singh *et al*., 9) also.

In the present investigation, the model was validated with i) the available information from experimental fields of Central Potato Research Station for two different seasons (summer and autumn) for three years under four different nitrogen levels, ii) Available yield data from different locations of Nilgiris obtained from State Department of Horticulture records.For the above data (Table 1), the parameters MBE, MB%E, R^2 and Modelling efficiency were calculated and found that the model predicts potato yields satisfactorily under different nitrogen levels in Nilgiri conditions for potato. The positive value for MBE indicates that the model has little over estimated the yields both in summer and autumn seasons. This is mainly because Nilgiris is prone to late blight disease as a regular phenomenon. The effect of late blight is not included in the model. That could be the probable reason for the over estimation. Otherwise, the modeling efficiency being positive and above one indicates that the model has good efficiency to predict the yields under Nilgiri conditions. The correlation coefficient values being almost nearer to 1, indicate that there is perfect correlation between observed and predicted values by the model (Table 2). Similarly, the predicted values were also verified with that of long term average yields (where ever data is available) of some of the locations from the records of State Department of Horticulture and a great degree of correlation was observed between them (Fig. 1).

The present DSS could clearly bring out the impact of season, altitude and also the harvesting date on attainable yields of potato (Kufri Jyoti) under different dates of planting as there are clear cut differences in attainable yields of same locations in different seasons at same elevations and differences were also observed between locations with different altitudes in the same season. Similarly, the yield differences were also noticed between different dates of planting at same location and also under different dates of harvest. The results of few representative areas for summer and autumn seasons, for 20th April and 20th August dates of planting respectively from the present DSS have been summarized in the Table 3. A DSS to be successful should give specific outputs without spending much time to run it. In this context, the strengths of this DSS are that from the farmers' point of view, even a person with a minimum knowledge of computer can use the DSS to generate the results. Further, this is very much useful for the researchers, policy makers and extension officials as it gives information on potato crop scheduling as well as harvesting schedule within no time. Further the different types of outputs makes it more useful, since, apart from planning the consequences of early and late planting as reflected on the yield in both the crop seasons it would enable the user to plan harvest schedule and thus plan his disposal strategy. Thus, this tool meets the criteria of good DSS as set out by earlier researchers (Magarey *et al*., 3) and is expected to be a useful aid for scheduling the potato crop according to local necessities in Nilgiris.

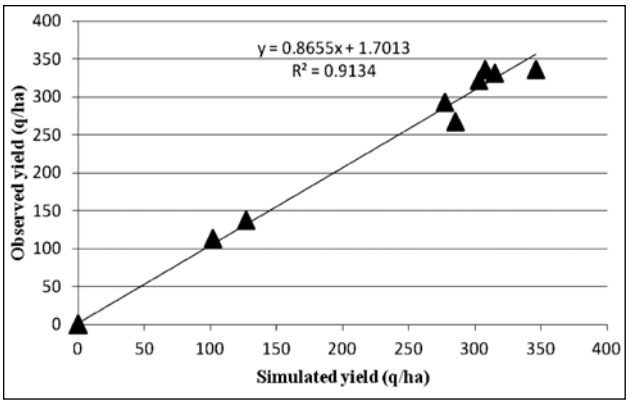

Fig. 1. Comparison of predicted yields (q ha^{-1}) with that of long term averages of different locations in Nilgiri hills.

Table 2. Model validation parameters for comparing simulated yields with observed yields under different nitrogen levels.

Season	MBE	MB%E	R	LR slope	Intercept	EF	CRM
Summer	18.8	9.3	0.9973	1.035	11.74	0.0936	-0.0945
Autumn	10.5	4.9	0.9984	0.9854	13.64	0.0202	-0.0488
General	14.6	7.04	0.9961	1.004	13.799	0.0404	-0.0707

Table 3. Simulated potato yield using INFO-CROP Potato model for different locations of Nilgiris.

Place			Summer season		
	Altitude (m)	Duration (days)	Yield (q/ha)	Duration (days)	Yield (q/ha)
Kallar	485	-99	-99	-99	-99
Barliar	799	82	68	94	102
Gudalur	996	101	110	109	127
Coonoor	1725	97	270	109	285
Kundah	1851	101	235	112	308
Kotagiri	1906	96	253	-99	-99
Ooty	2257	101	197	121	277
			Autumn season		
Kallar	485	-99	-99	-99	-99
Barliar	799	-99	-99	-99	-99
Gudalur	996	-99	-99	-99	-99
Coonoor	1725	99	302	110	315
Kundah	1851	99	335	117	346
Kotagiri	1906	81	287	-99	-99
Ooty	2257	100	292	116	303

-99 represents that the simulated crop has matured earlier to the said duration.

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Received : October, 2014; Revised : January, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00023.2

Indian J. Hort. 73(1), March 2016: 82-86

On-farm storage of table and processing potatoes in heaps

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ABSTRACT

Heap storage of potatoes is commonly used in many states of India to avoid distress sale at harvest, but the losses in stored potatoes are generally enormous. Spray application of CIPC (isopropyl N-(3-chlorophenyl) carbamate) at the time of storage has been recommended to inhibit sprouting and reduce total losses in potatoes up to 90 days of storage in heaps. Sprouting in tubers was inhibited and total losses in potatoes were reduced (by 58.7%) up to 90 days of storage (temp. 19-31°C, 55-90% RH) during March to June. The farmers could market 6.5% more weight of CIPC treated potatoes (cv. Kufri Pukhraj) compared to the control (untreated) tubers due to reduced total losses and fetch 55.3% higher market price than the price at the time of harvest. In processing cultivar, Kufri Chipsona-1, reducing sugar concentrations decreased from 188.1 to 22.5 mg/100 g fresh weight during storage up to 90 days and chip colour improved significantly. Stored potatoes were found highly acceptable for processing by an industry collaborator (M/s Satnam Agri Products Ltd., Jalandhar) and were used in making good quality flakes and French fries. Findings established that the improved storage technology can beneficially be used to increase remunerations from potato cultivation and to preserve the quality of processing potatoes for three months at lower storage cost.

Key words: Potato, heap storage, sprout inhibition, storage losses, processing quality.

INTRODUCTION

Heap storage of potato (*Solanum tuberosum* L.) is commonly used in many states of India to avoid distress sale at harvest. Sprouting of potatoes is the main problem under heap storage where losses due to shrinkage, sprouting and attack by microorganisms are generally enormous (15-25%). Spray application of CIPC @ 20 mg a.i. kg^{-1} tuber weight in heaps was effective in reducing sprouting, sprout weight and total losses up to 90 days of storage in nine potato varieties varying widely in dormancy period and storability (Mehta *et al*., 7). An on-farm heap storage technology integrating essential pre- and post-harvest measures with the use of sprout inhibitor CIPC (isopropyl N-(3-chlorophenyl) carbamate) was developed for short-term storage of table and processing potatoes (Mehta *et al.*, 8). Although the on-station trials provided broad guidelines for technology development, specific requirements needed to be devised in conjunction with the farmers and the potato industry by evaluating the improved technology in the climatic and physical conditions of beneficiaries farms and to monitor their experience in order to judge its acceptability. This investigation was, therefore, designed to evaluate on-farm storage in heaps at farmers' fields at four locations in Punjab during March to June, by determining losses in stored potatoes, their marketability as table potatoes and suitability for processing. Stored potatoes were also periodically tested by an industry collaborator to examine their suitability for processing into flakes and French fries.

MATERIALS AND METHODS

Farmers were randomly selected for the study. They were advised to follow essential pre harvest measures for raising the crop *viz*. tuber maturity and haulm cutting prior to harvest. The harvested tubers were kept in heaps covered with rice straw (*pural)* in their fields for 15-20 days to allow wound healing and curing of peel, prior to selecting undamaged and healthy tubers. Potatoes of cv. Kufri Pukhraj, a popular variety of the region, were stored for table purpose under the shade of trees in village Sultanpur (Jalandhar district) at three farms to determine storage losses and marketability of potatoes. Variety Kufri Chipsona-1 was stored in collaboration with the processing industry (M/s Satnam Agri Products Ltd., Pratapura, Jalandhar) under two storage conditions (high sheds made of asbestos sheets/shade of trees) in three villages of Punjab, *viz*. Badshahpur (Jalandhar district), Shamchaurassi (Hoshiarpur district) and Khatkar Kalan (Nawanshar district) to determine storage losses and the suitability of stored potatoes for processing.

Potatoes were treated with CIPC (20 mg a.i. kg^{-1} tuber weight) prior to storage and stored in heaps (Mehta *et al.*, 7) in tuber weight ranging between 20

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to 25 quintals at each place in the 1st week of March. Heaps of untreated control potatoes raised with all recommended pre- and post-harvest measures were laid nearby under the same storage environment. Ten kg of potatoes with three replications in each treatment packed in nylon bags were also placed in all heaps to record replicated data. Heaps laid in open were protected from rains during the storage period. Monitoring of tuber condition and processing quality evaluation of potatoes was done during storage and heaps were dismantled after 90 days of storage (DOS).

Temperatures and relative humidity was recorded daily at two locations, *i.e.* under the shade of trees and in the shed. Final observations on sprout weight (%), loss in weight due to tuber rotting and total weight loss (physiological + pathological + sprout loss) on fresh weight basis were recorded after storage. Tubers with slight evidence of decay were weighed to represent loss due to tuber rotting. Sprouting Index (SI) was recorded on 50 tubers per treatment after storage (Mehta *et al*., 7). At 0, 45 and 90 days of storage, tubers of only Kufri Chipsona-1 were analyzed for reducing sugars, sucrose and chip colour as per standard methods (Mehta and Singh, 6) as cv. Kufri Pukhraj is known for producing unacceptable colour chips due to higher reducing sugar contents (>250 mg/100 g fresh weight) in potatoes stored in heaps (Mehta *et al.*, 7). Chip colour was scored on 1-10 scale of increasing colour using the chip colour cards (Ezekiel *et al*., 2). Chip colour score up to and including 5 was considered acceptable. Potatoes were also periodically tested for dry matter and sugar level (by strip test) and chip colour in laboratory of the industry. Data was statistically analyzed using MSTAT 4.0C software following the method of Gomez and Gomez (3).

RESULTS AND DISCUSSION

Storage in heap reduced the range of variation in temperature while maintaining a high relative humidity. Storage ambience did not affect the environment inside the heaps. The temperatures ranged between 19-31°C in the heaps laid under sheds or trees compared to the ambient (17 to 44°C) during March to June. Relative humidity inside the heap remained consistently high (55-90%) compared to a wider variation and lower levels (26-75%) under ambient conditions. Temperatures and humidity in the heap were in the same range as reported in our on-station trials conducted at Jalandhar (Mehta *et al.*, 7).

Untreated (control) tubers of both the cultivars had 100% sprouting with multiple sprouts at the end of storage period. The treatment of potatoes with CIPC resulted in significant reduction of sprouting at all the four locations resulting in lower sprout weight, sprouting index and total weight loss irrespective of location (Fig. 1). Sprout inhibition effect of CIPC

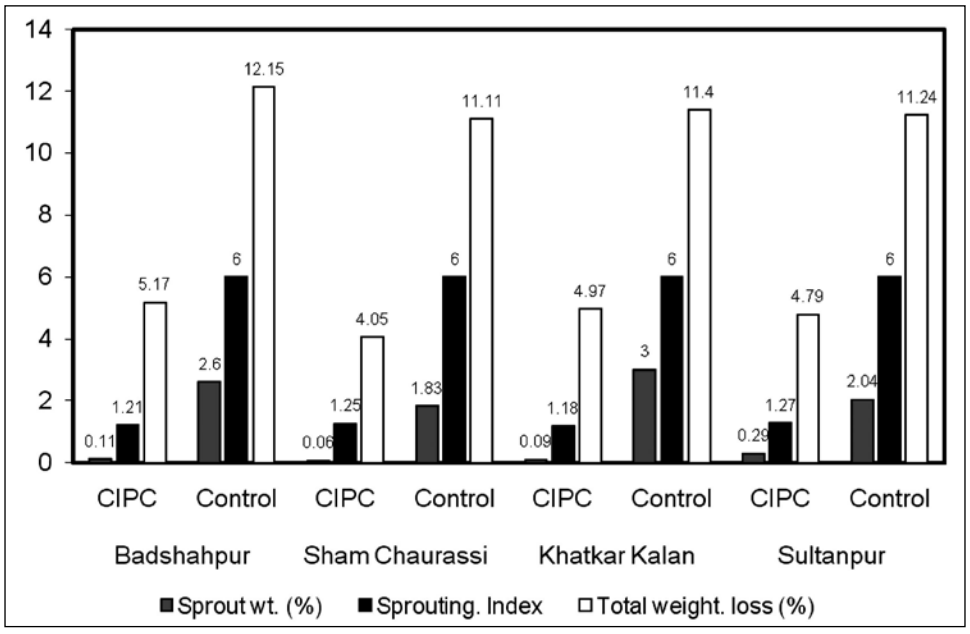

Fig. 1. Sprout weight (%), sprouting index and total weight loss in potatoes after 90 days of storage in improved heaps at four different locations in Punjab. [CD at 5%: Sprout weight: L = 0.29, T = 0.21, L × T = 0.42; Sprouting index: L = NS, T = 0.08, L × T = NS; Total weight loss: L = 0.68, T = 0.49, L × T = NS, where L = Location; T = Treatment; L × T = Location × Treatment; NS = Not significant].

was at par in the two cultivars and the two storage conditions. CIPC-treated tubers appeared firm whereas the control tubers with multiple sprouts had slightly shriveled appearance. There was no need to desprout the treated potatoes before sending to the market or the processing industry. CIPC spray is often applied to potatoes, which have already received one or two CIPC aerosol treatments, to ensure that the tubers remain sprout free during transit and fresh market utilization (Kleinkopf *et al.*, 4), but in this case even single spray application of CIPC remained effective up to 90 days of storage at higher temperatures.

CIPC treatment had no effect on pathological losses in tubers and the proportion of rotted tubers in heaps was negligible (0.11-0.42%) (data not included). There was no incidence of black heart generally reported under high temperature storage (Burton *et al.*, 1). Total weight loss in treated tubers was significantly reduced (4.74%) compared to control potatoes (11.47%) (Fig. 1). Tuber weight loss during storage is an important quality parameter for the potato industry. It results from processes like evaporation, respiration and sprouting. Evaporative loss is the major contributing factor, depending largely on the degree of suberization of the tubers, which reduces moisture loss during storage. Losses in this investigation were lower even in control potatoes as compared to traditional storage methods (15-40%) because the tuber skin was properly set by haulm cutting the crop 3 weeks prior to harvest and proper wound healing was also achieved by keeping harvested tubers in heaps covered with rice straw for 15 days prior to storage. However, evaporation is increased by sprout growth because the epidermis of sprouts is about 100 times more permeable to water than the tuber skin (Burton *et al.*, 1). Higher sprout weight was probably the main cause for higher tuber weight loss during storage in untreated tubers.

CIPC treated tubers with 4.79% weight loss, did not suffer sufficient moisture loss to affect tissue turgor and consequently the potatoes could be sold for prices comparable to tubers from refrigerated cold stores. The farmers could market 6.5% more weight of CIPC treated potatoes (Kufri Pukhraj) and fetch 21.6% higher price than the control (untreated) tubers, besides saving on desprouting labour cost (approx. Rs. 300 /tonne). CIPC treated potatoes after storage fetched Rs. 5,900 /tonne market price at par with the cold stored potatoes (Rs. 5,850 /tonne) and returned a 55.3% higher market price than the price at harvest (Rs. 3,800 /tonne). Untreated potatoes though fetched comparatively lower market price (Rs. 4,850 /tonne), but the price was 27.6% higher than that at the time of harvest. On-farm storage is less expensive compared to refrigerated storage (Rs. 350-400 /tonne v/s Rs. 1,600-1,800 /tonne) (Mehta *et al*., 8), thus, by incurring lower expenditure on storage, farmers could get higher net profit using this storage technology.

In processing cv. Kufri Chipsona-1, reducing sugar concentrations were higher (149.9-208.9 mg/100 g fresh weight) at all the three locations at 0 day of storage and chip colour was unacceptable (Table 1). Higher reducing sugar content at harvest is the result of prevailing low (<10°C) temperatures during later period of crop growth (Marwaha, 5). The concentration of reducing sugars in potato tuber is an important quality factor in potatoes, affecting the colour of processed products as frying at high temperature results in a typical Maillard's reaction between reducing sugars and free amino acids present in the tuber (Roe *et al.*, 10). Low sugar concentration is a desirable characteristic for potatoes meant for table purposes also to avoid an undesirable sweet flavour. Reducing sugar content in potatoes decreased up to 90 days of storage with more decrease recorded in CIPC treated potatoes (Table 1). High rates of starch resynthesis and respiration during higher temperature storage are responsible for this decrease (Mehta *et al*., 7). Sucrose content in potatoes increased during storage, may be due to the inhibition of invertase activity or synthesis of invertase inhibitor at higher temperature (Uppal and Verma, 11). Though sucrose is not directly involved in Maillard's reaction, there is some evidence of thermal decomposition of sucrose contributing to Maillard's browning (Mehta *et al*., 9).

Chip colour was unacceptable at 0 day of storage at all the three locations. The colour improved significantly during storage and potatoes became suitable for processing (Table 1). CIPC treated and untreated stored potatoes when tested in the industry for quality parameters, were remarked highly acceptable for processing after 45 days of storage (personal comm.). Our laboratory test also showed that CIPC treated potatoes from Shamchaurassi and Badshahpur produced acceptable colour chips after 45 DOS, while potatoes from Khatkar Kalan produced acceptable chips after 75 days (Table 1). The lighter chip colour after storage v/s before storage, attributable to the corresponding decrease in reducing sugars has been reported in our earlier study also (Mehta *et al.*, 7). Chipping quality of untreated (control) potatoes also improved to acceptable level after 45 DOS at Badshahpur and after 75 days of storage at other two locations. Stored potatoes (treated and untreated) were used in the industry for making good

Table 1. Processing quality of potatoes (cv. Kufri Chipsona-1) during storage at three locations in Punjab during March-June.

Location (L)	0 day	Storage days/ Treatment (T)					
		45 day		75 day		90 day	
		CIPC	Control	CIPC	Control	CIPC	Control
Reducing sugar (mg/100 g FW)							
Khatkar Kalan (Nawanshahar)	205.2	92.33	97.71	98.1	116.8	29.8	36.6
Sham Chaurassi (Hoshiarpur)	208.9	151.9	89.6	76.0	65.2	19.2	119.3
Badshahpur (Jalandhar)	149.9	41.8	52.6	25.4	35.6	18.5	25.5
Mean	188.1	95.3	80.0	66.5	72.5	22.5	60.5
CD at 5%		L = 9.5, T = 9.5, L × T = 16.4		L = 7.5, T = 7.5, L × T = 13.1		L = 16.7, T = 16.7, L × T = 29.0	
Sucrose (mg/100 g FW)							
Khatkar Kalan (Nawanshahar)	169.0	146.9	169.7	172.4	238.5	428.8	342.4
Sham Chaurassi (Hoshiarpur)	164.9	162.3	160.4	186.9	270.3	230.6	344.6
Badshahpur (Jalandhar)	170.9	120.5	168.7	170.6	190.5	288.9	304.9
Mean	167.9	143.2	166.3	183.3	265.5	316.1	330.6
CD at 5%		L = NS, T = 10.9, L × T = 18.9		L = NS, T = 31.6, L × T = NS		L = 37.5, T = 37.5, L × T = 64.8	
Chip colour score (On a 1-10 scale of increasing dark colour, score up to 5 was acceptable)							
Khatkar Kalan (Nawanshahar)	6.67	5.67	5.83	1.33	1.83	2.17	4.67
Sham Chaurassi (Hoshiarpur)	6.83	4.17	5.83	1.33	2.33	1.83	4.50
Badshahpur (Jalandhar)	6.33	1.30	3.50	1.17	1.33	1.67	2.33
Mean	6.61	3.72	5.06	1.28	1.83	1.89	3.83
CD at 5%		L = 0.30, T = 0.30, L × T = 0.50		L = 0.21, T = 0.21, L × T = 0.36		L = 0.30, T = 0.30, L × T = 0.50	

quality flakes and French fries up to 90 days of storage (Personal comm.).

In conclusion, the demonstrations validated the research findings. Thus, low-cost heap storage technology can profitably be used by the farmers to increase remunerations from potato cultivation and by the potato industry to maintain the desired quality in processing potatoes for 3-4 months after harvest at lower storage cost. The technology for storage of processing potatoes at elevated temperatures (10-12°C) is new to India and such stores till date are very few in many states. The growth and progress of the potato processing sector is therefore restricted due to lack of round the year availability of potatoes with low reducing sugars and acceptable chip colour. Potatoes stored on-farm in heaps by this low-cost method can meet the needs of the processing industry for short periods (March-June) and potatoes stored at 10-12°C can be used for additional period after June. Also, the potatoes for table purpose in India are generally stored along with seed potatoes in refrigerated stores maintained at 2-4°C, which results in low temperature sweetening. Short term on-farm storage can maintain good flavour of table potatoes and improve the farmers' sale price while incurring low storage cost.

ACKNOWLEDGEMENTS

Thanks are due to the Head, CPRS, Jalandhar for providing facilities, Mr. Yogesh Kumar Gupta (TO) and Mr. Kulwinder Singh (TA) for assistance and Mr. Raj Kumar (TO) for statistical analysis.

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Received : July, 2013; Revised : January, 2016;
Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00024.4

Indian J. Hort. 73(1), March 2016: 87-93

Comparative analysis of vegetable production, value-addition and marketing in National Capital Region

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ABSTRACT

Agriculture, especially vegetable sector needs streamlined supply chain in the form of well functioning marketing infrastructure to make 'farm' to 'fork' model as reality. In the present study, field survey was carried out to identify different existing marketing channels among vegetable producers and processors. Marketing efficiency and price spread of the identified marketing channels were analysed. Producer's share in consumer's price significantly differed among vegetable processors (61%) and producers (30%). Acharya's marketing efficiency analysis method showed that Channel I (Processor/ producer-Consumer) was most efficient marketing channel with maximum profit to both processors and producers. Most important motivating factor identified among processor to go for value addition of vegetable produce was price of value added products (Mean ranks of Friedman's test is 12.15). Financial constraint (mean rank of Kruskal-Wallis's test 33.35) to start and run a processing unit was the major constraint faced by the vegetable processors. Under that lack of price policy (mean ranks of Friedman's test is 7.65) was identified as the major constraint in the study area. Marketing related factor was (mean rank of Kruskal-Wallis's test 45.50) identified as major inhibitor among growers to undertake processing of vegetable by their own.

Key words: Economic analysis, motivators, constraints, vegetable production, value-addition.

INTRODUCTION

Indian agriculture and nation nutritional security has a strong linkage with vegetables, due to their progrssive yield, economic viability, nutitional prosperity and ability to generate on farm and off farm employment opportunities through production and value addition of produces. India ranked second in production of fruits and vegetables all over the world. Total area under horticultural crops is 24.19 million ha and production is 280.48 million tonnes (NHB, 6). Fruits and vegetables together contribute about 92% of the total horticultural production in the country. Presently, vegetable occupies 9.39 million hectares area with the annual production of 162.89 million tonnes (NHB, 6). Even if production and productivity of vegetables in India is in a remarkable position among other countries, post production scenario in India is not up to the mark. According to Sinha (7) losses after harvest due to poor infrastructure and unorganized retail lead India to experience some of the highest food losses in the world. Lack of cold storage and harvest spoilage causes over 35 to 40% loss in farmers' produce especially the perishable commodities like fruits and vegetables. A national level post-harvest losses study conducted by Nanda *et al*. (5) covered eight vegetables, *viz*., cabbage, cauliflower, green pea, mushroom, onion, potato, tapioca and tomato also revealed the same result. The overall total losses were observed to be 6.9% in cauliflower to 13% in tomato. Producer's share in consumer price is as low as 10 to 23% (Anon, 1) in India as against 60 to 81% in developed countries. This huge difference mainly occurs due to distress sale resulting from the lack of post-harvest managerial ability by farmers along with inefficiencies and interventions of middle men traders. Kader and Rolle (3) stated the 95% of the total research investment directed for enhancing the productivity and only 5% investment involved in postharvest loss reduction of the fruits and vegetables. Similar results were found by Kitinoja *et al.* (4). India has made desired strides on production front but appallingly wanting in the field of agricultural marketing, post-harvest management and value addition of agricultural commodities. In this context, present study was undertaken in peri-urban areas of National Capital Region (NCR) to identify different marketing channel existing among vegetable processors and vegetable growers and examine its efficiency. An attempt has also been made to identify motivating factors for taking post-harvest decision, the constraints faced by processors and the inhibiting factors among the vegetable farmers to undertake value-addition and post-harvest operations.

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MATERIALS AND METHODS

The present study was purposively conducted in Hapur and Sonipat districts, which are known as vegetable hubs of Uttar Pradesh and Haryana, respectively along with Delhi to study the difference in post-harvest management behaviour of processors and producers in peri-urban areas. To study the determinants and generalize the findings among vegetable processors and vegetable producers, five different vegetables (tomato, potato, green chilli, cauliflower and radish) and main processed products from these vegetables (pickle, chips, puree and sauce) were selected purposively. A random sample of 20 respondents comprising of 10 processors and 10 producers were selected. Data collected were analysed with the help of SPSS 20.0 software to draw valid conclusion. In order to find out the producers share in consumer price, farm gate price and consumer price were collected and analysed. For analysing the marketing efficiency of different channels identified in the study area, Shepherd's and Acharya's formulae for marketing efficiency index were used. For the identification of factors, which help to take post-harvest decision by the vegetable processors, constraints faced by them and factors inhibiting the vegetable growers from undertaking processing activities, validity and reliability tested Likert like rating scale was adopted. Data collected under these variables were analysed and interpreted on the basis of nonparametric tests, *viz*., Kruskal-Walli's one-way ANOVA and Friedman's two-way ANOVA.

RESULTS AND DISCUSSION

Huge fluctuation in vegetable price was observed at different point of time in one season, *viz*., tomato 4-9 Rs./kg, potato 2-8 Rs./kg, green chilli 10-30 Rs./kg, cauliflower 2-9 Rs./kg and radish 2-14 Rs./kg. Productivity of selected vegetables in the study area is as follows; tomato 62.5-65 t/ha, potato 50-60 t/ha, green chilli 20-22 t/ha, cauliflower 60-65 t/ha and radish 62.5-65 t/ha. On an average six rupee per kilogram was calculated as farm gate price for tomato, potato, radish and cauliflower. For chilli the average farm gate price is Rs. 20, which is almost three times the average market price of other vegetables selected for the study. However, the average productivity of chilli is one third of the average productivity of other selected vegetables. Hence, on an average the farm gate price of all the vegetables were considered as Rs. 6/ kg.

Price for the value added products from these vegetables also showed variations (50-55 Rs./kg as farm gate price and 150-200 Rs./kg as consumer price). For making one kilogram of value added products nearly 2-2.5 kilogram of raw vegetable is needed. All these parameters were considered while calculating cost and benefit of the processed vegetables and raw vegetables. Since the clients for farmer in Channel II and III in case of raw vegetables were, either wholesaler or retailer, and farmers were selling their produce to them in *Mandi* in same price, an average of five rupee per kilogram has been taken in to consideration, while calculating the gross returns to farmers in these channels.

Total cost of production of value-added products of vegetables from one hectare of land (Rs. 10,51,048.70 ± 22,508.00) was high ($t = 34.187$, $p \leq 0.05$) as compared to the total cost of production of vegetables in one hectare land (Rs. 2,62,400.90 ± 5,053.48) (from Table 1). At the same time the return side for the value added products (Rs. 6,02,551.30 ± 41,897.14) was also found significantly higher ($t = 11.770$, $p < 0.05$)as compared with vegetable cultivation (Rs. 1,05,999.10 ± 4,935.09) (from Table 1).

Study results showed that total of six (three in each group) different type of marketing channels were present in the study area in marketing of value-added vegetables and raw vegetables. But there was a remarkable difference in the marketing efficiency of the existing marketing channels due to the existance of different players, variations in marketing costs and marketing margins.

Table 1. Average production cost and average net income of vegetable processors and vegetable producers.

Cost/ Return (Rs./ha)	Vegetable	Mean	Std. Error	Levene's test for equality of variances	t-test for equality of means (equal variances)	t-test for equality of means (unequal variances)
				F (Prob. F)	t, DF (Prob. t)	t, DF (Prob. t)
Total cost	Processors	1051048.70	22508.00	9.249	34.19, 18	34.19, 9.91
	Producers	262400.90	5053.48	(p = 0.007)	(p < 0.001)	(p < 0.001)
Net returns	Processors	602551.30	41897.14	20.365	11.77, 18	11.77, 9.25
	Vegetable producers	105999.10	4935.09	(p < 0.001)	(p < 0.001)	(p < 0.001)

*DF = Degrees of freedom; F = Value of the F-statistic; t = Value of the t statistic

Identified channels among vegetable processors and vegetable growers were with almost same type of players. Channel I was with only two players, *i.e.* producer/ processor and consumer. Channel II includes market players, *viz.*, Producer/processor-wholesaler/ retailer-consumer. Whereas, channel III, the longest channel was with four different players or four different transfer of ownership of goods as producer/processor-wholesaler-retailer-consumer.

In value-added vegetable marketing and raw vegetable marketing, Channel I was efficient than Channel II and III with 100% of share in producers price by consumer. In the case of processed vegetables rate of change (decrease) of producers share in consumer's price has been found less as compared with the raw vegetables. In Channel II of value added vegetable nearly 28% of consumers price was taken up by the middle-men and not reaching to the farmers. Whereas, in Channel III, about 56% of consumers price was went to the intermediary people. In the case of raw vegetable, increase in number of middle-men in each channel resulted in more share of consumers' price to middle men. In Channel II producers share in consumers' price was about 33.33%, whereas in Channel III it was about 16.66%. From Table 2 it is clear that maximum profit/ margin was taken by retailers in all marketing channels. Acharya's marketing efficiency index also indicated that more efficient marketing channel was direct selling channel (Channel I) in both group (processor 2.74 and producer 3.47). Shepherd's marketing efficiency index indicated that when channel length increases, the margin taken up by the intermediaries will also increase.

Respondents were asked to mark their preference in a 3 point continuum with respect to the importance of selected 13 motivating factors in making post harvest decisions among them. These factors were compared using Friedman's two-way ANOVA. As the computed p-value ($p < 0.05$) is less than the significant level with test statistics $\chi^2 = 69.950$ with df = 12, it can be inferred that the level of influence of different factors to the post-harvest decision making among vegetable processors is different according to processor's perception.

The mean rank corresponding to 'price of value-added food' (11.80) has been greater than all other factors (Table 3). Processors were getting premium price and profit for the value-added products and this attracted them to take the post-harvest decisions. Since, majority of the respondents (vegetable processors) were selling their products in their own brand name through their own outlet or some specific sponsored outlets like Indian Agricultural Research Institute Farmer's Mall, they were getting more market margin. Because of these reasons, processors were

Table 2. Average price spread (Rs./ ha) in different marketing channel with respect to value-added vegetables and raw vegetables.

Particulars	Vegetable producer			Vegetable processor		
	Channel I	Channel II	Channel III	Channel I	Channel II	Channel III
1. Cost of production	2,62,400.9	2,62,400.9	2,62,400.9	10,51,048.7	10,51,048.7	10,51,048.7
2. Marketing cost of producer/ processor	13,433	13,433	13,433	16,533	16,533.0	16,533
3. Gross returns to producer/ processor	3,68,400	3,07,000	3,07,000	16,53,600	15,90,000	15,26,400
4. Net returns of producer/ processor (MM) (3-(1+2))	92,566.1	31,166.1	31,166.1	5,86,018.3	5,22,418.3	4,58,818.3
5. MC of wholesaler		15,783	15,783		18,133	1,813.30
6. MM of wholesaler (7-3+5)		5,98,217	3,06,567		6,17,867	45,467.0
7. Gross price to wholesaler		9,21,000	6,14,000		22,26,000	15,90,000
8. MC of retailer			4,559			13,026.5
9. MM of retailer (10-7+8)			9,16,441			18,94,973.5
10. Consumer price	3,68,400	9,21,000	18,42,000	16,53,600	22,26,000	34,98,000
11. Producers share in consumers price (3/10)*100	100%	33.33%	16.66%	100%	71.14%	43.63%
12. Marketing efficiency (Shepherd) [(V/TMC)-1]	26.4	30.52	53.53	99.08	63.21	72.34
13. Marketing efficiency (Acharya) [Gross return of producer/ (TMC + TMM)]	3.47	0.46	0.23	2.74	1.35	0.62

Table 3. Factors influencing post-harvest decision making among vegetable processors based on mean ranks of Friedman's test.

Factors for post-harvest decision making	Mean rank
Price of value-added food	11.80
High market margin obtained	10.50
Branding and new look of products	9.05
Increasing food demand in urban areas	8.40
Consumer satisfaction and loyalty	7.55
Labour availability	7.05
Rising disposable income in hand	6.65
Transportation facilities to market	6.10
Changing consumer needs and choice	6.05
To minimize wastage	5.70
Marketed and marketable surplus availability	4.20
Competition from the market	4.20
To avoid distress sale	3.75

ranked market margin obtained due to elimination of middle man (10.50) and branding and new look of products (9.05) as two important factors, which were motivating them to take postharvest decisions. Majority of the respondents were with small and marginal land holding. Even some of the respondents were not having farm land and they were purchasing the inputs from local market and making value-added products, like pickles, jam, jelly etc. Processors reopens were also evident to this because the factors like to minimize wastage (Mean rank 5.70) and marketed and marketable surplus availability (Mean rank 4.20) were identified as less important elements in post-harvest decision making.

Generalized category of four different constraints (technical and capacity building related, infrastructure related, financial and market related) were compared using Kruskal-Wallis's one-way ANOVA. The test ($\chi^2 = 17.283$, df = 3, $p < 0.05$) revealed a significant difference among the level of influence of different constraints.

The mean rank corresponding to 'financial constraints' (33.35) is more, hence it was the major constraint to existing post harvest management mechanism in vegetables. Least affecting constraint was market related constraints with mean rank 14.15 (Table 4). Further analysis of the each category of the constraints was conducted using the Friedman's test.

Friedman's test statistic for technical and capacity building constraint is $\chi^2 = 59.075$, df = 8, $p \leq 0.05$. The major constraint identified among the technical and capacity building constraint was 'high cost

Table 4. Major dimensions of constraints among vegetable Processors based on Kruskal-Wallis's one-way ANOVA.

Constraint	Mean rank
Technical and capacity building related	15.40
Infrastructure related	19.10
Financial	33.35
Market related	14.15

involved in purchase of suitable machineries' for the post harvest management or value addition of the vegetable with mean rank 8.45 (Table 5). This was followed by 'low cohesion in groups' (Mean rank 7.60); 'non availability of improved machineries for processing' (Mean rank 6.35). Even if many of the respondents for this study were women and they were also members in one or another groups like SHGs, they perceived that it has been very difficult to maintain the cohesion among members. Sometimes they needed to devote more time to resolve the problems in group and among the group members. It was found that this 'conflict in group' and 'time wastage to solve the problems' has been identified as a factor to reduce the profit which actually they could get. Least severe constraints identified under this category were lack of motivation (Mean rank 2.70) and inadequate technical capacity (Mean rank 2.70). Based on Friedman's test statistic for infrastructure related constraints ($\chi^2 = 41.691$, df = 8, $p < 0.05$) and the results presented in Table 5, 'non-availability of machineries in local places' (Mean rank 8.70) was the major infrastructure related constraints among the vegetable processors. Poor infrastructure for storage and lack of marketing yards/ places (Mean ranks 6.45 and 6.05, respectively) were identified as prominent constraints among them. Since the study area is in peri-urban areas and near to national capital, 'lack of proper roads and transportation' (Mean rank 2.85) was less affecting the processors in post-harvest management practices. Study results and analysis showed that financial constraints among vegetable processors also differed significantly (Friedman's test statistic is $\chi^2 = 55.207$, df = 8, $p < 0.05$). 'Lack of price policy by the government' with Mean rank 7.65 and high cost of skilled labour (Mean rank 7.40) were the two most severe financial constraints. High rate of interest for credits and lack of finance (Mean rank, 7.20 and 5.60, respectively) were also identified as prominent financial constraints. As per the Friedman's test statistic ($\chi^2 = 60.450$, df = 8, $p < 0.05$) market related constraints among the vegetable processors varied significantly. Among the listed nine marketing constraints 'lack of appropriate marketing

Table 5. Severity analysis of different constraints based on mean ranks of Friedman's test.

	Particulars	Mean rank
Technical and capacity building related	High cost involved in purchase of suitable machineries	8.45
	Low cohesion in groups	7.60
	Non availability of improved machineries for processing	6.35
	Lack of knowledge about trading options (future and forward)	6.35
	Lack of training programmes	4.05
	Lack of proper knowledge about harvesting time	3.55
	Lack of feedback/ success stories in media	3.00
	Inadequate technical capacity	2.95
	Lack of motivation	2.70
Infrastructure related	Non availability of machineries in local places	8.70
	Poor infrastructure for storage	6.45
	Lack of marketing yard/ place	6.05
	Lack of cold chain management	5.15
	Lack of proper packaging facilities	4.50
	Lack of proper grading facilities	4.15
	Lack of power and electricity	4.10
	Non availability of labour	3.05
	Lack of proper roads and transportation	2.85
Financial	Lack of price policy by the government	7.65
	High cost of skilled labour	7.40
	High rate of interest for credits	7.20
	Lack of finance	5.60
	High payback period in investment	5.55
	Lack of awareness about government support policies	3.40
	Distress sale of produce due to need of immediate liquid cash	3.30
	Lack of awareness about credit availability	2.75
	Lack of banking facilities near by	2.15
Market Related	Lack of appropriate marketing channel	8.85
	Large numbers of middlemen	6.95
	Lack of market intelligent and market facility	6.35
	Less knowledge about marketing strategies	6.25
	Inability to meet standards as prescribed	4.90
	Inability to find market for value added produce	3.30
	Difficulties of contract enforcement with wholesale processors	3.20
	Produce has low market value due to poor appearance	2.60
	Price risk and uncertainty (market value vary widely between the time of harvest and the time of local shortage)	2.60

channel' with mean rank 8.85 was identified as the most severe one. Large numbers of middlemen (Mean rank 6.95) and lack of market intelligent and market facility (Mean rank 6.35) were identified as moderately severe market related constraints among the vegetable processors.

Five different dimensions of major inhibiting factors (socio-psychological, technical, financial, marketing related and infrastructure related) were identified. Kruskal-Walli's one-way ANOVA test (χ^2 = 40.850, df = 4, $p < 0.05$) revealed that the level of influence of different inhibitors differed significantly.

The mean rank corresponding to marketing related factors (45.50) is more; therefore it was the most important inhibiting factor among the vegetable farmers. It was followed by technical factors with mean rank of 32.60. Least inhibiting factors for vegetable processing among farmers were socio-psychological factors (Mean rank 8.45). Further examination of the each group of the inhibitors was done using the Friedman's test.

Friedman's test statistic for socio-psychological inhibitors is χ^2 = 30.987, df = 6, $p < 0.05$. This indicates significantly different level of influence of different components under socio-psychological inhibitors among the vegetable growers. Lack of tolerance for ambiguity was identified as most severe inhibitor among the vegetable growers to undertake post-harvest management and value addition of vegetable (Table 7). Whereas, lack of urge for social status and lack of education (Mean ranks 2.05 and 3.20, respectively) were the two least severe inhibitors. Friedman's ANOVA statistic (χ^2 = 32.724, df = 6, $p < 0.05$) for technical inhibitors showed that there is a significant difference in influence of various components. Major identified inhibiting technical factor to undertake vegetable processing was high cost of processing activities and unavailability of processing machineries in the study area with mean rank 5.75 and 4.85, respectively. Least severe inhibiting factor was unavailability of raw materials year round (Mean rank 1.20). Lack of knowledge about processing standards, lack of knowledge about processing activities and unavailability of processing technologies were identified as moderately severe inhibiting factors. It is evident from Friedman's test statistic for financial inhibitors (χ^2 = 33.849, df = 6, $p < 0.05$) that significant difference was found among financial inhibitors. Lack of price policy by the government was perceived as most important inhibitors among financial factors with mean rank 6.30 (Table 7). It was followed by lack of awareness about government support policies and high initial investment to start value addition and processing of vegetables. Friedman's test statistics revealed a significant difference of influence of market related inhibitors among vegetable growers (χ^2 = 46.535, df = 6, $p < 0.05$). They identified lack of appropriate marketing channel (Mean rank 5.70) as the major market related inhibitor among them. This was followed by large number of middle man in marketing and distress sale of produce due to need of immediate liquid cash (Mean rank 5.40 and 5.35, respectively). Since, inability to find market for value added produce was identified as least severe inhibitor among the growers it was well evident that value-added products have a well recognized place in the competing market. It is well evident that (Table 5, Friedman's ANOVA statistic, χ^2 = 38.732, df = 6, $p < 0.05$) lack of storage facilities by own / in locality (Mean rank 6.45) and lack of cold storage for keeping the raw products (Mean rank 5.95) were bearing highest mean rank and hence those were the major infrastructure related inhibitors.

Farmers have the right to get more shares in consumer's rupee, but in India it is nearly about 16- 33% (Table 2). In order to increase this share (43 -71%, Table 2) and reducing the post harvest wastage, value addition is a valid option. Vegetable growers' main reason of inhibition to undertake post harvest management has been identified as their apprehension about the marketing of products, mainly because of the astringent incident they got from current vegetable marketing scenario (Tables 6 & 7). But the actual practicers identified market related constraints as less severe in post harvest management of vegetables (Table 4). Apprehension of grower is not an actual constraint in value addition of vegetables, especially in peri-urban areas because of its proximity to inputs, nearness to consumers and ever increasing demand of products.

ACKNOWLEDGEMENT

First author is thankful to Department of Science and Technology, Government of India for providing the financial assistance in the form of Inspire Fellowship.

Table 6. Identification of major dimensions of inhibitors among vegetable producer based on Kruskal-Walli's one-way ANOVA.

Inhibitor	Mean rank
Socio-psychological	8.45
Technical	32.60
Financial	14.50
Marketing related	45.50
Infrastructure related	26.45

Table 7. Severity analysis of different components of inhibiting factors based on mean ranks of Friedman's test.

	Particulars	Mean rank
Socio-psychological	Lack of tolerance for ambiguity	6.45
	Lack of independence in decision making	5.00
	Lack of proper direction in the needed way	4.15
	Lack of locus of control	3.80
	Negative attitude of the society	3.35
	Lack of education	3.20
	Lack of urge for social status	2.05
Technical	High cost of processing activities	5.75
	Unavailability of machineries in this place	4.85
	Lack of labour	4.65
	Lack of knowledge about processing standards	4.45
	Unavailability of processing technologies	4.35
	Lack of knowledge about processing activities	2.75
Financial	Unavailability of raw materials year round	1.20
	Lack of price policy by the government	6.30
	Lack of awareness about government support policies	4.85
	High initial investment	4.65
	High cost of raw materials	4.15
	High payback period in investment	3.65
	Lack of awareness about credit availability	2.20
	Unavailability of credits	2.20
Marketing related	Lack of appropriate marketing channel	5.70
	Large number of middle man in marketing of value added products	5.40
	Distress sale of produce due to need of immediate liquid cash	5.35
	Price risk and uncertainty	5.25
	Lack of market intelligent	2.85
	Lack of market facilities in this place	1.95
	Inability to find market for value added produce(lack of demand)	1.50
Infrastructure related	Lack of storage facilities by own/in locality	6.45
	Lack of cold storage for keeping the raw products	5.95
	Lack of space and building for processing	4.20
	High cost involved as *Mandi* charges	3.55
	Lack of electricity	3.15
	Lack of proper waste utilization / recycling facility	2.45
	Lack of good transportation facility	2.25

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Received : October, 2015; Revised : February, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00025.6

Indian J. Hort. 73(1), March 2016: 94-98

Effect of coloured shade net on production of *Dracaena fragrans*

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ABSTRACT

An experiment was conducted to study the effect of different coloured shade-nets on production and quality of *Dracaena fragrans*. Plants were grown under different coloured shade-nets, *viz.* red, green, black, and white with 50% shading intensity along with control (without shade net). Different weather parameters and plant growth parameters were measured at different crop growth stages. Coloured shade net exhibited special optical properties and also influenced the microclimate. Cut greens grown under coloured shade-nets gave better performance in terms of plant height, number of leaves, biomass, leaf area, photosynthetic rate, harvest index etc. compared to control. All the shade-nets reduced temperature, light intensity and improved relative humidity. Red and white shade-nets gave higher photosynthetically active radiation (PAR, µmol/m²/s) and transmittance than other coloured nets. Plants grown under red and white shade-nets exhibited better plant height, leaf number, leaf chlorophyll content, leaf area, fresh weight, dry weight, photosynthetic rate and transpiration. The harvest index was superior under red shade net. Red and white shade-nets were found superior in improving plant and weather parameters and hence they can be used in place of the commercially used green shade net for improved growth of dracaena.

Key words: *Dracaena fragrans*, shade-net, leaf area, transmittance, quality.

INTRODUCTION

Cut greens are an important component of the floricultural industry and are largely used for decoration as fillers in floral compositions. They provide freshness, colour and variety to arrangements and bouquets. Dracaena is one of the important cut greens and is used widely for its beautiful foliage. Nets are commonly used in agricultural crops for specific modification of sunlight, improving microenvironment, and providing physical protection. They not only decrease light quantity but also alters light quality to a varying extent and also change other environmental conditions (Smith *et al*., 11). Colour nets represent new agro-technological concept, which not only exhibit special optical properties that allow the control of light, but also have the advantage of influencing the microclimate to which the plant is exposed and offer physical protection against excessive radiation, insect pests and environmental changes (Shahak *et al*., 10).

The utilization of solar radiation by ornamental crops is based on selective filtration of light by different colour shade-nets with special optical properties that modify the quality of natural radiation. Use of these nets aim to optimize desirable physiological responses, resulting in substantial effect on shoot elongation, branching and flowering in ornamentals crops (Oren-Shamir *et al*., 9). The colour shade-nets approach is evaluated in ornamentals (Nissim-Levi, 8), vegetables (Fallik *et al*., 4) and fruit trees (Shahak *et al*., 10). Nettings, regardless of colour, reduce radiation reaching crops underneath which is directly proportional to the shade factor and modify micro-environment. Keeping these facts in view the present study was undertaken to observe the production and quality of dracaena under different colour shade-nets.

MATERIALS AND METHODS

A field experiment was conducted at the Research Farm of the Division of Floriculture and Landscaping, IARI, New Delhi during 2013-14 with *Dracaena fragrans*. The planting was done during September 2013 under four coloured shade-nets (white, red, black and green) with shading intensity of 50%. The micro-environment and production under these shade levels were compared with the outdoor environment (without shade net).

Weather parameters such as temperature and relative humidity were measured by pocket weather tracker. Light measurement was carried out periodically during different growth stages, to monitor the actual light conditions to which the plants were exposed. All the measurements were taken on clear days at mid-day. The light intensity was measured by digital light meter (Extech Instruments, 401025). Transmitted photosynthetically active radiation (PAR) as well as

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intercepted radiation by plant in each treatment was measured by the Line Quantum Sensor (LiCOR-3000), whereas transmittance inside the net was calculated as the ratio of the PAR radiation spectra inside net and outdoor. Canopy temperature was measured using infrared thermometer. Plant height (cm) was taken up-to the tip of 3rd leaf using standard scale and number of matured leaves was also counted during different growth stages. Leaf readings to determine chlorophyll content were taken with a chlorophyll meter (model SPAD-502) by averaging the 10-15 readings per plant. The photosynthesis/ CO_2 uptake rate (µmol $CO_2/m^2/s$), transpiration (µmol $H_2O/m^2/s$), stomatal conductance (µmol/m^2/s) and PS2 efficiency were taken by LiCor-6400 Leaf Gas Exchange instrument, *i.e*., Infra-red gas analyzer (IRGA) (Long *et al*., 7). Fresh leaf weight was taken, and then leaf area was measured using leaf area meter (Licor-3100). The specific leaf area (SLA) was computed using the fresh weight and leaf area. Harvest index (HI) was calculated as percentage of economic yield to the biological yield whereas vase-life was estimated by placing the stem along with leaves in test tube containing distilled water. The trial was laid out in randomized block design (RBD) and the data were analyzed accordingly.

RESULTS AND DISCUSSION

In the present investigation all the shade-nets reduced temperature, light intensity and improved humidity. Black shade net exhibited the lowest temperature, light intensity and highest RH levels. The light intensity was 44.77, 16.53, 34.15 and 40.55% of control for green, black, red and white nets, respectively (Fig. 1). Radiation is the major mode of energy exchange between plant and environment. Solar radiation provides the main energy input to plants, with much of this energy being converted to heat and driving other radiation exchanges and processes such as transpiration and photosynthesis, as well as being involved in determining tissue temperatures with consequences for rates of metabolic processes and the balance between them (Jones, 5). The main climatic parameter affected by shade net is solar radiation, which depends upon type of shade net and density. The coloured shade cloth is designed to modify light in either the ultra-violet, visible, or far-red spectral regions; the cloth also enhances the relative content of scattered vs. direct light and absorbs infra-red radiation (Shahak *et al*., 10).

The temperature under different coloured shade-nets varied and was found to be higher under control as compared to shade net. Temperature reduction was the highest in black shade net followed by green, white and red, while during winter months, temperature inside the nets was higher compared to control (Fig. 2). Relative humidity was higher under coloured nets even though temperature was low. Relative humidity was highest under black shade net, which was followed by green, red and white as compared to control (Fig. 3). Reduction in radiations resulting from netting affects temperature and RH (Stamps, 13). Reduced air temperature was in agreement with the result of Campanha *et al*. (1). The present study was in agreement with earlier studies that the temperatures reduced by 2-3°C under black shade net and this in turn affects plant processes (Smith *et al*., 11).

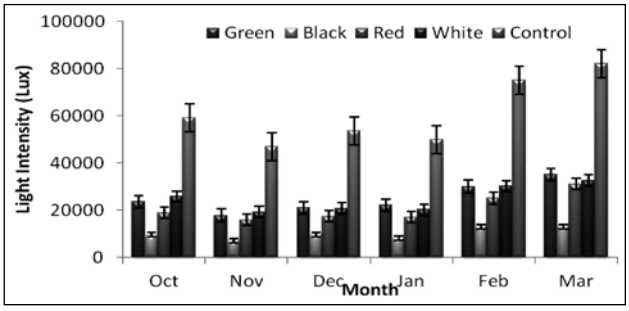

Fig. 1. Light intensity under different coloured nets during different months after planting.

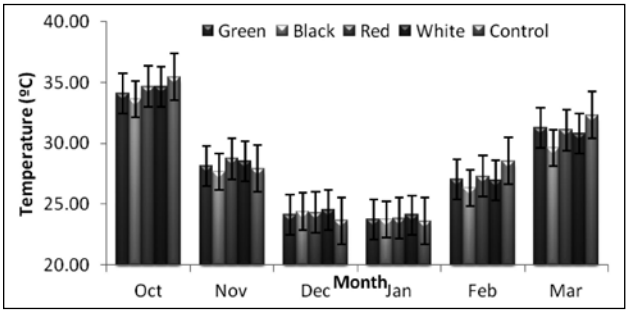

Fig. 2. Air temperature under different coloured nets during different months after planting.

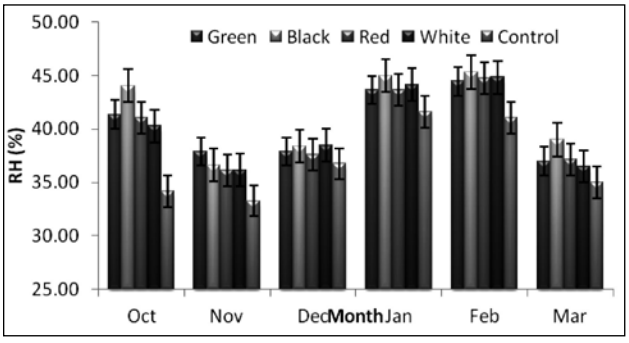

Fig. 3. Relative humidity under different coloured nets during different months after planting.

PAR and transmittance levels were significantly lower under different colour shade nets. White (519.80-648.20 µmol/m²/s) and Red (459.20-577.00 µmol/m²/s) shade-nets exhibited higher PAR and transmittance than other colour nets, while, it was minimum under black net (Fig. 4 & 5). The proportion of diffused to direct PAR was significantly higher under green shade net compared to black (Oren-Shamir *et al*., 9).

Canopy temperature under coloured shade-nets was lower as compared to control. Black shade-nets had lowest light, temperature, PAR and transmittance; as a result the plants grown under this had the lowest canopy temperature, fresh and dry weight. Black net (5.40-6.93°C) showed maximum reduction followed by green, white and red. Smith *et al*. (11) also observed that under shade-nets, canopy temperature were lower than those of control. In this study, all the nets improved leaf chlorophyll content. The SPAD reading was found to be significantly higher under coloured net when compared to control. It was found to be higher by 31.22% under white net and by 29.65% under red net compared to control (Table 2).

Chloroplasts were more numerous and larger in plants grown under shading, whilst the accumulation of chloroplastic starch grains was greater in plants grown under red shading or in full sunlight (Costa *et al.*, 2). Because of the above factor photosynthetic rates were higher in white shade net. The photosynthetic activity of the leaves was significantly higher by 41.92% and stomatal conductance under white net over control. The efficiency of photosystem 2 is directly related with CO_2 absorbance. It was maximum under white net as well as control. Transpiration rate was observed highest under white-net followed by red, control, black and green-nets. Overall all the gaseous exchange was found to be higher under white shade net.

Plants grown under different coloured nets had varying growth due to their spectral effect that influences the plant growth. Plants were found to be significantly taller by 24.80% over control with higher number of leaves under red net (Fig. 6 & 7). The present study was in agreement to Kawabata *et al.*

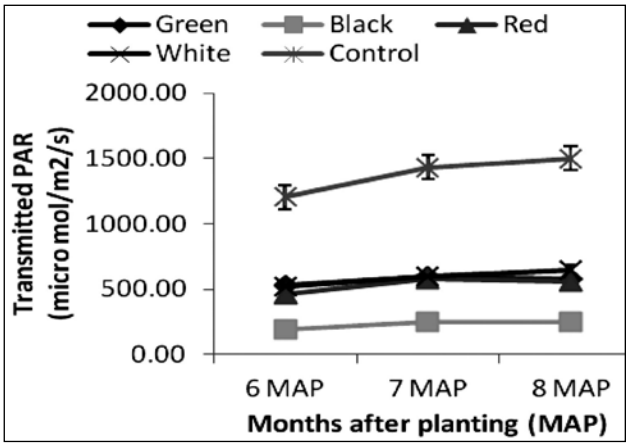

Fig. 4. Transmitted PAR under different coloured nets during different months after planting.

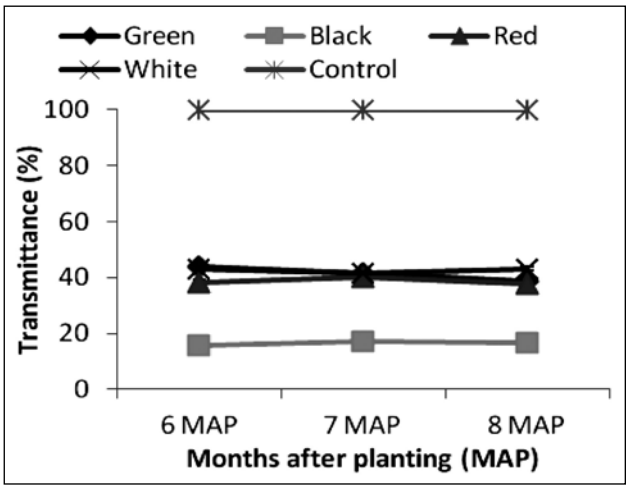

Fig. 5. Transmittance under different coloured nets during different months after planting.

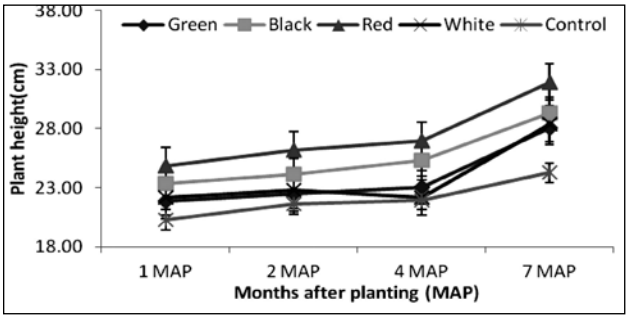

Fig. 6. Influence of coloured shade-nets on plant height at different months after planting.

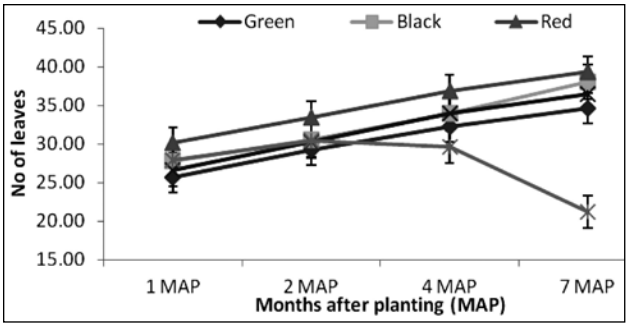

Fig. 7. Influence of coloured shade-nets on number of leaves at different months after planting.

Table 1. The influence of different coloured shade-nets on plant height, number of leaves, chlorophyll content (SPAD), canopy temperature, leaf area, leaf weight, Specific Leaf Area (SLA), gas exchange characteristics and vase-life of dracaena.

Treatment	Plant height (cm)	Leaf No.	SPAD reading	Canopy temp. (°C)	Leaf area (cm^2)	Fresh leaf wt. (g)	SLA (cm^2/g)
Green	23.86 ± 1.21	30.46 ± 1.88	34.87 ± 0.52	26.62 ± 0.10	508.60 ± 25.33	22.47 ± 2.14	22.73 ± 0.82
Black	25.54 ± 0.56	32.60 ± 4.01	37.77 ± 0.39	25.36 ± 0.08	455.45 ± 17.92	20.63 ± 1.62	22.40 ± 1.43
Red	27.51 ± 0.99	34.98 ± 4.68	39.56 ± 0.37	27.53 ± 0.06	543.18 ± 27.20	23.63 ± 2.93	22.70 ± 1.41
White	23.90 ± 1.55	31.86 ± 3.24	40.04 ± 0.94	27.18 ± 0.06	457.23 ± 39.49	20.98 ± 2.89	21.16 ± 0.92
Control	22.04 ± 0.74	27.29 ± 2.23	30.51 ± 0.09	30.81 ± 0.15	317.13 ± 33.15	15.46 ± 1.39	21.25 ± 1.01
$CD_{(0.05)}$	3.216	4.99	1.51	0.27	97.11	NS	1.33

contd...

Treatment	Dry leaf wt. (g)	Gas exchange characteristics				Vase-life (days)
		Photosynthesis rate (µmol $CO_2/m^2/s$)	Stomatal conductance (µmol $H_2O/m^2/s$)	Efficiency of photo system 2	Transpiration rate (µmol $H_2O/m^2/s$)	
Green	4.55 ± 0.50	2.36 ± 0.19	0.005 ± 0.000	0.169 ± 0.010	0.30 ± 0.03	11.50 ± 1.50
Black	3.32 ± 0.18	2.99 ± 0.76	0.005 ± 0.000	0.064 ± 0.015	0.32 ± 0.00	16.00 ± 0.00
Red	4.71 ± 0.68	3.99 ± 0.58	0.010 ± 0.002	0.193 ± 0.005	0.54 ± 0.09	11.50 ± 1.50
White	4.39 ± 0.92	7.59 ± 0.33	0.021 ± 0.001	0.198 ± 0.002	1.08 ± 0.03	19.00 ± 1.00
Control	3.16 ± 0.50	5.35 ± 0.22	0.008 ± 0.001	0.199 ± 0.007	0.38 ± 0.04	10.00 ± 0.00
$CD_{(0.05)}$	1.18	1.09	0.003	0.031	0.16	3.38

Note: Data (mean ± SE) at P<0.05

(6), who had also reported that red shade produced higher number of leaves in dracaena.

Leaf area is an useful parameter of growth as it interprets the capacity of a crop for producing dry matter in term of the utilization of intercepted radiation and amount of photosynthesis synthesized. Leaf area was calculated and results show that leaf area was lowest in control, followed by black, white, green and it was highest under red. The percentage increase in leaf area was nearly 71.28% under red as compared to control (Table 1).

The differences in fresh weight of leaves were insignificant, while the specific leaf area (SLA) value was significant when compared to control but not between green, black and red nets. It was highest under green followed by red and black shade net indicating thinner leaves in shade net compare to outdoor environment. Stomatal density and leaf thickness increased in plants maintained in full sunlight owing to the expansion of the abaxial epidermis and the spongy parenchyma. Dry leaf weight for dracaena was found to be significantly higher under red net and lowest under control. In accordance with study carried by Crowley (3) in the present investigation fresh and dry weight of leaves were highest under red shade net. Plants grown under red and white shade nets exhibited highest HI. Red and white shade-nets gave higher PAR and transmittance than other coloured nets. As a result plants grown under red and white shade nets exhibited better plant height, leaf number, leaf chlorophyll content, leaf area, fresh weight and dry weight, photosynthetic rate and transpiration and thus HI.

Table 2. The influence of different coloured shade-nets on the harvest index.

Treatment	Harvest index (%)
Green	66.06
Black	66.76
Red	70.52
White	68.58
Control	60.00

Vase-life determines the commercial value of cut greens and higher value is always preferred in trade. Vase-life was found higher under white shade net. It was higher by 90% when compared to control (Fig. 8). In the present study, vase-life was found to be superior under white and black coloured nets, which was in contradiction to earlier reports (Stamps and

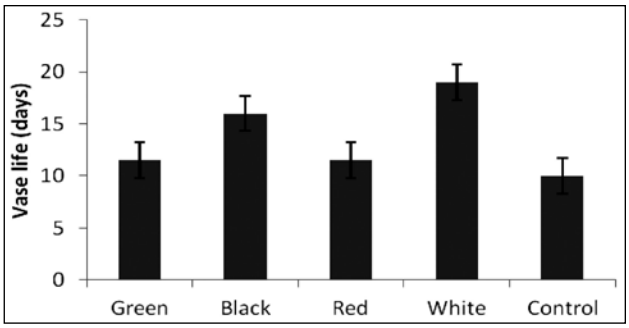

Fig. 8. Influence of coloured shade-nets on vase-life of dracaena cut foliage.

Chandler, 12). This may be due to better protection of the leaves by these nets from high light intensity and thereby improved quality of cut foliage.

Red and white coloured shade-nets were found to be superior in improving most of the plant parameters compared to green net, black net and control. Red shade net has been found to be effective for improving plant height, number of leaves, leaf area and Harvest Index (Table 2), while other important characteristics were superior under white. Hence, it can be recommended to use red and white nets for commercial production of dracaena in place of commonly used green net.

ACKNOWLEDGMENT

First author duly acknowledges ICAR, New Delhi for providing Junior Research Fellowship.

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Received : February, 2015; Revised : October, 2015; Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00026.8

Indian J. Hort. 73(1), March 2016: 99-103

Standardization of dehydration technique for greenhouse cut rose var. Shakira

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ABSTRACT

An investigation was conducted to evaluate optimum dehydration technique for greenhouse cut rose var. Shakira at different harvest stages, *viz.*, bud stage, 50% open and fully opened flowers. Flowers dried at 50% open stage showed higher retention in dry weight and flower diameter though flowers dried at fully opened stage took minimum time to drying process. Among various drying techniques, microwave oven with silica gel as embedding medium took minimum time in drying and showed higher moisture loss, lower moisture content and higher dry weight, flower diameter and petal pigment retention. Further, among all treatment combinations, flowers dried at 50% open stage in microwave oven embedded with silica gel as embedding media showed best quality in terms of higher retention in dry weight, flower diameter, petal pigments (anthocyanin) along with well-maintained shape, smooth petal texture and maximum dry flower longevity.

Key words: Rose, harvest stage, anthocyanin, dehydration techniques, microwave oven, silica gel.

INTRODUCTION

Dehydrated flowers and foliage are excellent owing to their special beauty, long lasting value and their enjoyment during various temperature extremes. Dried and preserved ornamental products offer a wide range of qualities like novelty, longevity, aesthetic properties, flexibility and year round availability (Joyce, 5). Dry flowers constitute more than two-thirds of the total floriculture exports and the demand for dry flowers is increasing at an impressive rate of 8-10 per cent annually, thus offering a lot of opportunities for the Indian entrepreneurs to enter the global floricultural trade. Rose is the top ranking cut flower in the world flower trade. Cut roses grown under protected cultivation are superior in quality. However, even quality cut roses suffer from price fluctuation during glut periods in flower market. The technology of flower dehydration offers good scope of marketing through value addition. Harvest stage and technique of flower drying have been known to influence dry flower quality (Sangama, 9; Singh *et al*., 12; Safeena *et al*., 8). Hence, an investigation was undertaken to standardize appropriate harvest stage and to evaluate optimum dehydration technique for greenhouse cut rose var. Shakira.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, NAU, Navsari. Six different dehydration techniques, *viz*., hang drying, room drying with silica gel (60-120 mesh) and sand (0.2 to 0.02 mm) as embedding medium, oven drying with silica gel and sand as embedding medium, microwave oven drying with silica gel as embedding medium and three harvest stages, *viz*., bud stage, 50% open and fully open flower were used to standardize the optimum dehydration technique and harvest stage of greenhouse cut rose var. Shakira. The experiment was laid out in completely randomized design with factorial concept and three repetitions. Observations were recorded on qualitative and quantitative parameters such as fresh weight, time taken for drying, moisture loss, moisture content, dry weight, weight loss, longevity of dried flower, change in flower diameter, pigment content (anthocyanins) after drying. Qualitative parameters (colour, shape and texture of flowers) were graded on visual basis.

RESULTS AND DISCUSSION

Harvest stage and dehydration techniques significantly influenced moisture loss and weight loss from the flowers as well as moisture content in dry flowers and time taken in drying process. Among harvest stages, flowers dried at fully open stage showed higher per cent moisture loss and per cent weight loss which was followed by 50% open flower, while it was minimum in bud stage (Table 1). Moisture content was also minimum in flowers dried at advanced stage as compared to early stage

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Table 1. Effect of drying techniques on moisture loss, weight loss and moisture content in greenhouse cut rose var. Shakira.

Drying technique	Moisture loss (%)				Weight loss (%)				Moisture content (%)			
	Bud stage (S_1)	50 % open (S_2)	Fully open (S_3)	Mean D	Bud stage (S_1)	50 % open (S_2)	Fully open (S_3)	Mean D	Bud stage (S_1)	50 % open (S_2)	Fully open (S_3)	Mean D
Hang drying (D_1)	57.75	66.22	74.25	66.07	63.00	66.98	69.13	66.37	29.37	24.35	19.88	24.53
Room + silica gel drying (D_2)	60.55	71.72	77.03	69.77	67.47	76.87	81.35	75.23	28.20	21.13	16.98	22.11
Room + sand drying (D_3)	59.30	68.38	76.42	68.03	65.92	74.12	79.78	73.27	29.45	23.87	18.20	23.84
Oven + silica gel drying (D_4)	65.93	74.25	80.88	73.69	69.83	78.17	83.33	77.11	24.15	19.17	14.27	19.19
Oven + sand drying (D_5)	62.62	68.48	79.70	70.27	68.37	76.05	82.70	75.71	26.57	23.52	15.27	21.78
Microwave oven + silica gel drying (D_6)	69.65	76.08	82.87	76.20	74.75	80.58	85.30	80.21	21.27	17.75	12.44	17.15
Mean S	62.63	70.86	78.52		68.22	75.46	80.27		26.50	21.63	16.17	
CD at 5%	S = 1.07, D = 1.51, S × D = 2.91				S = 2.40, D = 3.39, S × D = 5.11				S = 0.71, D = 1.00, S × D = 1.73			

(bud) as shown in Table 2. Petal arrangement at advanced flower stage (50% and fully open) being less compact created more availability of space for silica gel embedding media, which induced early drying process, higher moisture loss and weight loss and low moisture content and dry weight. Lower moisture content due to higher moisture loss in dried flowers has been known in chrysanthemum (Dahiya *et al*., 4) and in zinnia (Singh *et al*., 11).

Among different dehydration methods, higher per cent moisture loss and weight loss was observed in microwave oven drying with silica gel, which was statistically at par with oven drying with silica gel, while minimum moisture loss and weight loss was observed in hang drying. Further, moisture content was also minimum in microwave oven dried flowers. Flowers dried in microwave oven took minimum time in drying process, while hang drying process took maximum time. Drying process in microwave oven involves electronically produced microwaves which liberates moisture by agitating water molecules in organic substances and maintaining particular high temperature (with 900 watt creating frictional heat) that induces higher moisture loss and faster drying process as compared to other drying techniques (hang drying, room drying and oven drying). Further silica gel as embedding medium being highly hygroscopic in nature also contributes in faster drying process.

Table 2. Effect of drying techniques on time taken for drying, dry weight and pigment content in greenhouse cut rose var. Shakira.

Drying technique	Time taken for drying (h)				Dry wt. (g)				Anthocyanins (mg/g)			
	Bud stage (S_1)	50 % open (S_2)	Fully open (S_3)	Mean D	Bud stage (S_1)	50% open (S_2)	Fully open (S_3)	Mean D	Bud stage (S_1)	50% open (S_2)	Fully open (S_3)	Mean D
Hang drying (D_1)	383.67	359.67	335.67	359.67	0.69	1.44	0.91	1.01	0.41	0.52	0.54	0.49
Room + silica gel drying (D_2)	239.67	215.67	191.67	215.67	0.72	1.64	1.03	1.13	0.48	0.71	0.70	0.63
Room + sand drying (D_3)	263.67	239.67	215.67	239.67	0.76	1.63	1.02	1.14	0.43	0.59	0.57	0.53
Oven + silica gel drying (D_4)	35.67	27.67	27.67	30.33	0.73	1.64	1.02	1.13	0.44	0.64	0.60	0.56
Oven + sand drying (D_5)	47.67	35.67	35.67	39.67	0.73	1.61	1.03	1.12	0.42	0.58	0.55	0.52
Microwave oven + silica gel drying (D_6)	0.05	0.04	0.03	0.04	0.77	1.65	1.04	1.15	0.51	0.75	0.73	0.67
Mean S	161.73	146.40	134.39		0.73	1.60	1.01		0.45	0.63	0.62	
CD at 5%	S = 0.94, D = 1.33, S × D = 2.31				S = 0.04, D = 0.05, S × D = NS				S = 0.01, D = 0.02, S × D = 0.03			

Hang drying and room drying with sand at bud stage took maximum time for drying, which may be due to slower rate of moisture loss at room temperature. Microwave oven with silica gel has earlier been reported as the quick method for drying flowers (Paparozzi and McCallister, 6), while flower drying process under shade requires longer time (Singh *et al*., 12; Sangama, 9).

Dry weight and petal pigment retention were maximum in flowers dried at 50% open stage with microwave oven drying method (Table 2). Change in flower diameter was also minimum in flowers dried at 50% open stage and with microwave oven drying method (Table 3). Minimum dry weight and pigment retention with higher reduction in flower size after drying was observed in bud stage and hang drying method. Higher dry weight at 50% open harvest stage may be due to carbohydrate accumulation, which is more at 50% open stage in rose (Safeena and Patil, 7) as compared to fully open and early stage. Further, Sangama (9) also observed low dry weight retention in dried flowers of chrysanthemum harvested at early stage. Since microwave oven drying process is speedy and is known to retain higher nutritional value (Shams *et al*., 10), the degradation of other cellular components may be less that retained higher dry weight as compared to other drying techniques. Protective influence of sugar on anthocyanin pigmentation through tonoplast stabilization has been known (Van Doorn, 13). Carbohydrates being major constituent of dry weight in flowers, may have contributed in higher pigment retention. Change in flower diameter is a result of moisture loss from cells causing cell shrinkage and wrinkling of petal (with rough petal texture) after drying. The minimum change in flower diameter in microwave oven with silica gel at 50% open flower stage may be due to fast liberation of moisture and embedding medium silica gel absorbing moisture uniformly without affecting the flower form, and restricting the flower to get shriveled as also observed in zinnia (Singh *et al*., 12) and in chrysanthemum (Aravinda and Jayanthi, 1). Hang dried flowers showed higher decrease in flower size due to absence of support provided by embedding medium and slow and uneven drying process that caused more cell shrinkage and petal wrinkling.

Maximum dried flower longevity (days) was observed in rose flowers dried at 50% open stage with microwave oven drying method, which was followed by oven drying method and minimum dried flower longevity was found in flowers dried at bud stage with room drying having sand and hang drying method (Table 3). Dry flower longevity has been found to be dependent on moisture content (Bhutani, 2). Deterioration in dry flower occurs due to physiological and microbial activities that are dependent upon availability of moisture in the cell. Thus, in order to induce higher longevity, moisture has to be reduced to optimum level where such activities could be minimized or brought to a standstill. Thus, moisture content in flowers dried at advance stage (50% and fully open) with microwave oven drying method being low resulted into enhanced dry flower longevity.

Dry flower quality in terms of colour, shape and petal texture recorded maximum grade score on visual basis in flowers dried at advance stages (50% and fully open) with microwave oven drying method as observed on 60th day after drying (DAD). Flowers dried at bud stage with hang drying method were subjected to higher loss in colour, shape with rough petal texture. Colour retention in microwave oven method was a result of retained petal pigments and speedy drying process. Further, browning in

Table 3. Effect of drying techniques on change in flower diameter and longevity of dried flowers in greenhouse cut rose var. Shakira.

Drying technique	Change in flower dia. (cm)				Longevity of dried flowers (days)			
	Bud stage (S_1)	50% open (S_2)	Fully open (S_3)	Mean D	Bud stage (S_1)	50% open (S2)	Fully open (S_3)	Mean D
Hang drying (D_1)	0.80	0.93	0.95	0.89	52.33	54.67	53.33	53.44
Room + silica gel drying (D_2)	0.48	0.38	0.53	0.46	55.00	91.00	88.67	78.22
Room + sand drying (D_3)	0.51	0.40	0.55	0.49	48.00	83.00	93.67	74.89
Oven + silica gel drying (D_4)	0.54	0.32	0.47	0.44	63.00	98.67	91.67	84.44
Oven + sand drying (D_5)	0.54	0.34	0.50	0.46	58.00	94.67	96.00	82.89
Microwave oven + silica gel drying (D_6)	0.37	0.26	0.42	0.35	77.67	105.67	95.33	92.89
Mean S	0.54	0.44	0.57		59.00	87.94	86.44	
CD at 5%	S = 0.02, D = 0.02, S × D = 0.04				S = 2.14, D = 3.02, S × D = 5.23			

Table 4. Effect of drying techniques on colour, shape, intactness and texture of cut rose var. Shakira under greenhouse.

Treatment			Colour	Shape	Texture
Bud stage	Hang drying	(S_1D_1)	1	2	1
	Room + silica gel drying	(S_1D_2)	1	2	2
	Room + sand drying	(S_1D_3)	1	2	2
	Oven + silica gel drying	(S_1D_4)	1	3	2
	Oven + sand drying	(S_1D_5)	1	2	2
	Microwave oven + silica gel drying	(S_1D_6)	3	4	3
50% open	Hang drying	(S_2D_1)	1	2	2
	Room + silica gel drying	(S_2D_2)	4	4	4
	Room + sand drying	(S_2D_3)	3	3	4
	Oven + silica gel drying	(S_2D_4)	3	4	4
	Oven + sand drying	(S_2D_5)	3	4	4
	Microwave oven + silica gel drying	(S_2D_6)	4	4	4
Fully open	Hang drying	(S_3D_1)	1	2	1
	Room + silica gel drying	(S_3D_2)	4	2	4
	Room + sand drying	(S_3D_3)	3	2	4
	Oven + silica gel drying	(S_3D_4)	3	4	3
	Oven + sand drying	(S_3D_5)	3	3	3
	Microwave oven + silica gel drying	(S_3D_6)	4	4	4

(Grades for colour, shape and texture: 4 = Highly acceptable; 3 = Acceptable; 2 = Less acceptable; 1 = Not acceptable)

hang dried flowers and discoloration in oven dried flowers was observed with increase in storage period due to oxidation reaction associated with loss of compartments within the cell during the desiccation of the tissue as also explained by Safeena and Patil (7) in rose and Singh *et al*. (12) in zinnia flower. Uniform drying of the entire flower with microwave oven method with silica gel embedding at advanced harvest stage (50% open and fully open) along with low moisture content in flowers after drying resulted in well maintained flower shape and petal texure (Table 4). In support to this, Chen *et al*. (3) reported stiffer and stronger petal in flowers having low moisture content. Similar results have been recorded by Paparozzi and McCallister (6) in statice and by Arvinda and Jayanthi (1) in chrysanthemum. Thus, cut rose sticks harvested at 50% open stage and dried using microwave oven with silica gel as embedding medium retain good dry flower quality in terms of flower colour, shape, pigment retention with higher longevity.

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Received : April, 2013; Revised : October, 2015;
Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00027.X

Indian J. Hort. 73(1), March 2016: 104-108

Organic nutrition of *Chlorophytum borivilianum* for higher yield, quality and soil properties

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ABSTRACT

Present investigation was carried out with five doses of farmyard manure (FYM) as 0, 5, 10, 15 and 20 t ha^{-1} to assess the effect on yield, quality, nutrient content and uptake along with soil properties. The highest yield of *C. borivilianum* roots was obtained with 15t FYM ha^{-1} and it was at par with 10 and 20 t FYM. The per cent yield increased with each level of FYM was 30.0, 61.5, 69.6 and 61.7 over control. The N, K, protein and sapogenine content in *musli* enhanced by application of FYM. The Fe, Zn and Mn contents increased up to 15 t FYM and Cu up to 10 t FYM and reduced thereafter. Uptake of macro-and micro-nutrients were also more with FYM. Organic carbon content, per cent porosity and water holding capacity of the soil increased and bulk density of the soil decreased with increasing the FYM levels. The ratio of N: P uptake remained constant with application of FYM. However, uptake ratio of P: K increased with FYM doses. The uptake ratio indicates that N requirement of the crop is approximately five times and K requirement is 1.2-1.8 times more to P. Uptake ration of micro-nutrients reflects that Fe, Zn and Mn requirement of the crop was approximately 110, 6 and 4 times more than Cu. Hence, crop-specific formulations of could be prepared precisely based on nutrient content and uptake rather indiscriminate use of manures and fertilizers.

Key words: *Chlorophytum borivilianum,* nutrient content, farmyard manure, soil properties, macro-nutrients, micro-nutrients.

INTRODUCTION

Chlorophytum borivilianum (Santpau and Fernandes) is commonly known as *safed musli* in India. It is an important medicinal plants used in Indian System of Medicine (ISM). The fleshy roots containing saponins are used in the preparation of Ayurvedic tonics and nutraceuticals. The drug is used as a valuable nervine and general tonic for improving strength and vigour. During the survey of *safed musli* in the natural habitat, it was observed that it produces healthy growth over humus and where leaf litter has accumulated in between the stony gaps in the substratum. Application of vermi-compost and bio-fertilizers improved the yield and quality of *musli* (Gaikwad *et al.*, 5). However, 5t poultry manure gave the best results followed by 10 and 20 t FYM (Patel *et al.*, 11). Though the crop is brought under commercial cultivation, yet the manures and nutritional requirements are not known along with soil test value. Being a high value crop farmers are applying huge amount of organic manures and chemical fertilizers without the knowledge of nutritional requirement of this crop. Therefore, it is essential to know the nutrient removal by the crop so as to avoid the excessive use of manures and fertilizers, which leads to environment implications as underground water pollution, eutrofications *etc.* Hence, the study has been undertaken.

MATERIALS AND METHODS

The field experiments were carried out under the Fluventic Ustochrepts soil during *kharif* season in two consecutive years at NRC on MAP, Boriavi, Anand situated at 22.5°N latitude and 73°E longitude. The treatments consisted of five doses of farmyard manure (FYM) 0, 5, 10, 15 and 20 t ha^{-1}, were arranged in a randomized block design with four replications. Fasciculated roots @ 2.5 q ha^{-1} were planted at 45 cm × 20 cm on ridges in second week of June by making holes with narrow wooden wedge. As per the treatment, well rotten FYM was incorporated in the experimental plots. Cultural practices were uniformly followed during the growing seasons in both the years. A light irrigation was given prior to dig-out the roots. The crops were harvested after 240 days of planting and yield of dried root was recorded.

Soil samples were collected before planting and after the harvest of both year crops from the surface (0-15 cm depth). Samples were air-dried and powdered with wooden mortar and pestle and passed through a 2 mm stainless steal sieve. Experimental soil was analyzed for texture (international pipette method), EC and pH (Richard, 13), organic carbon content by rapid chromic titration (Walkley and

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Black, 15), available N by alkaline permanganate (Subbiah and Asija, 14), available P by 0.5 M $NaHCO_3$ extractable P (Olsen *et al.*, 10), available K by 1N NH_4OAc extract method (Jackson, 7), micro-nutrients by DTPA method (Lindsay and Norvell, 8), CEC by neutral normal ammonium acetate (Piper, 12) and water holding capacity (w/w basis). The pre cent porosity and bulk density were also assessed. Initial characteristics of the experimental soil are given in Table 1. The net available N, P and K had been calculated by subtracting the initial level of these nutrients from the available nutrients after the crops.

The plant samples were collected after the harvest of crops from all the treatments and their replications. Root samples were successively washed with tap water, 0.1 M HCl and distilled water and dried at 70°C. After proper drying, samples were powdered in wiley mill and passed through the 20 mesh steel sieve. Nitrogen was estimated by Kjeldahl method (Piper, 12). The samples were digested in nitric and perchloric acids (10:4) for the estimation of P (Chapman and Pratt, 4) and K (flame photometer). Total protein was estimated by nitrogen fraction basis and sapogenine by Mishra (9). From the digested plant material micro-nutrients content were estimated by atomic absorption spectrophotometer and uptake was calculated as multiplication of content with yield. Leaves of this crop are very succulent and got decomposed immediately. After the senescence of crop and roots are only part remaining to calculate nutrient content and uptake.

Farmyard manure and the underground water used for irrigation were also analyzed for its chemical composition to work out the actual conditions of experimental trials. The elemental compositions of farmyard manure and irrigation water are given in Table 2. The irrigation water was dominated with residual sodium carbonate.

The data of two years followed the similar trend for almost all the parameters; hence both years data were pooled and analyzed by ANOVA and treatment differences are expressed for least significant differences (LSD) at 5% probability to determine the significance among the treatment means (Gomez and Gomez, 6).

Table 1. Initial properties of the experimental soil.

Parameter	Value	Parameter	Value
Soil type	Fluventic Ustochrepts	Zinc (mg kg^{-1})	2.50
Texture	Sandy loam	Manganese (mg kg^{-1})	10.80
pH	8.05	Copper (mg kg^{-1})	1.01
EC (dSm^{-1})	0.17	Bulk density (Mg m^3)	1.27
Organic carbon (%)	0.18	Porosity (per cent)	50.40
Available N (kg ha^{-1})	108.10	Water holding capacity (%)	43.10
Available P (kg ha^{-1})	14.30	CEC [C mol (P^+) kg^{-1}]	11.90
Available K kg ha^{-1})	144.40	Exchangeable sodium (%)	5.20
Iron (mg kg^{-1})	5.30	Calcium carbonate (%)	6.70

W = weight, v = volume, N = normal (normality), CEC = Cation Exchange Capacity

Table 2. Quality of underground water, elemental composition of farmyard manure used for irrigation and organic input, respectively.

Parameter	Underground water quality			Composition of FYM	
	Value	Parameter	Value	Parameter	Value
EC (dSm^{-1})	0.86	HCO_3 (me l^{-1})	9.80	Nitrogen	9.50
pH	8.00	Cl (mg l^{-1})	3.90	Phosphorus	4.30
Ca (me l^{-1})	1.50	SO_4 (mg l^{-1})	0.80	Potassium	10.10
Mg (me l^{-1})	2.10	NO_3 (mg l^{-1})	6.50	Iron	0.65
Na (me l^{-1})	7.10	PO_4 (mg l^{-1})	0.05	Zinc	0.16
K (me l^{-1})	1.50	RSC (me l^{-1})	10.40	Manganese	0.20
CO_3 (me l^{-1})	4.20	Fluoride (mg l^{-1})	1.50	Copper	0.15

RESULTS AND DISCUSSION

The root yield of *C. borivilianum* enhanced significantly with application of FYM over the control (Table 3). The magnitude of yield enhancement was 30.0, 61.5, 69.6 and 61.7 per cent with 5, 10, 15 and 20 t of FYM over the control, respectively. The highest root yield was obtained at 15 t FYM ha^{-1} and it was at par with 10 and 20 t FYM ha^{-1} in both the years. The yield enhancement might be attributed due to improvement in soil physical properties and availability of nutrients.

The protein and sapogenine content in *C. borivilianum* was also significantly higher with 10, 15 and 20 t ha^{-1} FYM over the control (Table 4). The total protein content in *C. borivilianum* ranged from 12.96 to 13.44 per cent with different FYM levels. Forest dwelling tribals are using *C. borivillianum* for making milk pudding for women after their delivery of child for fast recovery of health. This may be due to good source of protein and iron for the health restoration and sapogenine as nutraceuticals.

The nitrogen content in root was significantly more with 10, 15 and 20 t ha^{-1} of FYM over control (Table 3). While, P content was not statistically influenced by applied FYM. Potassium content and N uptake increased in successive levels of 5, 10, and 20 t ha^{-1} of FYM. However, 10 and 15 t ha^{-1} FYM and 15 and 20 t ha^{-1} FYM were at par with respect to K content and N uptake. The P uptake increased with increased levels of FYM up to 15 t and reduced thereafter. However, K uptake increased up to 10 t FYM ha^{-1} and other higher levels of FYM were at par with this level. Uptake of N, P and K was more with higher doses of FYM, it is obvious that yield was higher with FYM resultant higher uptake. The uptake ratio of N: P remained

Table 3. Influence of farmyard manure on yield, content and uptake of nutrients by *safed musli*.

Treatments (t ha^{-1})	Yield (kg ha^{-1})	N content (%)	P content (%)	K content (%)	N-uptake (kg ha^{-1})	P-uptake (kg ha^{-1})	K-uptake (kg ha^{-1})
Control	132.9	2.07	0.43	0.50	2.75	0.56	0.67
5	172.8	2.10	0.43	0.73	3.63	0.75	1.26
10	214.6	2.12	0.43	0.77	4.54	0.93	1.64
15	225.4	2.12	0.43	0.79	4.79	0.98	1.78
20	214.9	2.15	0.44	0.81	4.62	0.94	1.74
LSD(*P = 0.05*)	19.6	0.04	NS	0.026	0.48	0.10	0.15

Treatments (t ha^{-1})	Micro-nutrients content (mg kg^{-1})				Micro-nutrient uptake (g ha^{-1})			
	Iron	Zinc	Manganese	Copper	Iron	Zinc	Manganese	Copper
Control	442.0	20. 5	15.8	3.5	62.1	2.7	2.1	0.5
5	539.9	25.9	18.3	5.9	99.6	4.5	3.2	1.0
10	512.4	38.3	19.2	6.3	130.6	8.2	4.1	1.3
15	585.3	40.4	21.5	4.9	139.6	9.1	4.9	1.1
20	516.1	33.9	18.4	4.7	116.3	7.3	3.9	1.0
LSD(*P = 0.05*)	63.46	3.7	2.6	1.1	16.8	1.1	0.7	0.2

NS = Not significant

Table 4. Influence of farmyard manure on macro-and micro-nutrient uptake ratio in *Safed Musli*.

Treatment	Protein content (%)	Sapogenine content (%)		Uptake ratio of	
				N : K : P	Fe : Zn : Mn : Cu
Control	12.96	1.25	Control	4.9 : 1.2 : 1.0	132.0 : 5.7 : 4.5 : 1.0
5 t FYM	13.12	1.45	5 t FYM	4.9 : 1.7 : 1.0	98.6 : 4.5 : 3.1 : 1.0
10 t FYM	13.23	1.48	10 t FYM	4.9 : 1.8 : 1.0	97.6 : 6.1 : 3.1 : 1.0
15 t FYM	13.25	1.48	15 t FYM	4.9 : 1.8 : 1.0	125.6 : 8.2 : 4.4 : 1.0
20 t FYM	13.44	1.48	20 t FYM	4.9 : 1.8 : 1.0	114.6 : 7.2 : 3.9 : 1.0
LSD(*P=0.05*)	0.26	0.17	Mean	4.9 : 1.7 : 1.0	110.8 : 6.4 : 3.7 : 1.0

constant with the doses of FYM (Table 4). However the uptake rations of K: P increased with increase in FYM levels, which indicates the luxury consumption of K due to enhancement in availability of K. The uptake ration also showed that N requirement of the crop is approximately five fold and K requirements 1.5 – 1.75 times with those towards P. The five-fold requirement of N is due to higher protein content in *musli* than the other tuber crops.

The content of iron, zinc, manganese and copper were more with the application of FYM (Table 3). However, iron, zinc and manganese contents were highest at 15 t FYM and copper at 10 t FYM ha^{-1} and reduced thereafter. This might be due to the fact that experiments were carried out on the soil having higher pH and slightly calcareous in nature and also have appreciable amount of exchangeable sodium per cent, leads to increase in carbonates and bicarbonates of sodium in soil solution and these ions may put hindrance on absorption and translocation of these nutrients. Apart from the soil pH, the crops irrigated with underground water were having high residual sodium carbonates (RSC) 10.4 me l^{-1}. The lime induced chlorosis was also observed in *C. borivilianum* and other medicinal and aromatic plants with such high levels of RSC of underground water by Aishwath (1) and in other field crops (Aishwath, 2). Therefore, higher the carbonates and bicarbonates lesser the uptake and translocation of micronutrients and divalent cations (Ca and Mg) are also affect the uptake of these elements with higher soil pH. The uptake of micro-nutrients enhanced with FYM, might be due to the more content and yield with FYM (Table 3). However, uptake of iron, zinc and manganese were highest at 15 t FYM and copper at 10 t ha^{-1} FYM and reduced thereafter. This might be due to lower down of content and yields with that level of FYM.

The uptake ratio of micro-nutrients indicated that iron uptake was as high as >100 times compared to copper (Table 4). However, uptake ratio of zinc and manganese was approximately six and four times more to that of copper, respectively. In general uptake ratio of micro-nutrients was highest in control and lowest at 5 t ha^{-1} FYM. This indicates that organic manure narrow down the uptake of micronutrients. However, the beneficial effect of FYM was noticed as a critical level at 15 t ha^{-1} in slightly calcareous soil having low organic matter and higher pH. The uptake ratio of micro-nutrients also showed the requirement of individual micro-nutrients with others. Uptake ratio revealed that iron requirement of *C. borivilianum* is highest than the other micronutrients, hence many of the places iron chlorosis has been observed in this crop.

In agro-ecosystem soil receives considerable input of organic matter, which has the direct relationship with soil organic carbon. In this study the organic carbon significantly built up in the soil with each successive levels of FYM (Table 5). The organic carbon built up in the soil ranged from 0.18 to 0.31 per cent, which was about to double to the 20 t ha^{-1} FYM over the control. Many studies revealed that direct input of organic matter or through left over by the crops showed linear relationship with organic carbon built up in the soil (Aishwath *et al*., 3). Bulk density of the soil decrease with each higher levels of FYM, while per cent porosity and water-holding capacity of soil increased with higher doses of FYM (Table 5). This is because of increased soil organic carbon, induces aggregates stability, moisture retention capacity and infiltration rate of the surface soil and reduces bulk density.

Soil available N and K increased with each successive levels of FYM. It is obvious that incorporation of FYM contributed for more availability of N and K in the soil (Table 5). Similarly, phosphorus also improved may be due to contribution by FYM and solublization of native P. The net improvement in available N with 5, 10, 15 and 20 t ha^{-1} FYM was 15.3, 30.2, 44.3 and 57.2 kg ha^{-1}, respectively. The phosphorus availability was improved with the

Table 5. Soil physical properties, organic carbon and available NPK in soil after the *Chlorophytum borivilianum* crop.

Treatments (t ha^{-1})	Bulk density (cm^3)	Porosity (%)	Water holding capacity (%)	Organic carbon (%)	Available N, P and K in soil (kg ha^{-1})		
Control	1.26	51.2	43.0	0.18	103.5	13.9	142.8
5	1.21	53.0	43.5	0.22	118.8 (15.3)	17.8 (3.9)	158.5 (15.7)
10	1.18	54.4	44.5	0.25	133.7 (30.2)	20.4 (6.4)	172.8 (30.0)
15	1.15	55.5	45.8	0.28	147.8 (44.3)	23.1 (9.1)	189.8 (47.1)
20	1.13	55.9	47.6	0.31	160.7 (57.2)	25.7 (11.8)	207.8 (65.0)
LSD (*p = 0.05*)	0.02	0.6	0.4	0.012	6.1	0.4	11.6

The value given in the parenthesis is net improvement of available N, P and K in soil by farm yard manure.

successive levels of FYM was 3.9, 6.4, 9.1 and 11.8 kg ha^{-1}. Similarly, potassium availability was also enhanced by 15.7, 30.0, 47.1 and 65.0 kg ha^{-1} with corresponding levels of FYM as 5, 10, 15 and 20 t ha^{-1}, respectively.

The yield, quality and uptake of nutrients in *C. borivilianum* were highest at 15 t ha^{-1} FYM. Application of FYM positively enhanced content of N, K, Fe, Zn, Mn and Cu. Uptake ratio indicated that N and K requirement of the crop was five and two times more than P. However, Fe, Zn and Mn requirement was approximately 110, 6 and 4 times more than copper. Highest yield, quality and uptake of nutrients were with 15 t ha^{-1} FYM. Physical and chemical properties of the soil improved with application of FYM. Based on the contents, it is a good source of iron and protein and sapogenine as nutraceuticals. The nutrient requirement of this crop is very low and it could come up very well even in low fertile of soil. Based on the content crop specific nutrients formulations could be prepared and indiscriminate use of manures and fertilizers may be avoided.

ACKNOWLEDGEMENT

The authors are grateful to the Director, Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat for facilities.

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Received : July, 2011; Revised : October, 2015;
Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00028.1

Indian J. Hort. 73(1), March 2016: 109-113

Response of 'Royal Delicious' apple to the staggered pre-harvest fruit-bagging

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ABSTRACT

Experiment was conducted to study the effect of staged fruit bagging on 'Royal Delicious' apples. For this, apples were bagged on three different dates, *i.e.*, 60, 75 and 90 days after full bloom (DAFB). Observations were recorded on the incidence of insects, diseases and different quality attributes at harvest and during storage at ambient conditions (32 ± 2°C and 80 ± 5% relative humidity). Our results revealed that the incidence of Sanjose scale insects (2.8 ± 0.2 %), scab (4.2 ± 0.2 %), sooty mold (0 ± 0%) and fly speck (2.2 ± 0.3%) was the least in apples bagged on 60 DAFB than those bagged on 75 DAFB or 90 DAFB or non-bagged apples. Apples bagged on an early date (60 DAFB) developed excellent colour (Hunter 'a' value = 62 ± 2.1) than those bagged on later dates or those which were non-bagged (Hunter 'a' value = 36 ± 1.2). Apples bagged on 60 DAFB were less firm, their total anthocyanin content were high (288.5 ± 14.1 mg kg^{-1} FW) than non-bagged apples (252 ± 20.3 mg kg^{-1} FW) but they exhibited high lipoxygenase (LOX) activity (0.462 ± 0.03▲OD min^{-1} g^{-1}FW) than those bagged on 75 or 90 DAFB or non-bagged apples. The total phenolic contents (TPC) of 'Royal Delicious' apples were low (8.3 ± 0.1 mg 100 g^{-1} GAE) in apples bagged on 60 DAFB than non-bagged apples (9.5 ± 0.2 mg 100 g^{-1} GAE) but they exhibited quite high antioxidant (AOX) activity (12. 4 ± 0.1 µmol Trolox g^{-1} FW) than apples bagged on later dates or non-bagged apples, which decreased substantially during storage. However, bagging date has no significant influence on eating quality attributes.

Key words: Apple, diseases, fruit bagging, quality parameters.

INTRODUCTION

Apple is the 5th most important fruit crop in India where it is grown in hills ranging from 1200-3500 m above mean sea level. From hills, apples are transported to plains for marketing or storage (Chadha and Awasthi, 2). Usually, red-coloured apples are preferred in the market as they attract the consumers. However, at lower elevations, colour development is not adequate, and hence, majority of the farmers spray ethrel (2-chloroethyl phosphonic acid) for attractive colour development (Sharma *et al*., 11). Although, ethrel spray helps in developing attractive red colour in apples but it also enhances fruit drop and pre-mature leaf-fall. In addition, ethrel-treated apples are of poor keeping-quality (Sharma *et al*., 11). Hence, efforts world over have been started to find out some alternative approaches for better colour development and reduction in the incidence of insects, diseases and disorders in fruits including apple (Sharma *et al*., 13).

In the recent years, fruit bagging has emerged as one of the best GAPs (Good Agricultural Practices) in different parts of the world. It does not only improve visual quality of fruits by promoting fruit coloration and internal fruit quality but also protects fruits from the damage caused by several insects and pathogens (Kitawaka *et al*., 6; Sharma *et al*., 10; Sharma *et al*., 12). This technique is being commercially used for growing apple, pear, peach, grape and loquat in different countries for improved fruit finish, reduction in the incidence of insect-pests and diseases (Sharma *et al*., 10; Sharma *et al*., 12). Although, several experiments have been conducted by the scientists World over to standardize bagging-date for different fruits but contradictory reports on the effects of date of fruit bagging on different fruits have appeared in the literature. For example, Fan and Mattheis (4) reported that covering 'Fuji' apple fruit in paper bags 60 days after full bloom (DAFB) delayed and reduced red colour development, but the skin colour changed significantly within the first 4 d after the bags were removed. Teixeira *et al.* (14) reported that bagging 'Fuji Suprema' apples 40 days after flowering (DAF) helped in reducing the incidence of scab and storage disorders. Liu *et al*. (8) achieved maximum accumulation of anthocyanin in 'Golden Delicious' and 'Granny Smith' apples when bagged 40-45 DAF. Hence, we attempted to observe the effects of staggered bagging with non-woven spun-bound fabric bags on fruit maturity, peel colour and postharvest quality of 'Royal Delicious' apple, which is most abundantly grown variety in India.

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MATERIALS AND METHODS

25-year-old ten-trees of 'Royal Delicious' apple cultivar were randomly selected in a private orchard, located at Kullu, Himachal Pradesh (India). These trees were raised on MM-109 rootstock, trained on central leader system with a planting density of 278 trees per hectare (6 m × 6 m). In the fruiting season of 2012 and 2013, 20 fruits were randomly bagged on the selected trees with non-woven spun-bound light-yellow coloured bags on three different dates, *i.e.*, 60, 75 and 90 days after full bloom (DAFB). Similarly, 20 randomly selected fruits were tagged at each date to be used as control. Tags were put on all the apples bagged on different dates and also on apples which were not bagged. Thus, fruits of all bagging dates and non-bagged ones existed on all the trees under experimentation. Furthermore, every possibility was ensured for uniform distribution of bags in all the tree-directions and canopy heights. During the period of bagging, the trees were subjected to recommended cultural practices. The bags were uniformly removed 5 days before the expected date of harvest. The bagged as well as non-bagged fruits were sampled from all tree heights and directions. After harvesting fruits at full maturity, fruits of different bagging dates and the non-bagged ones were kept separately by making 4-lots (each containing 200 fruits), were packed separately in corrugated fibre board (CFB) boxes and transported to the Division of Food Science and Postharvest Technology, IARI, New Delhi for further experimentation and observations.

After harvesting, observations on incidence of Sanjose scales, and diseases such as scab, sooty mold, and fly speck, and fruit colour, firmness, total anthocyanin content (TAC), lipoxygenase (LOX) activity, antioxidant (AOX) activity, and fruit quality attributes such as soluble solids content (SSC), total phenolic contents (TPC), and ascorbic acid contents (AAC) were recorded in bagged and non-bagged apples at harvest. Then, apples were stored under ambient conditions (32 ± 2°C and 80 ± 5% relative humidity) for 42 days. At the end of storage period, observations were recorded on fruit colour, firmness, TAC, LOX activity, AOX activity and fruit quality attributes such as SSC, TPC, AAC. The shelf-life of apples bagged on different dates was determined by taking observations on fruit firmness, LOX activity and sensory evaluation at weekly intervals.

The incidence of Sanjose scale, scab, sooty mold and fly speck was expressed as the percentage of the fruit affected. All the fruits of each lot were used to study the extent of damage caused either by pest or a specific disease. Fruit colour in apple peel was measured from 10 randomly selected apples per lot. The parameters CIELab: L*, a* and b*, were measured with a CR-200b tristimulus reflectance colorimeter (Minolta, Osaka, Japan). The parameter L* indicates brightness or lightness (0 = black, 100 = white), a* indicates chromaticity on a green (-) to red (+) axis, and b* indicates chromaticity on a blue (-) to yellow axis (+). Fruit firmness was determined randomly selected 10 fruits with peel on both cheeks, using a texture analyzer (model: TA+Di, Stable micro systems, UK) using compression test and represented as N (Newton) (Sharma *et al*., 11). Each run was a replicate of 3 samples. The TAC of fruit peel was determined in 10 randomly selected fruits per lot, using a UV-visible spectrophotometer by the pH-differential method (Sharma *et al*., 11) and expressed in mg kg^{-1} FW of peel. The TPC were determined in 10 randomly selected apples per lot by the method of Sharma *et al.* (9) and expressed in mg gallic acid equivalents (GAE) 100 g^{-1} FW. The AOX capacity was determined following the CUPRAC method (Apak *et al*., 1), and was expressed in µmol Trolox g^{-1} FW. The SSC values of randomly selected 20 fruits were estimated using a Fisher hand-held refractometer on a scale of 0-50. Values were expressed in ºBrix at 20°C. The AAC values were determined by standard procedures and expressed in mg ascorbic acid 100 g^{-1} FW fruit pulp. The substrate and crude enzyme for the determination of LOX activity has been prepared by the method of Sharma *et al*. (11) with minor modifications, and LOX activity was expressed as µmoles linolenic acid oxidised g^{-1} FW min^{-1}.

The experiment was laid out in a completely randomised block design (CRBD). Analysis of variance using one-way ANOVA followed by Duncan's test was performed to test the significance of differences between means obtained among the treatments at the 5% level of significance.

RESULTS AND DISCUSSION

The incidence of Sanjose scale insects, and diseases such as scab, sooty mold and fly speck was significantly influenced by bagging date. The incidence of Sanjose scale (2.8 ± 0.2%), scab (4.2 ± 0.2%), sooty mold (0 ± 0%) and fly speck (2.2 ± 0.3%) was the least in apples bagged on 60 DAFB than those bagged on 75 DAFB or 90 DAFB or non-bagged apples (Table 1). Pre-harvest fruit bagging maintains physical separation between pathogens and the host, and fruits covered on different dates might have provided protection against insects and diseases for differential time (Sharma *et al*., 10). Furthermore, variation in the incidence of insects and diseases among different bagging dates may due to differences in the period for which apples remained covered with bags (Kitagawa *et al*., 6; Sharma *et al*., 10).

Table 1. Effect of date of bagging on the incidence of insect and diseases in 'Royal Delicious' apple*.

Bagging date	Sanjose scale (%)	Scab (%)	Sooty mold (%)	Fly speck (%)	Shelf-life (days)
60 DAFB	2.8 ± 0.2[a]	4.2 ± 0.2[a]	0.0 ± 0.0	2.2 ± 0.3[a]	21± 1.7[a]
75 DAFB	6.3± 0.2[b]	7.6 ± 0.2[b]	0.0 ± 0.0	2.6 ± 0.1[a]	28 ± 1.5[b]
90 DAFB	8.6± 0.4[c]	9.5 ± 0.3[c]	0.0 ± 0.0	2.4 ± 0.2[a]	35± 1.5[c]
Non-bagged	18.4 ± 0.3[d]	18.3 ± 0.2[d]	18.5 ± 0.2[a]	12.3 ± 0.3[b]	35 ± 1.2[c]

*Means within the column with the same alphabet are not significantly different by Duncan multiple range test at P ≤ 0.05.

Bagging date has significantly influenced colour development in 'Red Delicious' apples. Apples bagged on an early date (60 DAFB) developed excellent colour (Hunter 'a' value = 62 ± 2.1) than those bagged on 75 DAFB (Hunter 'a' value = 55 ± 2.1) or 90 DAFB (Hunter 'a' value = 52 ± 1.5) or those which were not bagged (Hunter 'a' value = 36 ± 1.2) (Table 2). During storage, there was slight increase in Hunter 'a' value in bagged or non-bagged apples. Fruit bagging has pronounced effect on colour development of apple primarily because bagged apples become highly sensitive to anthocyanin accumulation once exposed to sunlight (Ju, 5). The differences in Hunter 'a' value in apples bagged on different dates may be due to differences in period for which they remained covered and the extent of their sensitivity to anthocyanin accumulation so caused by bagging time. Thus, apples covered on early date might have become more sensitive to anthocyanin accumulation than those bagged on later dates or those which were not bagged at all. Ju (5) has demonstrated that fruit colour development in bagged apples is the result of sensitivity caused by bagging to UV light. Better colour development after pre-harvest fruit bagging has also been reported in apple (Fan and Mattheis, 4; Liu *et al*., 8).

Apples bagged on 60 DAFB were less firm (28.4 ± 1.4 N) than those bagged on later dates or those which were not bagged (35.4 ± 0.5 N) although, differences in fruit firmness between apples bagged on 90 DAFB (35.3 ± 0.7 N) and non-bagged apples (35.4 ± 0.5 N) were not significant (Table 2). Fruit firmness decreased substantially during storage. Apples bagged on early dates must be more senescent, exhibiting high LOX activity than those bagged on later dates, and hence they had low firmness. Decrease in firmness in apples during storage may be due to conversion of starch to simple sugars at a higher rate and dissolution of middle lamella of cells, which is responsible for maintaining the integrity of cells and thereby firmness (Sharma *et al.*, 10). A significant effect of bagging date was observed on LOX activity of apples at harvest and during subsequent storage at room temperature. Apples bagged on 60 DAFB exhibited high LOX activity (0.462 ± 0.03▲OD min^{-1} g^{-1}FW) than those bagged on 75 or 90 DAFB or non-bagged apples (0.398 ± 0.02▲OD min^{-1} g^{-1}FW). LOX activity increased during storage both in bagged and non-bagged apples (Table 2) due to increase in senescence with storage period. Higher LOX activity in apples bagged on early date may be due to low fruit firmness of fruits exhibited by increased senescence and maturity. Increase in LOX activity during storage may be due to increased senescence. Lower LOX activity in bagged 'Royal Delicious' apples than non-bagged apples has been reported by Sharma *et al*. (9).

The TAC of 'Royal Delicious' apples were significantly influenced by date of bagging, being high in apples bagged on 60 DAFB (288.5 ± 14.1 mg kg^{-1} FW) and low in non-bagged apples (252 ± 20.3 mg kg^{-1} FW) and increased slightly during storage (Table 3). Several studies have indicated an increase in TAC by bagging although this increase is influenced by the time of re-exposure of fruits to sunlight (Ju, 5). High TAC in apples bagged on early dates than those bagged on later dates or non-bagged ones may be because of increased ability of such

Table 2. Fruit colour, fruit firmness and the LOX activity of 'Royal Delicious' apples at harvest and during storage (32 ± 2°C and 80 ± 5% relative humidity) which were bagged on-the-tree on different dates.

Bagging date	Hunter 'a' value		Fruit firmness (N)		LOX (▲OD min^{-1} g^{-1}FW)	
	At harvest	After storage	At harvest	After storage	At harvest	After storage
60 DAFB	62 ± 2.1[a]	64 ± 0.6[a]	28.4 ± 1.4 [a]	16.3 ± 0.6[a]	0.462 ± 0.03[a]	0.412 ± 0.01[a]
75 DAFB	55 ± 2.1[b]	58 ± 2.7[b]	32.5 ± 1.5[b]	22.5 ± 0.3[b]	0.434 ± 0.01[b]	0.384 ± 0.01[b]
90 DAFB	52 ± 1.5[b]	57 ± 1.5[b]	35.3 ± 0.7[c]	28.2 ± 0.3[c]	0.412 ± 0.01[c]	0.332 ± 0.02[c]
Non-bagged	36 ± 1.2[c]	42 ± 2.3[c]	35.4 ± 0.5[c]	28.6 ± 0.2[c]	0.398 ± 0.02[d]	0.242 ± 0.02[d]

*Means within the column with the same alphabet are not significantly different by Duncan's multiple range test at P ≤ 0.05.

apples for synthesizing total anthocyanins after re-exposure of such fruits to sunlight. Interestingly, no-significant difference occurred in TAC of non-bagged apples and those bagged on 90 DAFB, indicating that covering apples for more time than re-exposing to sunlight helps in synthesizing high amount of TAC. Date of bagging has significant influence on the TPC of 'Royal Delicious' apples being low (8.3 ± 0.1 mg 100 g^{-1} GAE) in apples bagged on 60 DAFB and quite high in non-bagged apples (9.5 ± 0.2 mg 100 g^{-1} GAE), which increased substantially during storage (Table 3). Several studies have indicated that TPC usually decrease by pre-harvest fruit bagging primarily because such fruits are devoid of light, which is essentially required for the synthesis of secondary metabolites (phenolics). In this study, there was insignificant difference in TPC among apples of different dates perhaps because apples of all dates were re-exposed to sunlight for 5 d, a time quite sufficient for the synthesis of such compounds. Yet differences in TPC of apples bagged on different dates may be because of the differences in the time period for which apples remained covered with bags. Chen *et al.* (3) have reported decline in the TPC of bagged 'Golden Delicious' and 'Royal Gala' apples. The AOX activity of 'Royal Delicious' apples were significantly influenced by date of bagging. The AOX activity was high (12. 4 ± 0.1 µmol Trolox g^{-1} FW) in apples bagged on 60 DAFB than those bagged on later dates or non-bagged apples (9.2 ± 0.1 µmol Trolox g^{-1} FW) which decreased substantially during storage (Table 3). These results are contradictory to some findings but increase in the AOX activity in-spite of decline in the TPC may be due to accumulation of higher amount of the TAC, resulting in higher AOX activity. The AOX activity decreased during storage inspite of increase in the TAC and the TPC, primarily because of decline in the AAC which also contributes to the AOX activity. However, in contrast, Xu *et al.* (16) reported that total phenolics and flavonoid concentrations and total anti-oxidant activity in loquat fruit decreased following bagging treatments.

There was no significant difference in the AAC among apples bagged on different dates but the AAC was higher in bagged apples than non-bagged ones (28.4 ± 0.9 mg 100^{-1} g pulp). However, there was a sharp decline in the AAC during storage and it was least in non-bagged apples (18.6 ± 0.2 mg 100^{-1} g pulp) (Table 4). The AAC has been reported to be high in bagged 'Red Fuji' apples (Li *et al.,* 7), mango (Watanawan *et al.*, 15) and loquat (Xu *et al.*, 16). Date of bagging has significant influence on the SSC of 'Royal Delicious' apples, which was significantly higher (16.2 ± 0.2%) in apples bagged on 60 DAFB than those bagged on 75 DAFB (15.8 ± 0.1%), 90 DAFB (14.5 ± 0.2%) or non-bagged ones (13.8 ± 0.1%) (Table 4). The SSC showed insignificant increment during storage both in bagged and non-bagged apples. Improvement in SSC due to bagging has also been reported in grapes (Zhou and Guo, 17),

Table 3. Total anthocyanin content, phenolic content, and antioxidant activity of 'Royal Delicious' apples as influenced by the date of bagging at harvest and during storage at ambient conditions (32 ± 2°C and 80 ± 5% relative humidity) for 42 days*.

Bagging date	TAC (mg kg^{-1} FW)		TPC (mg 100 g^{-1} GAE)		AOX activity (µmol Trolox g^{-1} FW)	
	At harvest	After storage	At harvest	After storage	At harvest	After storage
60 DAFB	288.5 ± 14.0 [a]	294.2 ± 14.2[a]	8.33 ± 0.1[a]	8.66 ± 0.5[a]	13.8 ± 0.4[a]	12.4 ± 0.1[a]
75 DAFB	276.4 ± 16.4[b]	288.2 ± 16.0[b]	8.54 ± 0.1[b]	8.78 ± 0.3[b]	12.8 ± 0.2[b]	11.6 ± 0.2[b]
90 DAFB	264.4 ± 7.2[c]	280.5 ± 24.3[c]	9.22 ± 0.1[c]	9.45 ± 0.2[c]	12.6 ± 0.2[c]	11.2 ± 0.4[c]
Non-bagged	202.8 ± 20.2[d]	232.6 ± 13.8[d]	9.53 ± 0.2[d]	9.88 ± 0.3[d]	11.2 ± 0.2[d]	9.2 ± 0.1[d]

*Means within the column with the same alphabet are not significantly different by Duncan's multiple range test at P ≤ 0.05.

Table 4. Fruit quality attributes and shelf life of 'Royal Delicious' apples as influenced by the date of bagging at harvest and during storage at ambient conditions (32 ± 2°C and 80 ± 5% relative humidity) for 42 days*.

Bagging date	Ascorbic acid content (mg 100^{-1} pulp)		Soluble solids concentrate (°Brix)		Shelf-life (days)
	At harvest	After storage	At harvest	After storage	
60 DAFB	29.8 ± 0.7 [a]	22.8 ± 0.8[a]	16.2 ± 0.2[a]	16.6 ± 0.2[a]	21± 1.7[a]
75 DAFB	29.5 ± 0.9[a]	22.8 ± 0.7[a]	15.8 ± 0.1[a]	16.6 ± 0.1[a]	28 ± 1.5[b]
90 DAFB	29.3 ± 0.7[a]	22.3 ± 0.6[a]	14.5 ± 0.2[a]	15.4 ± 0.1[b]	35± 1.5[c]
Non-bagged	28.4 ± 0.9[a]	18.6 ± 0.2[a]	13.8 ± 0.1[c]	14.2 ± 0.1[c]	35 ± 1.2[c]

*Means within the column with the same alphabet are not significantly different by Duncan's multiple range test at P ≤ 0.05.

mango (Watanawan *et al.*, 15), loquat (Xu *et al.*, 16) and 'Granny Smith' and 'Golden Delicious' apples (Liu *et al.*, 8).

Apple bagged on an early date (60 DAFB) exhibited short stay (21 ± 1.7 d) at room temperature than those bagged on later dates (75 DAFB and 90 DAFB) or those which were not bagged (35 ± 1.2 d) (Table 4). Short shelf-life of apples bagged on early date than those bagged on later dates, may be due to their high senescent nature and soft texture created by high LOX activity or other enzymes (Sharma *et al*., 10).

Thus, it can be concluded that pre-harvest fruit bagging on different dates influences colour development, fruit firmness, shelf-life and different fruit quality attributes. Hence, by staged bagging, we can achieve desirable effects and can regulate market by supplying apples on different dates and variable quality.

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Received : January, 2015; Revised : February, 2016;
Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00029.3

Indian J. Hort. 73(1), March 2016: 114-119

Short communication

Effect of packaging films on shelf-life and quality of bell pepper under super and ordinary market conditions

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ABSTRACT

Bell pepper (*Capsicum annuum* L.) fruits of cv. Indra were harvested at commercial maturity and packed in different packaging films, *viz*. heat shrinkable film (15 µ), cling film (15 µ) and low density polyethylene film (LDPE 25 µ) and thereafter stored under two different conditions, *i.e.* super-market conditions (18-20°C; 90-95% RH) and ordinary market conditions (28-30°C; 60-65% RH). The shrink film significantly checked the loss in weight (3.38%), firmness (1330.5 g force) and decay incidence (0%), maintained qualities attributes like ascorbic acid (20.70 mg/100 g) and chlorophyll content (0.066 mg/100 g), respectively during 10 days of storage under super market conditions. The in-package gaseous composition (O_2 and CO_2) in shrink film packed fruits was found to be 3.55 and 17.10% after 10 days of storage. The overall sensory quality of the packed bell pepper during retailing was found to be better than control fruits. The shrink and cling film prolonged the shelf-life and maintained the quality of bell pepper fruits for 10 and 7 days under super market conditions and ordinary market conditions, respectively as against 5 and 2 days only in case of unpacked control fruits.

Key words: Bell pepper, packaging films, quality, storage conditions.

INTRODUCTION

Bell pepper (*Capsicum annuum* L.) is an important commercial vegetable crop grown in India. It is a nutritious vegetable and is known to contain biologically active compounds such as antioxidants, vitamins and other phytochemicals (Marin *et al*., 10). In Punjab, extreme weather conditions under open field conditions are the major limiting factors for achieving higher yield and better quality of vegetables. Under such circumstances, protected cultivation, *i.e.* naturally ventilated poly-house technology is the best option. Therefore, the farmers of Punjab are switching over to this technology for cultivation of bell pepper and other vegetables such as cucumber and tomato.

Bell pepper is a perishable crop and is liable to spoilage like all fruits and vegetables due to inadequate packaging techniques and improper storage conditions. The major postharvest problem with this crop are excessive softening due to water loss that lead to shriveling, wilting and pathogenic disorders, which severely reduce the quality and acceptability of the produce (Rao *et al.*, 12). Generally, the growers and traders keep perishables under ambient conditions, where the quality of produce deteriorates rapidly.

The concept of super market is fast gearing up in Indian markets and selected Indian and exotic vegetable and fruits are being displayed in these out-lets for attracting upper-end consumers. Packing of vegetables in polymeric film creates modified atmospheric conditions around the produce inside the package allowing lower degree of control of gases, which can interplay with physiological processes of commodity resulting in reduced respiration rate, transpiration and other metabolic processes of fresh produce (Zagory and Kader, 16). Thus, the aim of this study was to investigate the effects of different packaging films on post-harvest performance of bell pepper under super-market conditions (SMC) and ordinary market conditions (OMC).

MATERIALS AND METHODS

The fruits of bell pepper cv. Indra grown under naturally ventilated poly-house were harvested at commercial maturity. Three types of packaging films commercially available in the market, *viz.* heat shrinkable film (15 µ), cling film (15 µ) and low density polyethylene film (LDPE, 25 µ) were used for packaging of bell pepper fruits in paper moulded trays (22 cm × 13 cm). The fruit were packed in trays (6 fruits in each tray) and tightly sealed with different packaging films. However, the shrink film wrapped packs were passed through a shrink wrapping machine (Model BS-450, Samrath Engineers, India) at 165°C for 10 sec. Thereafter, the packed fruits as well as control (non-packed) fruits were stored at 18-20°C

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and 90-95% RH under super market conditions (SMC) and 28-30°C and 60-65% RH under ordinary market conditions (OMC).

The physiological loss in weight (PLW) of stored fruit was calculated by subtracting final weight from the initial weight of the fruits and expressed in per cent. Firmness of fruit was measured with the help texture analyser (Model TA-HDi, Stable Micro System, England). The bell pepper fruit was kept on the platform of the instrument and compressed to a distance of 5 mm with 75 mm diameter compression probe (p/75). The results were expressed as g force of compression. The decay percentage of treated and untreated fruit was calculated as the number of decayed fruit divided by initial number of all fruit multiplied by hundred. The overall organoleptic rating of the fruit was done by a panel of ten judges using 9-point Hedonic scale (Amerine *et al.*, 1). The ascorbic acid and chlorophyll content were estimated as per standard procedures (AOAC, 2). The in-package gaseous composition (CO_2 and O_2 conc.) of sealed fruit package was monitored at periodic intervals with the help of portable Head Space Gas Analyzer (Model: GS 3/P, Make: Systech Instruments, UK). A sample of 0.5 ml was automatically withdrawn from the headspace atmosphere with a pin-needle connected to the injection system. Gases were analyzed with inbuilt sensors for CO_2 and O_2. The instrument was calibrated towards air.

The experiment consisted of 4 treatments and 3 storage intervals for SMC experiment and 4 treatments and 4 storage intervals for OMC experiment. Both experiments were laid out in completely randomized design with three replications for each treatment and each storage interval. Each replication was comprised of 6 fruit. In total there were 216 fruits in SMC experiment and 288 fruits in OMC experiment. The experiments were conducted for two seasons (2012-13 and 2013-14). The data were pooled for both the seasons and analyzed for variance by using the SAS (V 9.3, SAS Institute Inc., Cary, NC, USA) package.

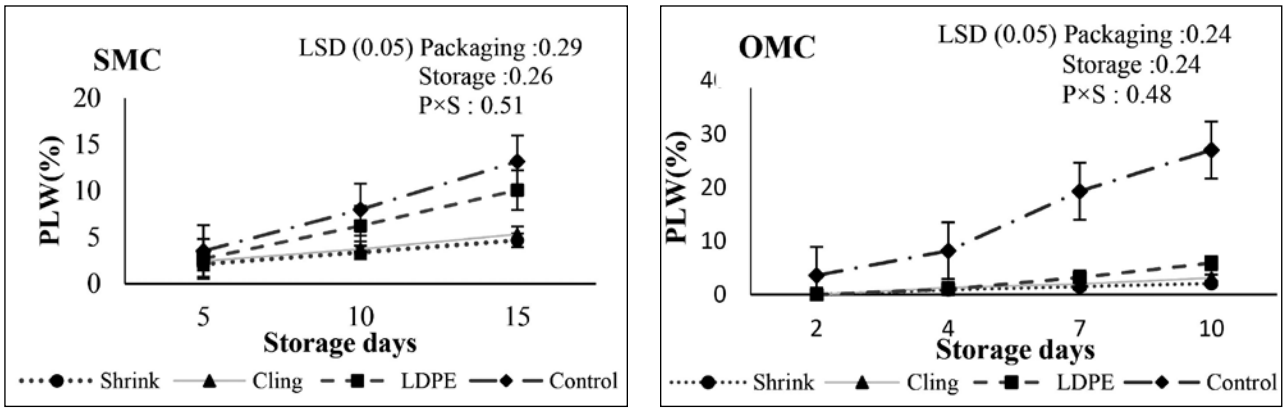
RESULTS AND DISCUSSION

The percent PLW, in general, increased with the advancement of storage period rather slowly in the beginning but at a faster pace as the storage period advanced (Fig. 1A). It was noticed that shrink film packed fruits registered the lowest average PLW (3.39%) and ranged between 2.13 to 4.67 per cent from 5 to 15 days of storage as compared to control, where PLW was found to be the highest and ranged between 3.53 to 13.18 per cent under SMC. Similarly, under OMC, the lowest mean PLW (1.11%) was observed in fruits packed in shrink film and the highest (14.51%) was observed in control fruits. Water loss is a critical factor in shortening the storage life and increasing deterioration of many fruit during storage, which reduce both market value and consumer acceptability. The reduction in weight loss in film-packed fruits is attributed to restricted respiratory process of fruits inside the packaging films (Ben Yehoshua, 3). The positive role of shrink film in reducing the PLW of papaya has been reported (Singh and Rao, 15).

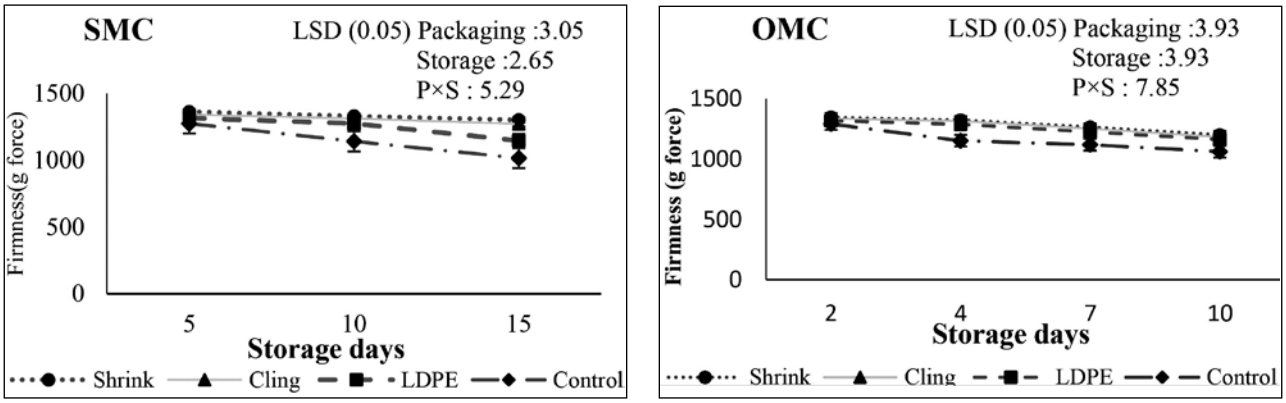
The firmness, in general, followed a declining trend commensurate with advancement in storage period (Fig. 1B). The fruits packed in shrink packaging film maintained the highest average firmness (1332.33 g force) and control fruits registered the lowest mean firmness (1144.50 g force) under SMC. The firmness of fruits in shrink film declined slower and steadily and ranged between 1363.83 to 1302.83 g force from 5 to 15 days of storage interval, whereas in case of control fruits, the decline in firmness was found to be abrupt and sharp and ranged between 1275.83 to 1015.83 g force, thereby leading to excessive softening and shriveling of fruits. Under OMC, the shrink film packed fruits recorded the highest average firmness (1282.92 g force) and ranged between 1346 to 1200 g force from 2 to 10 days of storage. However, in control, the fruits experienced a faster loss of firmness during storage and ranged between 1290 to 1061 g force with the average fruit firmness of 1154.92 g force. The texture, in particular, crispness of pepper is an important quality attribute to the consumer. Flaccidity development appears to be directly associated with water loss in pepper (Lownds *et al.*, 7). The maintenance of higher firmness with heat shrinkable packaging film has been noticed in apple and pear fruits (Sharma *et al.*, 14).

The spoilage of bell pepper fruit under both storage conditions was found to be lower in shrink and cling packaging film (Fig. 1C). Under SMC, the average fruit decay was observed to be 0.99 and 1.34 percent in shrink and cling films, whereas in LDPE film the level of decay was quite high (9.15%). Similarly, under OMC the shrink and cling films recoded negligible fruit decay. On the other hand the level of decay was the highest in LDPE film (7.31%). In the present studies, the LDPE film packed fruits recorded the highest spoilage of fruits, even higher than unpacked control fruits under both the storage conditions. The occurrence of higher decay incidence in LDPE film might be due to accumulation of excessive water vapour inside the package, because of restricted movement of water through the film as the water vapour transmission rate of LDPE has been reported to be higher as compared to shrink and cling film (Robertson, 13). Shrink film wrapped

A. Physiological loss in weight (PLW)



B. Firmness



C. Decay

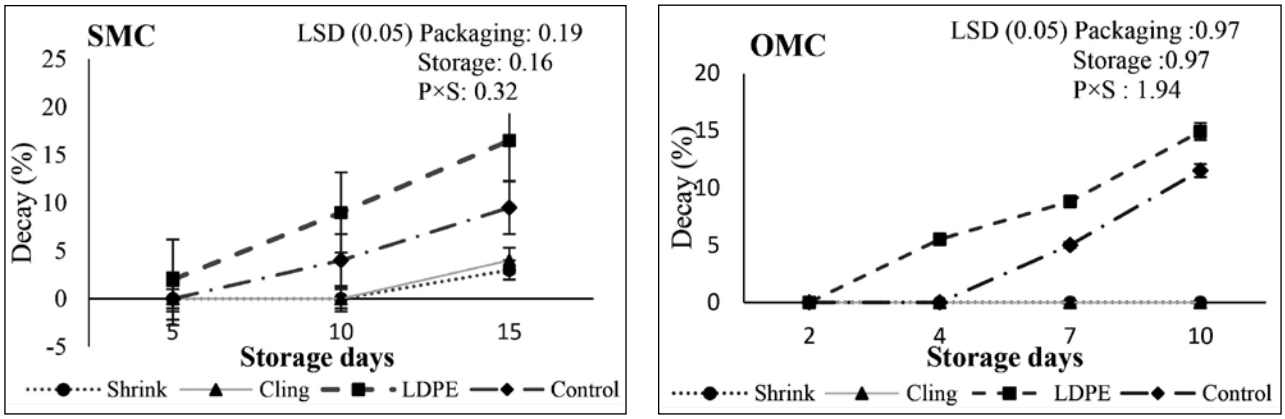


Fig. 1. Effect of different packaging films on PLW (A), firmness (B) and decay (C) of bell pepper under super market conditions (SMC) and ordinary market conditions (OMC).

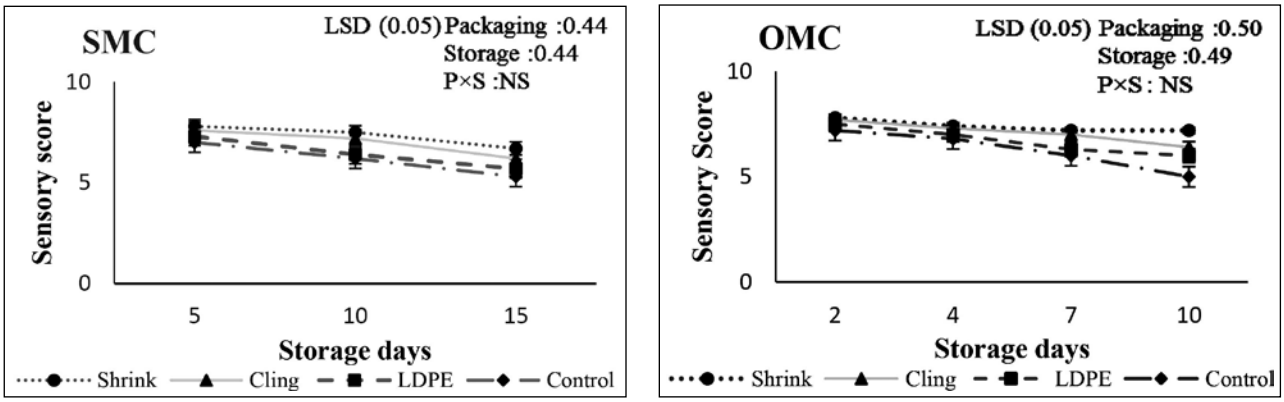
cucumber exhibited lower decay incidence and better retention of green colour and other physico-chemical attributes during storage as compared to unwrapped cucumber (Dhall *et al.*, 4).

A gradual decline in sensory score was noticed in shrink and cling film packed fruits as compared to control where decline was sharp under both the storage conditions (Fig. 2A). The maximum average sensory score was shown by fruits packed in shrink film (7.3 and 7.2) followed by cling film (7.0 and 7.0) under both the marketing conditions. The control fruits registered the minimum sensory

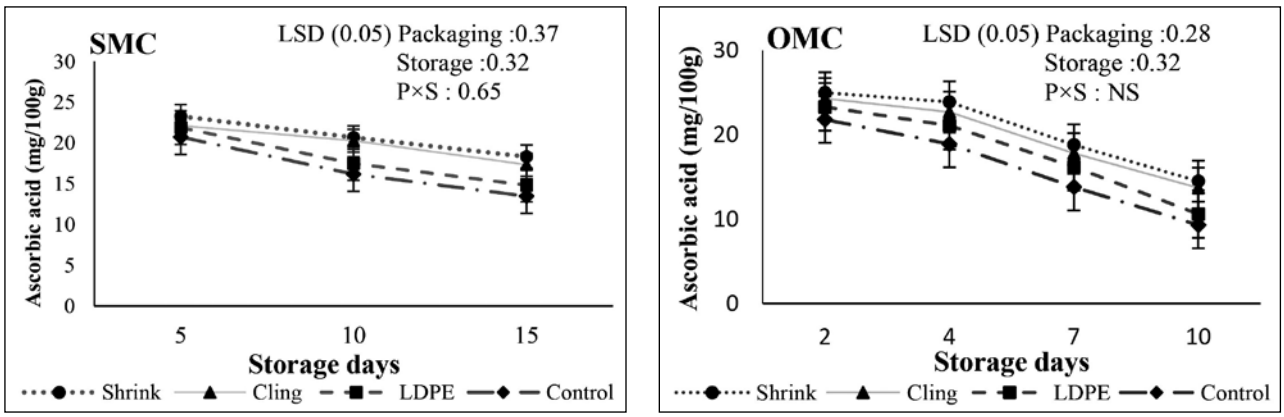
score (6.1 and 6.2). Successful control of respiration of vegetable and fruit by modified atmosphere packaging (MAP) can result in maintaining high organoleptic quality of produce. Shrink film packed peach fruits successfully maintained freshness, crispness and aroma during storage (Mahajan *et al.*, 8).

The ascorbic acid of bell pepper fruits experienced a linear decline as the storage period advanced (Fig. 2B). It was noticed that shrink film packed fruits

A. Sensory quality



B. Ascorbic acid



C. Chlorophyll

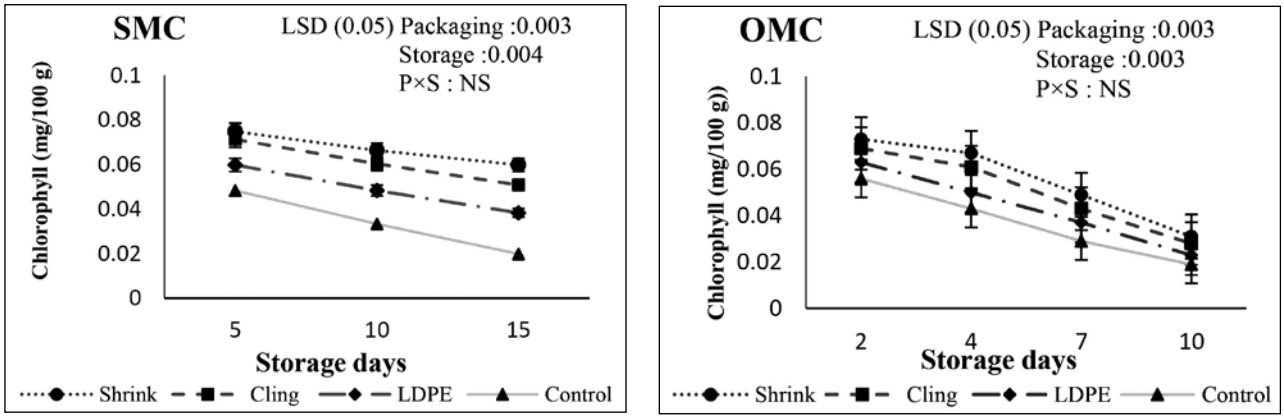


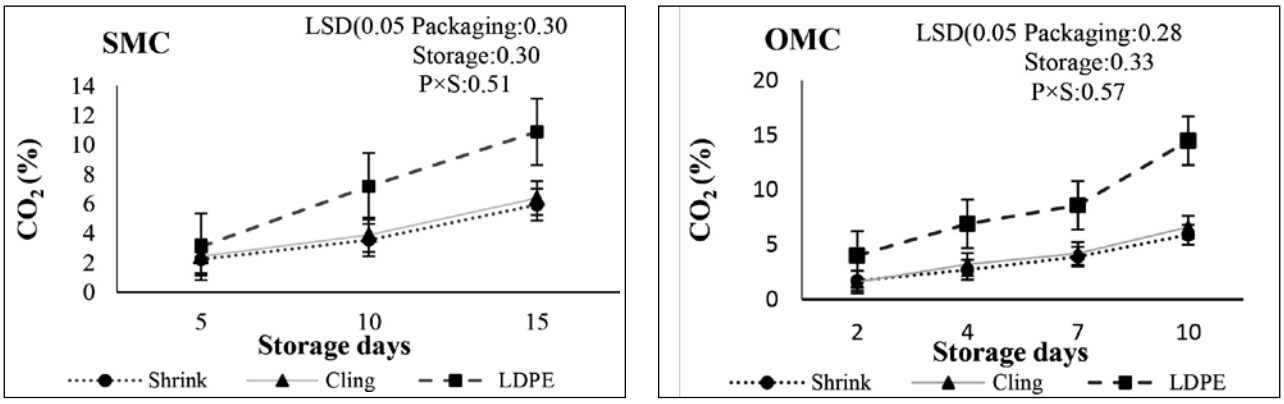
Fig. 2. Effect of different packaging films on sensory quality (A), ascorbic acid (B) and chlorophyll content (C) of bell pepper under super market conditions (SMC) and ordinary market conditions (OMC).

showed higher ascorbic acid over the other treatments throughout the storage period and recorded mean ascorbic acid (20.77 mg %) followed by cling film packed fruits (19.92 mg %). The control fruits showed the lowest mean ascorbic acid (16.80 mg %) under SMC. The ascorbic acid content in shrink and cling film packed fruits, ranged between 23.28 to 18.33 and 22.13 to 17.33 mg %, respectively from 5 to 15 days of storage as compared to control, where ascorbic acid was found to be the lowest and ranged between 20.73 to 13.48 mg %. Under OMC, the highest mean ascorbic acid content (20.56 mg %) was observed in shrink film packed fruits and it was closely followed by cling film packed fruits (19.63 mg %). On the other hand, the lowest mean acidity (15.97 mg %) was observed in unpacked control fruits. The decrease in ascorbic acid during storage may be due to the oxidation of L-ascorbic acid into dehydroascorbic acid (Mapson, 9). The influence of heat shrinkable films on maintaining higher ascorbic acid content in citrus fruits has also been reported (Ladaniya and Singh, 6).

The chlorophyll content of the bell pepper fruit declined during storage irrespective of different packaging films (Table 2C). However, the shrink film packed fruits maintained the highest chlorophyll content followed by cling film as compared to control fruits under both the storage conditions. The decrease in chlorophyll during storage is expected due to chlorophyll degradation as a result of chlorophyllase enzyme activity leading to senescence (Gong and Mattheis, 5). The maintenance of green colour in modified atmosphere packaged cucumber during storage has been reported by Dhall *et al.* (4).

During storage a decrease in O_2 and an increase in CO_2 levels occurred for passive modified atmospheric packaging (MAP) in all the three films under SMC as well as under OMC (Fig. 3A and 3B). However, gaseous composition in-side the package was significantly different depending on the type of film used. The heat shrinkable packaging film registered a gradual increase in CO_2 and decrease in O_2 concentration within the package where as LDPE

A. Carbon dioxide (CO_2)



B. Oxygen (O_2)

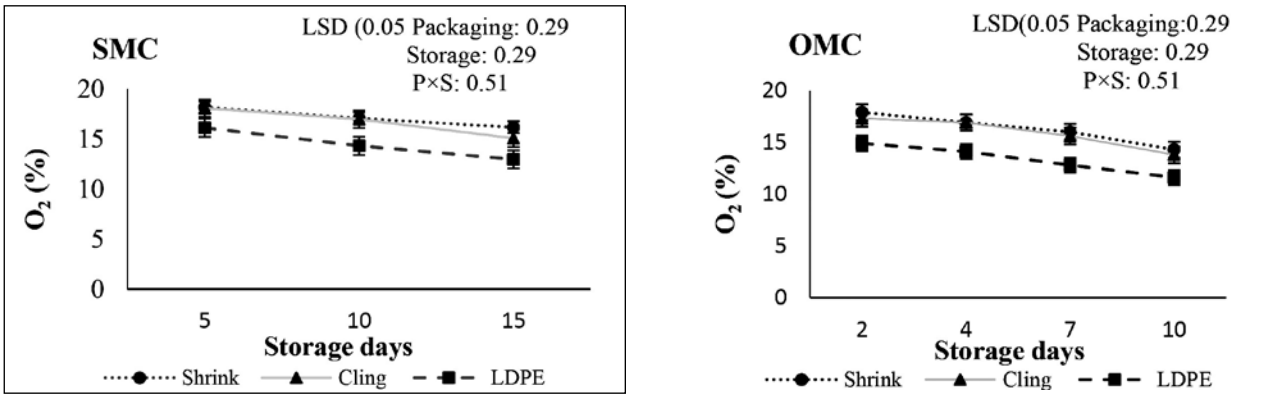


Fig. 3. Effect of different packaging films on CO_2 level (A) and O_2 level (B) of bell pepper under super market conditions (SMC) and ordinary market conditions (OMC).

film recorded a sharp increase in CO_2 and decrease in O_2 concentration inside the package. Reduced respiration rate in shrink packed pomegranate due to impressive gas barrier properties of heat shrinkable film has been reported (Nanda *et al.*, 11). Modification of the atmosphere around the fresh produce in the package made of flexible plastic films has been confirmed (Zagory and Kader, 16). Packaging of bell pepper fruits in paper moulded trays followed by wrapping with heat shrinkable film or cling film seems to hold promise in improving the shelf-life and maintaining the quality under super market and ordinary market conditions by 10 and 7 days, respectively as against 5 and 2 days in case of unpacked control. This technology can be helpful in minimizing the postharvest losses of bell pepper during retail marketing.

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Received : April, 2015; Revised : November, 2015;
Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00030.X

Indian J. Hort. 73(1), March 2016: 120-123

Short communication

In vitro studies on callus induction and shoot regeneration from leaf explants of *Prunus avium* L. rootstock 'F12/1'

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ABSTRACT

To obtain regeneration from leaf explant of 'F12/1' (*Prunus avium* L.) rootstock, young leaves from 4-week-old *in vitro* shoot cultures were wounded by transverse cuts three times along the midrib and incubated with abaxial side facing up on Woody Plant Medium (WPM). Shoot regeneration was obtained with benzyladenine (BA) both in dark incubated cultures (5% at 2.5 µM) and non-dark incubated cultures (5% at 10 µM). Thidiazuron (TDZ) containing medium also yielded shoot regeneration (5% at 9.08 µM) in cultures that were kept in continuous light. Overall, TDZ pre-treatment and dark incubations improved callus formation frequencies and callus colony numbers. Regenerates were rooted on WPM containing 9.08 µM IBA, acclimatized and then transferred to greenhouse conditions.

Key words: Benzyladenine, cherry rootstock, dark incubation, organogenesis, thidiazuron.

INTRODUCTION

Conventional improvement of *Prunus* rootstocks is a long, and expensive process due to polyploidy, heterozygosity, long breeding cycles and field trials. Genetic transformation is an alternative tool to accelerate the breeding process. However, efficient transformation protocols have not yet been reported for most of the *Prunus* species due to their recalcitrance to regeneration (Canli and Tian, 2). 'F 12/1' (*Prunus avium* L.) is a traditional cherry rootstock and development of a genetic transformation protocol for this rootstock largely depends on the availability of an efficient regeneration protocol. Regeneration of shoots from leaf tissues of *P. avium* were reported, but these regeneration protocols were either for forest timber trees (Yang and Schmidt, 7) or established only for a limited number of cultivars (Peer *et al*., 4) and had generally low regeneration rates (Tang *et al.*, 5; Hammatt and Grant, 3).

Genetic transformation of 'F 12/1' has not been attempted till date due to the lack of an efficient regeneration protocol. Therefore, the objective of this study was to investigate the effect of cytokinins, dark treatments and pre-treatments on callus formation and regeneration from leaf tissue of 'F 12/1' rootstock.

MATERIALS AND METHODS

Shoot tips of 'F12/1' rootstock were harvested in the spring of 2012 from Egirdir Horticultural Research Station. The shoot tips were cut into about 5 cm pieces and were cleaned with tap water. Sliced stem pieces were sterilized twice with 0.8% sodium hypochlorite for 15 min., each followed by a 15 min. rinse using sterile double-distilled water. After surface sterilization, stems of 'F12/1' were cut into about 2 cm sections containing one or two buds and explanted on Murashige and Skoog (MS) medium containing 0.54 µM NAA, 3 µM BA, 30 g/l sucrose and 7 g/l agar-agar. The pH was adjusted to 5.5 before autoclaving at 18 atm at 121°C for 30 min. Then the medium was distributed into 9 × 25 × 150 mm culture tubes. The cultures tubes were transferred in a growth room kept at 16/8-h (light/dark) photoperiod supplied by cool-white fluorescent lambs (131 µM m^{-2} s^{-1}) and 24 ± 1°C. After bud break, newly emerged shoots were excised and were explanted into jars containing 30 ml of the same medium described above. Then, shoot cultures were sub-cultured onto the same medium every 5 weeks.

After shoot cultures reached the necessary population size to conduct the regeneration experiments, young leaves were harvested from cultures and wounded by transverse cuts three times along the midrib. Then the leaves were incubated with abaxial side facing up in petri dishes containing 30 ml of the regeneration medium consisting of Woody Plant Medium (WPM), 30 g/l sucrose, 7 g/l agar-agar, 0.54 µM NAA and either BA (0, 1.25, 2.5, 5, 10, or 20 µM) (Table 1) or TDZ (0, 0.57, 1.35, 2.27, 4.54, 6.81 and 9.08 µM) (Table 3). The petridishes were placed in the culture room conditions described above.

In dark treatment experiments, wounded leaf explants were placed abaxial side facing up on the same regeneration media containing either BA (Table 2) or TDZ (Table 4) as described above.

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Acknowledgments : This research was funded by a grant from Suleyman Demirel University Scientific Research Projects Coordination Unit (SDU-BAP 3504-YL1-13).

Table 1. Effects of different concentration of benzyladenine (BA) on regeneration and callus formation from leaf explants of 'F12/1' *Prunus* rootstock.

BA (µM)	Callus formation (%)	No. of callus colony[y]	Regeneration (%)
0	0.00c	0.00c	0b[z]
1.25	50.0a	1.00a	0b
2.5	57.5a	0.97a	0b
5	15.0b	0.22b	0b
10	12.5b	0.12b	5.0a
20	10.0b	0.10b	0b

[z]Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y]Calli smaller than 3 mm in diameter were not included

Table 2. Effects of different concentration of benzyladenine on regeneration and callus formation from dark incubated leaf explants of 'F12/1' *Prunus* rootstock.

BA (µM)	Callus formation (%)	No. of callus colony[y]	Regeneration (%)
0	12.5c	0.22b	0b[z]
1.25	42.5ab	0.92a	0b
2.5	55.0a	1.22a	5.0a
5	52.5ab	0.95a	0b
10	40.0ab	0.40b	0b
20	35.0b	0.35b	0b

[z] Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y] Calli smaller than 3 mm in diameter were not included

Table 3. Effects of different concentration of thidiazuron (TDZ) on regeneration and callus formation from leaf explants of 'F12/1' (*Prunus avium* L.) rootstock.

TDZ (µM)	Callus formation (%)	No. of callus colony[y]	Regeneration (%)
0	2.50d[z]	0.05d	0b[z]
0.57	22.5d	0.32cd	0b
1.35	22.5d	0.27cd	0b
2.27	30.0cd	0.60c	0b
4.54	60.0ab	1.12b	0b
6.81	52.5bc	1.12b	0b
9.08	87.5a	1.92a	5.0a

[z] Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y] Calli smaller than 3 mm in diameter were not included

Table 4. Effects of different concentration of thidiazuron (TDZ) on regeneration and callus formation from dark incubated leaf explants of 'F12/1' (*Prunus avium* L.) rootstock.

TDZ (µM)	Callus formation (%)	No.of callus colony[y]
0	7.5d[z]	0.07d
0.57	37.5cd	0.60c
1.35	45.0bc	0.47c
2.27	72.5ab	1.50a
4.54	80.0a	1.2ab
6.81	76.6ab	1.30ab
9.08	67.5ab	1.07b

[z] Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y] Calli smaller than 3 mm in diameter were not included

These petridishes were kept in the darkness for the first ten days of culture by wrapping petri-dishes with aluminum foil. After ten days, aluminum foils were removed and then petri dishes were subjected to the culture room conditions described above.

After determining the effects of TDZ, BA and dark incubation treatments, effects of BA and TDZ pre-treatments on callus formation and shoot regeneration were evaluated. In these pre-treatment experiments, the leaf explants were first incubated 1 h in a liquid containing different levels of BA (0, 2.2, 4.4, 6.6 and 8.8 µM) or TDZ (0, 0.23, 0.46, 1.14, and 2.28 µM), then leaves were wounded and transferred onto the regeneration medium consisting of WPM, 30 g/l sucrose, 7 g/l agar-agar, 0.54 µM NAA and either 10 µM BA (Table 5) or 9.08 µM TDZ (Table 6).

Regenerates were excised from leaves and rooted on MS medium containing 9.08 µM IBA. The plantlets about 3-4 cm in size were transferred to pots (8 × 8 cm) containing a potting mix of peat moss and perlite (1:1) and were acclimatized by covering pots with a transparent lid to provide humidity for the first week. After a month of acclimation in culture room, the plantlets were transferred to greenhouse conditions.

The experiments were conducted in a completely randomized design. Each treatment had three replicates (petri dishes) and each petri dish had ten leaf explants. Only calli larger than 3 mm in diameter were included in data collection. Frequency data (callus formation) was transformed using Arc Sin square root transformation before statistical analysis. Data were analyzed using ANOVA and means were separated by LSD test using SAS (SAS 9.1, SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

In the continuous light regime, the effect of BA treatments on regeneration, callus formation and

Table 5. Effects of benzyladenine (BA) pre-treatments on regeneration and callus formation from 'F12/1' (*Prunus avium* L.) rootstock on Woody Plant Medium (WPM) containing 10 µM BA.

BA pre-treatment (µM)	Callus formation (%)	No. of callus colony[y]
0	12.5ab[z]	0.12a
2.2	10.0b	0.06a
4.4	6.6ab	0.16a
6.6	16.6a	0.20a
8.8	12.5ab	0.12a

[z] Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y] Calli smaller than 3 mm in diameter were not included

Table 6. Effects of thidiazuron (TDZ) pre-treatments on regeneration and callus formation from 'F12/1' (*Prunus avium* L.) rootstock on Woody Plant Medium (WPM) containing 9.08 µM TDZ.

TDZ pre-treatment (µM)	Callus formation (%)	No. of callus colony[y]
0	35.0d[z]	0.67c
0.23	47.5cd	0.92bc
0.46	62.5bc	1.32b
1.14	72.5ab	1.27b
2.28	82.5a	2.17a

[z] Different letters in the same column represent significant differences at $P \leq 0.05$ by LSD test
[y] Calli smaller than 3 mm in diameter were not included

number of callus colony were significant (Table 1). Only explants cultured with 10 µM BA regenerated shoots and none of the other levels of BA induced shoot formation under light conditions (Fig.1 a-b). The highest callus formation frequency and callus colony numbers were obtained from 1.25 and 2.5 µM BA treatments (Table 1).

In the dark-incubated explants, the effects of BA treatments on regeneration, callus formation, and number of callus colony were significant (Table 2). Shoot regeneration was observed only from 2.5 µM BA (5%) treatment. All BA concentrations had higher callus formation frequencies than control treatment. Compared to the non-dark incubated explants (Table 1), dark incubated explants had higher callus formation frequencies and higher callus colony numbers at mid- and high levels of BA (Table 2).

Thidiazuron significantly affected regeneration and callus formation frequency and number of callus colonies in non-dark incubated cultures. Regeneration was obtained only from 9.08 µM TDZ concentration at 5% (Fig.1 c). None of the other concentrations induced regeneration. Callus formation rate and number of callus colonies increased as the TDZ concentration increased. The highest callus formation rate and the highest callus colony formation were obtained from the 9.08 µM TDZ treatment (Table 3). Thidiazuron significantly affected the callus formation frequencies and the callus colony numbers in dark incubated cultures, but no regeneration was obtained in dark incubation experiments. The highest callus formation frequency was obtained at 4.54 µM TDZ and the highest callus colony number was obtained from 2.27 µM TDZ treatment (Table 4).

In the current study, there were no differences between BA and TDZ treated explants with regard to shoot regeneration frequencies. BA and TDZ were equally effective in promoting shoot regeneration in *Prunus* species (Yang and Schmidt, 7). There are reports that TDZ was more effective than BA in promoting shoot regeneration in *Prunus* species (Hammatt and Grant, 3; Ainsley *et al.*, 1; Canli and Tian, 2). Quite the reverse, BA was more effective than TDZ (Tang *et al*., 5) in inducing regeneration. Therefore, the effect of TDZ is specific to genotype and explant.

Although dark incubated leaves did not produce higher regeneration frequencies than non-dark incubated ones, dark treatments significantly improved the callus formation efficiency from leaves of 'F12/1' rootstock in mid and high levels of BA (Table 1 versus Table 2) and in most TDZ levels (Table 3 versus Table 4). Similarly, callus formation and/or shoot regeneration of cotyledon explants were promoted by dark incubation in *P. dulcis* (Ainsley *et al.*, 1) and *P. avium* (Canli and Tian, 2).

The effects of BA pre-treatment on callus formation and callus colony numbers were not significant and none of the BA pre-treatments were effective. No regeneration was obtained in the BA pre-treatment experiment (Table 5). TDZ pre-treatments significantly increased callus formation rates and callus colony numbers when compared to control, but no regeneration was obtained in TDZ pre-treatment experiment (Table 6). The callus formation rate and callus colony numbers increased as the TDZ concentration was increased. The highest callus formation rates and callus colony numbers were obtained from the highest level of TDZ pre-treatment (Table 6). Regenerates were successfully rooted with IBA (Fig.1 d), acclimatized (Fig.1 e), and transferred to greenhouse conditions (Fig.1 f).

Although TDZ pre-treatments did not enhance regeneration frequencies, the callus formation efficiency of explants was increased by TDZ pre-treatments in this study (Table 6). Similarly, TDZ

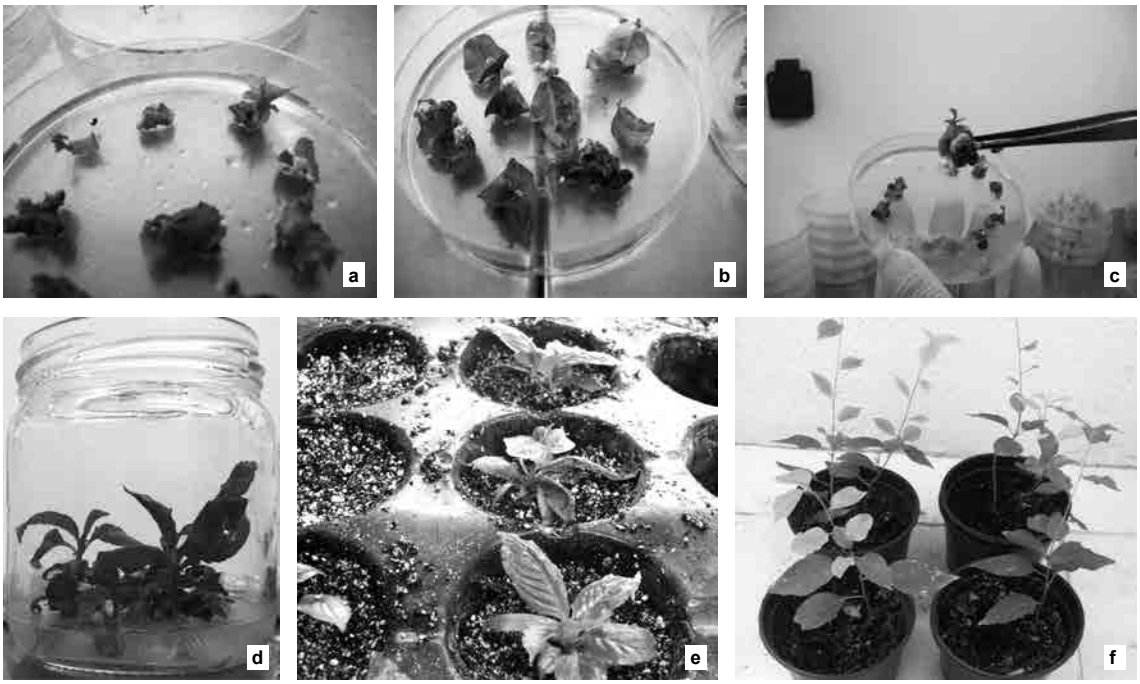

Fig. 1. Regeneration of adventitious shoots from leaf segments of 'F12/1' (*Prunus avium* L.) rootstock; (A-B) Shoot regeneration from leaves at 10 µM BA; (C) Appearance of adventitious shoots from leaves at 9.08 µM TDZ; (D) Rooted plantlets; (E) Acclimatization of regenerates; (F) Acclimatized 'F12/1' rootstock plantlets transfer to pots.

pre-treated explants did not yield higher regeneration frequencies than the controls in sweet cherry (Canli and Tian, 2). Regeneration studies in *Prunus* species showed that there is no such communal explant type or protocol, which could be effectively used for all *Prunus* species and success of regeneration is largely reliant on explant type and genotype in these species (Canli and Tian, 2).

In conclusion, regeneration protocols are pre-requirements for the improvement of known varieties via genetic transformation. The shoot regeneration protocol developed for 'F12/1' rootstock may provide useful and important information towards the development of its genetic transformation systems.

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Received : September, 2014; Revised : October, 2015;
Accepted : November, 2015

DOI : 10.5958/0974-0112.2016.00031.1

Indian J. Hort. 73(1), March 2016: 124-127

Short communication

Distribution of *Citrus tristeza virus* in the Darjeeling hills and their biological symptoms in mandarin orchards

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ABSTRACT

Mandarin (*Citrus reticulata* Blanco), a traditional fruit crop growing at altitude from 500 to 1500 m in the Darjeeling hills of Northeastern Himalayan regions of India. *Citrus tristeza virus* (CTV), member of a phloem limited *Closterovirus* and transmitted by brown citrus aphid, is one of the important virus causing losses in mandarin orchard in the Darjeeling hills. A survey was conducted in mandarin orchards of 30 locations covering all the Taluk/block in this region during April-July, 2011 and biological symptoms, incidence and distribution of CTV were studied. A general decline symptom along with chlorosis, poor as well as growth stunting was recorded observed in majority of the mandarin orchards. ELISA showed that mandarin samples of 29 locations were CTV positive showing considerable virus titer (OD value) of 3-10-fold compared to healthy control. The ELISA positive samples were further confirmed by RT-PCR. The present study concluded that CTV is widely distributed in all the mandarin growing areas in the Darjeeling hills and CTV isolates occurring in this region are declining inducing isolates.

Key words: *Citrus tristeza virus*, *C. reticulata*, Darjeeling hills, disease incidence, ELISA, RT-PCR.

Mandarin (*Citrus reticulata* Blanco), a traditional fruit crop growing in the Darjeeling hills of Northeastern Himalayan region of India, plays an pivotal role in the economy of the poor farmers of this hilly regions. Mandarin is cultivated in the area located at altitude from 500 to 1500 Mt which receives an annual rainfall of 2500-3000 mm. Because of the poor investment in the cultivation and maintenance, and affect of several biotic and abiotic factors, the mandarin orchards are gradually being wiped out, resulting in drastic reduction of fruit production in this area (Mukhopadhyay *et al*., 12; Ahlawat and Raychaudhuri, 2; Biswas *et al*., 4). *Citrus tristeza virus* (CTV), a phloem limited, aphid (*Toxoptera citiricidus*) transmitted *Closterovirus*, is one of the important factors causing decline of mandarin in the Darjeeling hills (Ahlawat and Raychaudhuri, 2; Chakraborty *et al*., 11; Biswas, 4). CTV is a destructive virus causing decline and death of about 100 million citrus trees worldwide including more than one million trees in India (Bar-Joseph and Dawson, 3; Ahlawat, 1). CTV contains long flexuous filamentous particles of 2000 x 11 nm in dimension and ssRNA molecules of 19.3 kb size (Bar-Joseph and Dawson, 3; Biswas *et al*., 7).

Citrus is cultivated almost in all the citrus-growing geographical regions of India, Central, South, Northwest and Northeast and CTV infects most of the cultivated citrus species (Ahlawat 1; Biswas, 4), with CTV incidence of 26.3% in Vidarbha (Central India), 47.1-56.0% in Northeast (Assam, Meghalaya, Sikkim and the Darjeeling hills), 36-50% in South (Andhra Pradesh and Karnataka) and 16-60% in North-Northwest (Uttarakhand, Delhi and Punjab) (Biswas *et al*., 9).

The virus in this region has been characterized based on biological reaction and sequencing of viral genome, and several CTV variants have been reported from Northeast regions of India (Biswas, 4; Biswas, 6; Biswas *et al*., 7,8; Tarafdar *et al*., 13). Biological host range and complete genome analysis revealed that CTV in the Darjeeling hills is likely to be decline inducing virus (Biswas *et al*., 8).

Although, occurrence of CTV has been reported and many virus isolates has been characterized, distribution of this disease in all the blocks (Taluk) under three subdivisions, Kalimpong, Karseong and Darjeeling in the Darjeeling hills has not been determined properly. Therefore, effort has been made in the present study to for an extensive survey of mandarin orchards covering all the blocks in this hill regions and subsequently, CTV has been diagnosed exploiting direct antibody coated indirect-ELISA (DAC-ELISA) using specific antisera and reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primers for the purpose of identification of disease-free mother stock for further development of healthy planting materials for the hill regions.

Surveys were conducted in mandarin orchards of 30 locations covering all the blocks (Taluk) in the

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Darjeeling hills during the month of April to July, 2011. About 3-11 samples (twigs of the tree), from each location were randomly collected and brought to laboratory for diagnostic assay. Virus crude extracts were prepared in extraction buffer at ratio of 1:10 (w/v) macerating 500 mg of tender bark tissues of citrus samples accordingly the method described earlier (Clark and Bar-Joseph, 10). DAC-ELISA using CTV specific antisera and measuring serological colour reaction was carried out using the protocol used earlier (Biswas, 4). Samples of healthy and CTV infected mandarin trees maintained in insect-proof greenhouse were used as negative and positive control, respectively. The samples were considered to be positive if the absorbance value in ELISA reader was at least two times more than that of the value of negative control. For RT-PCR, isolation of total plant RNA and synthesis of cDNA was carried out using the protocol used earlier (Biswas, 4, 6). The PCR was performed using the method described by Biswas (6) using specific primer pair KLM 543 and KLM544 for amplification of complete CP gene of CTV genome.

The biological reactions of Indian CTV isolates in Darjeeling hills were recorded and per cent plant infection was estimated (Table 1). In the present study, highest disease incidence was observed

Table 1. Disease incidence and general symptoms caused by *Citrus tristeza virus* in mandarin orchards in different locations of the Darjeeling hills.

Area			No. tree infected/ No. tree tested through ELISA	OD in ELISA (x fold)	RT-PCR positive	Symptoms
Sub-division	Block/Taluk	Location				
Kalimpong	Gorubathan	Upper Gorubathan	3/6	1.12-1.53 (4-5)	Yes	Decline and poor growth
		Damlin	6/10	1.34-2.12 (4-7)		
		Pate Gaon	3/6	1.31-1.89 (4-6)		
	Kalimpong	IARI-RS, Kalimpong	2/5	2.25-2.93 (8-10)	Yes	Decline, chlorosis, stunting, poor growth
		Reily road	7/11	1.15-2.54 (4-8)		
		Icchay Basti	10/10	1.55-2.45 (5-8)		
		Chibbo Basti	9/11	1.11-1.89 (4-6)		
	Algarah	Pedong	4/5	1.55-2.03 (5-7)	Yes	Decline, poor growth, chlorosis, stunting
		Sakyong	3/7	0.98-1.57(3-5)		
		13th mile	4/7	0.85-2.13 (3-7)		
Darjeeling	Takdah	Lower Glenburn	5/5	1.01-1.07 (3)	Yes	Decline and chlorosis
		Glenburn	2/3	1.06-1.21 (3-4)		
		Bazaar Gaon	0/3	0.41-0.48 (<1)		
		Singrintam-1	2/3	1.18-1.49 (4-5)		
		Singrintam-2	3/4	1.21- 1.52 (4-5)		
		Takling	4/6	1.59-2.43 (4-5)		
		Soreng	6/6	1.23-2.08 (4-9)		
		Mangowa	3/4	1.53-1.87 (5-6)		
	Bijanbari	Upper Rondok Basti	6/9	1.24-1.62 (4-5)		Decline, chlorosis, stunting
		Lower Rondok Basti	4/7	1.08- 1.31 (3-4)		
		Ging TE	10/10	1.82-2.85 (6- 9)	Yes	
		Lebong	3/7	0.98-1.23 (3-4)		
	Sukhia-pokhari	Upper Sukiapokhri	3/6	1.28-1.39 (4-5)	Yes	Poor growth, chlorosis, stunting
		Magarjung	3/7	0.87-1.66 (3-6)		
Karseong	Mirik	Upper Mirik	6/9	0.80-1.81 (3-6)	Yes	Poor growth, chlorosis, stunting
		Soureni	5/8	0.83-1.56 (3-5)		
	Karseong	Lower Monpu	3/4	1.95-2.90 (6-9)		Poor growth, chlorosis, stunting

Average OD values of positive, healthy and buffer control are 2.92, 0.32 and 0.28 respectively. OD Value taken at 405 nm; x fold titer values were calculated compared with the OD values of infected with healthy control

in Kalimpong taluk, where the mandarin orchards were found to be mosty affected by CTV showing symptoms including prominent chlorosis, poor growth and stunting of plant. A comparatively lesser disease infection was observed in the mandarin orchards of Darjeeling taluk during the period of survey. The prevalence of aphid vector, *T. citricidus* was observed in many mandarin orchards, particularly in the areas located at lower altitude. Occurrence of the aphid vector is common in the mandarin orchards, with an exception in a few areas located at higher altitude above 1,500 m in the Darjeeling hills (Mukhopadyay *et al*., 12; Biswas, 4). The *T. citricidus* transmits CTV efficiently to citrus species in the Darjeeling hills (Biswas, 4).

In the present study, ELISA results showed that mandarin samples of 29 locations out of total 30 locations were CTV positive with considerably higher virus titer (OD value). Only samples from Bazaar Gaon were found to be ELISA negative but the orchards in these regions are truly free from CTV infection is yet to be confirmed by further studies and analysis. Infected citrus samples showed different titer values ranging from 3 to 10-folds compared to healthy control in ELISA reader (Table 1). The virus infection detected by ELISA was further confirmed by RT-PCR. Total number of seven ELISA positive citrus samples was randomly tested and all of them were found to be RT-PCR positive amplifying desired size of 672 nucleotide sequence band of CP gene from the CTV genome (Fig. 1). The present results showed that per cent tree infection in some orchards were high, for instance, all the test samples from orchards of Lower Glenburn and Soreng in Takda block, Icchay Basty in Kalimpong block and Ging TE in Bijanbari block were found to be CTV positive. The previous (Biswas, 4) and present studies concluded that CTV is widely distributed in all the mandarin growing areas of eight blocks in the Darjeeling hills.

In the present study it was observed that CTV causes a general decline symptom along with chlorosis, poor growth and stunted growth of the mandarin tree in the majority of the orchards in the Darjeeling hills. Previously, Indian isolate Kpg3 has been described earlier to be a declining inducing CTV isolate in the Darjeeling hills (Biswas *et al*., 7). In the present study, stem pitting symptoms was not observed in any of the orchard in this region surveyed. Therefore, based on symptomatology, the previous (Biswas *et al*., 7) and the present study concluded that CTV isolates infecting mandarin orchards in the Darjeeling hills are declining inducing isolates.

Mandarin orchards in the Darjeeling hills are not receiving adequate scientific attention for proper rejuvenation and expansion, thus the total area under mandarin cultivation that was estimated to be 4,000 acres during the year 1984-85 (Mukhopadhyay *et al*., 12) remains same or reduced in the present condition (personal communication). Replanting of orchards has not been taken place properly resulting in appearance of age-old diseased trees in majorities of the orchards. Mandarin orchards in the Darjeeling hills appeared to be declining during 1960s and several orchards started to be wiped out during the year of 1980s causing enormous losses to the growers. Use of planting material without proper seed certification and then locally transmission of this disease through aphid vectors are the main reason for wide distribution of CTV in this hill region. Therefore, use of virus free planting materials and control of insect vectors are

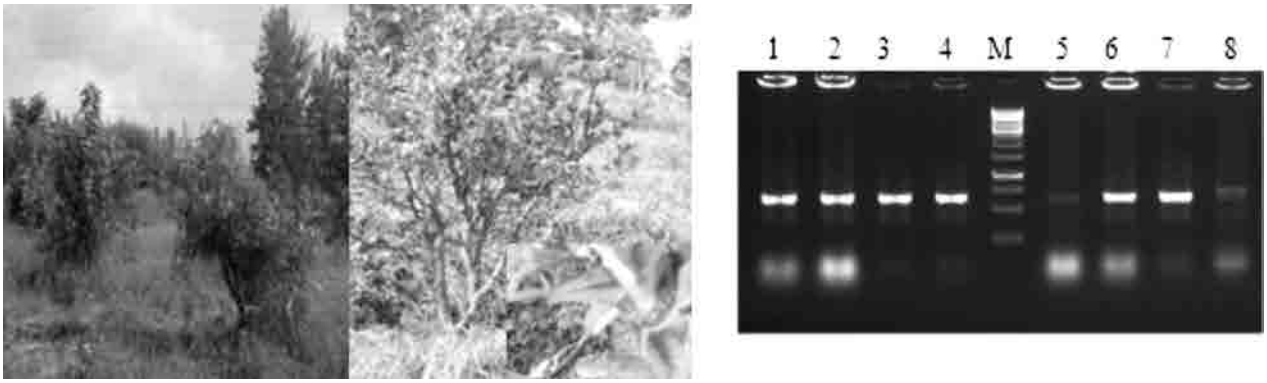

Fig. 1. a. Mandarin orchard showing decline symptoms with chlorosis and poor growth in the Darjeeling hills; b. Gel electrophoresis analysis showing RT-PCR amplification of CP gene of CTV genome of different citrus samples, Lane M: 1 kb DNA ladder, lane 1 = CTV infected green house mandarin plant as positive control, lane 2 = sample of Gorubathan, 3 = IARI-RS, Kalimpong, 4 = Pedong, 5 = Lower Glenburn, 6 = Ging TE, 7 = Upper Mirik and 8 = Upper Sukhiapokhri.

essential that would keep citrus industry more viable and profitable. A strategy for production of CTV free mandarin planting materials for the Darjeeling hill have been developed (Biswas *et al*., 5). The present study focused on the use of bud wood certification program and thereby production and supply of CTV-free planting materials, which are the universally recognized method for replenishing of the declined citrus orchards.

ACKNOWLEDGEMENTS

The authors duly acknowledge Dr R.K. Jain, former Head, Division Plant Pathology, and Joint Director, IARI for providing facilities. Authors are thankful to Mr. Ramu Meena, Technical Assistant, IARI-RS Kalimpong and Mr. B. Chettri, Kalimpong Krishak Kalyan Sanghathan, Kalimpong.

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Received : April, 2013; Revised : October, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00032.3

Indian J. Hort. 73(1), March 2016: 128-132

Short communication

Integrated nutrient management in *ber* (*Zizyphus mauritiana* Lamk.) cv. Gola under Malwa Plateau conditions of Madhya Pradesh

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ABSTRACT

A field experiment was carried out with different treatment combinations of organic manures (FYM @ 53, 39.75 and 26.5 kg, vermicompost @ 26.5, 19.86 and 13.25 kg), inorganic fertilizers (full dose of recommended fertilizer, 50% dose of recommended fertilizer and 25% dose of recommended fertilizer) and bio-fertilizers (*Azotobacter* and PSB) on five-year-old ber trees cv. Gola under Malwa plateau conditions. The results revealed that the application of 50% recommended dose of fertilizer through vermicompost + 50% RDF through NPK + 50 g *Azotobacter* + 50 g PSB (T_7) significantly increased the plant height (2.43 m), canopy volume (9.56), number of primary (16.93) and secondary branches (24.92) per shoot, fruit set (7.30%) and fruit retention (40.95%), fruit length (3.58 cm) and diameter (3.31 cm), fruit volume (22.25 ml), pulp weight (20.06 g), stone weight (1.91 g), average fruit weight (21.97 g), yield (34.14 kg/tree), TSS (20.85°Brix), ascorbic acid (74.04 mg/100 g pulp), reducing sugar (5.15%), non-reducing sugars (4.74%), total sugars (9.89%), TSS/acid ratio (160.38) and chlorophyll content in leaves spad value (71.00).

Key words: *Ber*, integrated nutrient management, quality, yield.

Ber (*Zizyphus mauritiana* Lamk.), the poor man's apple, is an important drought hardy fruit crop, which can be grown under hostile agro-climatic conditions of the arid region. Since it is hardy and salt tolerant, the tree can be grown even in marginal lands. *Ber* grows in wild and cultivated forms in India mostly in the marginal soils. To achieve the better yield of good fruit quality, INM plays a significant role in maintaining soil fertility and plant nutrient supply to optimum level. Vermicompost has been advocated as good source of organic manures for use in integrated nutrient management practices of fruit crops. Objectives of integrated plant nutrient management are to reduce inorganic fertilizer requirement, to restore the organic matter in soil and to increase nutrient use efficiency, to maintain quality in terms of physical, chemical and biological properties of soil, to maintain the nutrient balance between the supplied nutrient and nutrient removed by plant and to improve soil health and productivity on sustainable basis. In current scenario of organic agriculture, bio-fertilizers, more commonly known as microbial inoculants are choice of the farmer's because of its impact on soil fertility and crop productivity. Bio-fertilizers not only provide growth promoting activity to the plant by enhancing the nutrient uptake but also provide strength against soil borne diseases. The role of nutrient elements either alone or in combination with other sources (organic manures/ fertilizers) has been well established in many fruit crops; while such studies are very meagrely available in *ber*. Therefore, present investigation was undertaken to evaluate the effect of integrated plant nutrient management in *ber*.

A field experiment was conducted during 2012-13 at Department of Fruit Science, College of Horticulture, Mandsaur (M.P.) on five-year-old trees of *ber* cv. Gola. Initial properties of the soil were pH-7.5, EC-0.33, available nitrogen (207 kg/ha), available P_2O_5 (9.90 kg/ha) and available K_2O (784.0 kg/ha). Three levels of inorganic and organic sources of NPK *i.e.*, 424 : 315 : 327 g NPK (100% NPK), 212 : 157.5 : 163.5 g NPK (50% NPK) and 106 : 78.7 : 81.7 g NPK (25% NPK) ; three levels of FYM *i.e.*, 53 kg FYM (100% FYM), 39.7 kg FYM (75% FYM) and 26.5 kg FYM (50% FYM) and three levels of vermicompost, *i.e.*, 26.5 kg vermicompost (100% vermicompost), 19.8 kg vermicompost (75% vermicompost) and 13.25 kg vermicompost (50% vermicompost), *Azotobacter* and PSB inoculation (50 g each) were employed in the ber tree having uniform growth and vigor while the control plants received no fertilizer, inoculation and manure treatment.

The experiment was laid out in randomized block design with three replications. The full dose of FYM and vermicompost were applied at onset of monsoon. Then required doses of fertilizers were applied in two split doses in the month of July and August and then bio-fertilizers were applied one week after each

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application of inorganic fertilizer. The observation on plant height, canopy volume, number of primary and secondary branches, fruit set, fruit retention, fruit drop, length and diameter of fruit, fruit volume, pulp thickness, stone weight, pulp weight, average fruit weight, yield/kg, acidity, TSS, TSS/acid ratio, sugars (total, reducing and non-reducing), ascorbic acid in fruits and chlorophyll content in leaves were recorded. All of the data were analysed using one-way ANOVA tests for means comparisons, with standard errors. Data on fruit quality and nutritional analyses are reported as means of the three harvest times.

It is evident from the data presented (Table 1) that the application of nutrient including 50% RDF through VC + 50% RDF through NPK + PSB + *Azotobacter* (T_7) increased plant height, canopy volume, number of primary and secondary branches on selected shoot, fruit set , fruit retention and reduced the fruit drop as compared to remaining treatments in *ber*. The significant improvement in plant growth on account of vermicompost application along with inorganic sources of NPK and biofertilizers might have attributed due to the translocation of nutrients from soil and enhanced supply of nutrients during entire growth seasons and microbial decomposition. The increase in tree height, canopy volume, number of shoot emergence per branch could be attributed to the simulative activity of microflora in the rhizosphere leading to increased nutrient availability and hence vigorous plant growth. These findings are in accordance with the results given by Aseri *et al.* (1). The application of biofertilizers also helps the plants to increase the dehydrogenase, alkaline phosphatase, nitrogenase and hydrolysis enzyme activities mainly due to increase in the rhizosphere microbial population as a consequence of the inoculation treatments. The free living N_2 fixer can affect plant growth not only by fixing N_2 but also by altering microbial balance, solubilizing fixed soil phosphorus, suppressing pathogenic microorganisms and by producing metabolites that stimulate plant development. The highest fruit set and retention might be due to supply of nutrients in adequate proportion right from starting of the experimentation to the harvesting of crop, which induces the more flowering and retention of fruit due to production and supply of photosynthates at critical requirement. The results are also in close conformity with the findings of Goswami *et al*. (4), in guava and Singh *et al.* (7), in *ber*.

The result obtained in present investigation (Table 2) revealed that application of 50% RDF through vermicompost + 50% RDF through NPK + PSB + *Azotobacter* (T_7) was found best treatment as compared to T_0, T_2, T_3, T_8, T_9, T_{10} and T_{11} and

Table 1. Effect of integrated nutrient treatments on growth and reproductive parameters of *ber*.

Treatment		Plant height (m)	Canopy vol. (m^3)	Leaf length (cm)	Leaf width (cm)	No. of primary branches	No. of secondary branches	Fruit set (%)	Fruit drop (%)	Fruit retention (%)
Control	T_0	1.35	1.90	0.48	0.40	12.42	10.42	5.44	62.50	28.72
100% RDF through NPK	T_1	2.07	5.62	1.25	1.10	15.00	20.08	6.32	55.61	36.63
100% RDF through FYM	T_2	1.53	2.82	0.51	0.68	13.83	13.92	5.49	61.42	30.31
100% RDF through VC	T_3	1.71	3.50	0.76	0.75	13.77	15.50	5.78	60.44	32.42
50% RDF through FYM + 50% RDF through NPK	T_4	2.08	5.79	1.30	1.15	15.08	22.80	6.39	55.27	37.16
50% RDF through VC + 50% RDF through NPK	T_5	2.16	6.65	1.40	1.20	15.50	23.33	6.68	52.26	38.47
50% RDF through FYM + 50% RDF through NPK + PSB + *Azotobacter*	T_6	2.30	8.65	1.65	1.30	16.00	23.70	7.08	51.32	40.47
50% RDF through VC + 50% RDF through NPK + PSB + *Azotobacter*	T_7	2.43	9.56	1.70	1.40	16.93	24.92	7.30	50.72	40.95
75% RDF through FYM + 25% RDF through NPK	T_8	1.72	3.56	0.80	0.80	14.17	17.92	5.90	59.92	32.11
75% RDF through VC + 25% RDF through NPK	T_9	1.82	4.08	0.84	0.90	14.25	18.50	5.96	58.55	33.12
75% RDF through FYM + 25% RDF through NPK + PSB + *Azotobacter*	T_{10}	1.88	4.62	1.01	0.95	14.50	19.25	6.24	56.84	34.72
75% RDF through VC + 25% RDF through NPK + PSB + *Azotobacter*	T_{11}	1.98	5.03	1.10	1.00	14.95	19.75	6.27	56.48	35.15
CD at 5%		0.39	0.55	0.49	0.54	1.79	1.56	1.05	2.51	5.24

(The data given for plant height, canopy volume, leaf length and width are the increment during investigation period)

Table 2. Effect of integrated nutrient treatments on physical and yield attributes of *ber* at harvest.

Treatment		Fruit length (cm)	Fruit diameter (cm)	Average fruit weight (g)	Fruit volume (ml)	Pulp thickness (cm)	Stone weight (g)	Pulp weight (g)	No. of fruit/tree	Yield per tree (kg)
Control	T_0	3.26	2.91	15.03	15.83	0.90	1.29	13.74	1520	20.20
100% RDF through NPK	T_1	3.50	3.17	19.51	19.83	1.10	1.48	18.03	1531.67	30.15
100% RDF through FYM	T_2	3.38	2.91	16.40	17.21	0.93	1.34	15.06	1496.67	23.99
100% RDF through VC	T_3	3.38	2.93	16.72	17.58	0.95	1.37	15.35	1504	25.92
50% RDF through FYM + 50% RDF through NPK	T_4	3.51	3.21	19.88	20.17	1.13	1.57	18.31	1541.67	30.99
50% RDF through VC + 50% RDF through NPK	T_5	3.55	3.22	20.53	20.83	1.14	1.59	18.94	1553.33	31.29
50% RDF through FYM + 50% RDF through NPK + PSB + *Azotobacter*	T_6	3.55	3.23	21.40	21.83	1.17	1.65	19.75	1561.67	33.96
50% RDF through VC + 50% RDF through NPK + PSB + *Azotobacter*	T_7	3.58	3.31	21.97	22.25	1.21	1.91	20.06	1571.67	34.14
75% RDF through FYM + 25% RDF through NPK	T_8	3.39	3.02	17.76	18.33	0.99	1.40	16.36	1508.67	27.99
75% RDF through VC + 25% RDF through NPK	T_9	3.41	3.04	18.13	18.54	1.01	1.42	16.71	1513.33	28.47
75% RDF through FYM + 25% RDF through NPK + PSB + *Azotobacter*	T_{10}	3.41	3.08	18.54	19.25	1.01	1.43	17.11	1515.67	29.35
75% RDF through VC + 25% RDF through NPK + PSB + *Azotobacter*	T_{11}	3.42	3.08	19.20	19.58	1.06	1.45	17.75	1521.67	29.59
CD at 5%		0.10	0.18	2.63	1.51	0.04	0.06	1.79	16.18	2.59

VC = Vermicompost; RDF = Recommended dose of fertilizers

statistically at par with T_6, T_5, T_4 and T_1 with respect to physical characteristics and yield parameters of ber fruit. The increase in fruit size (length and width), weight and volume during the investigation period might be due to the increased photosynthetic ability of plants supplied with *Azotobacter* + vermicompost which in turn might have favoured and increased the accumulation of dry matter. Fruit size, weight and volume are highly correlated with dry matter content and balanced level of hormone. Nitrogen fixers are known for accumulation of dry matter and their translocation as well as favours synthesis of different growth regulators (Awasthi *et al.*, 3). The enhancement in yield by these treatments was mainly due to proper supply of nutrients and induction of growth hormones, which stimulated cell division, cell elongation leads to increase in number and weight of the fruits, better root development, and better translocation of water and deposition of nutrients. These findings are in accordance with the Singh *et al.* (6) in *aonla*.

The qualitative parameters of ber fruit were affected by different treatments (Table 3). The results obtained from the study revealed the maximum total soluble solids, TSS/acid ratio, sugars, ascorbic acid in fruits, chlorophyll content in leaves and minimum acidity in fruits with the application of T_7 (50% RDF through vermicompost + 50% RDF through NPK + PSB + *Azotobacter*), which was at par with T_6, T_5, T_4 and T_1. The increased fruit quality may be explained from the fact that the different sources of nutrients enhance the nutrient availability by enhancing the capability of plants for better uptake of nutrients from rhizosphere. These results are in conformity with the findings as reported by Korwar *et al.* (5), Singh *et al*. (6), and Athani *et al.* (2). The decrease in acidity of fruits may be attributed to their conversion into sugars and their derivatives by the reactions involving reversal of glycolytic pathway or might be used in respiration or both. An increase in TSS and total sugars contents with *Azotobacter* and vermicompost application may be attributed due to the quick metabolic transformation of starch and pectin into soluble compounds and rapid translocation of sugars from leaves to the developing fruits, conversion of complex polysaccharides into simple sugars. These findings are in agreement with the result of Athani *et al.* (2) in guava. The maximum amount of ascorbic acid content was recorded in fruits produced from the plants fertilized with vermicompost + NPK + *Azotobacter* + PSB this result got the support with the findings of Yadav *et al.* (8) in strawberry. The respective increase in ascorbic acid content might be due to the increased efficiency of microbial inoculants

Table 3. Effect of integrated nutrient treatments on chemical composition of *ber*.

Treatment		Acidity (%)	TSS (°Brix)	TSS/ acid ratio	Total sugars (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ascorbic acid content (mg/100 g pulp)	Chlorophyll content (Spad value)
Control	T_0	0.22	14.92	67.82	7.95	4.45	3.50	64.57	54.76
100% RDF through NPK	T_1	0.15	20.00	133.33	9.35	4.89	4.46	70.23	63.47
100% RDF through FYM	T_2	0.22	15.33	69.68	8.60	4.62	3.98	65.25	55.38
100% RDF through VC	T_3	0.19	16.54	87.05	8.65	4.82	3.83	68.04	58.28
50% RDF through FYM + 50% RDF through NPK	T_4	0.15	20.00	133.33	9.03	4.95	4.09	72.05	64.30
50% RDF through VC + 50% RDF through NPK	T_5	0.14	20.02	143.00	9.47	4.99	4.48	72.29	65.01
50% RDF through FYM + 50% RDF through NPK + PSB + *Azotobacter*	T_6	0.14	20.17	144.07	9.56	5.06	4.49	73.32	68.29
50% RDF through VC + 50% RDF through NPK + PSB + *Azotobacter*	T_7	0.13	20.85	160.38	9.89	5.15	4.74	74.04	71.00
75% RDF through FYM + 25% RDF through NPK	T_8	0.17	16.88	99.29	8.69	4.71	3.98	68.99	58.23
75% RDF through VC + 25% RDF through NPK	T_9	0.16	18.00	112.50	8.79	4.79	3.99	69.48	60.97
75% RDF through FYM + 25% RDF through NPK + PSB + *Azotobacter*	T_{10}	0.16	18.83	117.69	8.99	4.99	4.00	69.99	61.45
75% RDF through VC + 25% RDF through NPK + PSB + *Azotobacter*	T_{11}	0.16	19.50	121.88	9.16	4.86	4.31	70.10	62.12
C.D. at 5%		0.04	2.02	9.68	0.29	0.37	0.34	3.73	4.84

VC = Vermicompost; RDF = Recommended dose of fertilizers

to fix atmospheric nitrogen, increase in availability of phosphorous and secretion of growth promoting substances which accelerates the physiological process like carbohydrates synthesis, etc.

A treatment adjudged effective technically might not be economical if costs are more than benefits obtained. Therefore, economic analysis is the ultimate yardstick to recommend a technology. The economics worked out for this experiment indicated (Table 4) that maximum net income was obtained from application of 50% recommended dose of fertilizer through vermicompost + 50% RDF through NPK + 50 g *Azotobacter* + 50 g PSB (T_7) among all treatment. Although the benefit : cost ratio is at par with T5 and T1. The net income obtained from T1 (100% RDF through NPK) remained low when compared with T7 but the benefit : cost ratio is higher in T1. The benefit cost ratio of T1 is not consistent in successive years while vermicompost and FYM has long term effect in preceding crops, thus, the use of 50% recommended dose of fertilizer through vermicompost + 50% RDF through NPK + 50 g *Azotobacter* + 50 g PSB (T_7) proved the most economical treatment when adjudged through yardstick of BCR for long term effect.

Based on the above results, application of 50% RDF through vermicompost + 50% RDF through NPK + PSB + *Azotobacter* registered significantly higher plant growth, reproductive, yield and quality attributes in *ber*.

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Table 4. Economics of the different treatments (Rs. per ha.)

Treatment		Treatment cost	Treatment cost / ha	Total expenditure (ha)	Yield/ plant (kg)	Gross income	Net income	Benefit: Cost ratio
Control	T_0	0	0	17803.12	20.20	76356.0	58552.88	4.3
100% RDF through NPK	T_1	29.02	7835.40	25638.52	30.15	113967.0	88328.48	4.4
100% RDF through FYM	T_2	106.00	28620.00	46423.12	23.99	90682.2	44259.08	2.0
100% RDF through VC	T_3	79.50	21465.00	39268.12	25.92	97977.6	58709.48	2.5
50% RDF through FYM + 50% RDF through NPK	T_4	67.51	18227.70	36030.82	30.99	117142.2	81111.38	3.3
50% RDF through VC + 50% RDF through NPK	T_5	54.26	14650.20	32453.32	31.29	118276.2	85822.88	3.6
50% RDF through FYM + 50% RDF through NPK + PSB + *Azotobacter*	T_6	83.51	22547.70	40350.82	33.96	128368.8	88017.98	3.2
50% RDF through VC + 50% RDF through NPK + PSB + *Azotobacter*	T_7	70.26	18970.20	36773.32	34.14	129049.2	92275.88	3.5
75% RDF through FYM + 25% RDF through NPK	T_8	86.65	23395.50	41198.62	27.99	105802.2	64603.58	2.6
75% RDF through VC + 25% RDF through NPK	T_9	66.70	18009.00	35812.12	28.47	107616.6	71804.48	3.0
75% RDF through FYM + 25% RDF through NPK + PSB + *Azotobacter*	T_{10}	102.65	27715.50	45518.62	29.35	110943.0	65424.38	2.4
75% RDF through VC + 25% RDF through NPK +PSB+ *Azotobacter*	T_{11}	82.70	22329.00	40132.12	29.59	111850.2	71718.08	2.8

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Received : November, 2014; Revised : November, 2015; Accepted : December, 2016

DOI : 10.5958/0974-0112.2016.00033.5

Indian J. Hort. 73(1), March 2016: 133-136

Short communication

Identification of genic-SSR markers for diversity analyses in vegetable brassica *viz.-a-viz*. related species

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ABSTRACT

Genetic diversity in any crop species is critical for sustaining and thus continuing our efforts towards successful development of desirable varieties. Among the several methods available to assess diversity among germplasm, the SSR markers based DNA profiling is one of the most reliable approaches to assess differences across accessions or varieties unambiguously. In the present study we identified genic-SSR markers in the vegetable *Brassica* through transferability studies. The identified genic-SSR markers were also tested for their reproducibility across a panel of related but different *Brassica* species. The genic-SSR markers showing polymorphism across different *Brassica* species were employed in understanding relationship of vegetable *Brassicas* with that of both related and distant *Brassica* species.

Key words: Genic-SSR, diversity, vegetable *Brassica*.

Among *Brassica, the Brassica rugosa (Brassica juncea var. rugosa)* also known as cabbage leaf mustard is an important source of vegetable. The leaves and young stem are eaten raw as salad or cooked individually or mixed with other vegetables. In *Brassica* genetic enhancement effort, the important consideration of conservation of plant genetic resources and its utilization requires an understanding of genetic diversity both within and between *Brassica* species. The three indispensable components of human diets comprise starch, protein and oil and all of them being sourced from vegetable sources for large proportion of human population. *Brassica* comprises large group of plants among botanical families that supplies vegetables, oils, spice and condiments. All the available diversity in a plant species is an extremely important resources to support the program of crop improvement. Since all the diversity doesn't exist in a single individual of a particular plant species, we need a constant program of collection, evaluation, conservation and ultimately utilization of accessions (indigenous and exotic) in genetic enhancement of traits of economic importance. All the plant germplasm, collected at indigenous and exotic locations, serves as reservoir of entire spectrum of diversity. In the present study gene-based SSR markers (genic-SSR) were identified in the vegetable *Brassicas* through transferability approach. Additionally, we also studied the interrelation or relatedness of such vegetable *Brassicas* with that of other *Brassica* species.

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Altogether, 31 accessions of *Brassica* and related species were undertaken in this study (Table 1). We also included in our study three accessions of *Perilla*, an oilseed plant, as an outgroup. The genomic-DNA was isolated from germinating seedling by 2x CTAB method (Lukowitz *et al*., 3). Briefly, 100 mg of seedling tissue were crushed in chilled mortar & pestle. To the fine paste of sample added 500 µl of 2x CTAB and incubated for 15 min. at 65°C. The subsequent process followed the standard protocol, however, after ethanol (70%) precipitation, the pellet was dissolved in 150 µl of TE buffer and stored at 4°C. The thermal cycle for PCR amplification included touch-down method and the temperature programme was initial denaturation at 94°C for 5 min., five cycles of 30s at 94°C, 45s at 61°C with a 1°C decrease in annealing temperature per cycle and 1 min. at 72°C; 30 cycles at 30s at 94°C, 45s at 57°C and 1 min. at 72°C and final extension at 72°C for 10 min. (An *et al*., 1). The SSR markers undertaken in the current study were made available from publicly available sequence information, *i.e.* PlantGDB-assembled unique transcripts (PUTs) of *Brassica* species (An *et al*., 1). These SSR markers represent different functional alleles and therefore also designated as genic-SSR markers. Briefly, 1 µl of dissolved g-DNA was used as template in 20 µl of reaction volume containing 1U *Taq* DNA polymerase (Fermentas), 1x PCR buffer (Fermentas), 0.5 mM primers (IDT) and 0.2 mM of dNTP mix (Takara). The total volume was adjusted with nuclease free water (Gibco). The PCR products were mixed with 1x loading dye (Orange-G) and 5 µl of resultant samples were electrophoresed

Table 1. Details of *Brassica* species (the alphabet in capital indicates nature and ploidy of respective genome).

Species	Common name	Variety/ Accession	No.	Uses
Brassica rapa (syn. *B. campestris*) (A)	Brown sarson	Pusa Kalyani	1	Cooking oil, condiments, Spice
		KOS-1	2	
		TL-17	3	
		KS-101	4	
		HPSB-1	5	
	Yellow sarson	Jhumka	6	
		NRCYS-05-02	7	
		Pusa Gold	8	
		YSH-401	9	
		YST-151	10	
	Toria	TH-68	11	
		TL-15	12	
Brassica napus (AC)	Rapeseed/Gobhi sarson	GSC-5	13	Cooking oil, Biofuel
		Hyola-401	14	
		Teri Unnat	15	
		NUDB-26-11	16	
Brassica juncea (AB)	Indian mustard	Ashirwad	17	Cooking oil, condiments
		CS-52	18	
		CS-243-2	19	
Brassica carinata (BC)	Ethiopian mustard/ Karan rai	JTC-1	20	Biofuel
		Kiran	21	
		PC-5	22	
		Pusa swarnim	23	
Eruca sativa(E)	Garden rocket/ Taramira	Narendra Tara	24	Spice, condiments
		ITSA	25	
		PUT 93-1	26	
Sinapis alba (syn. *B. hirta*)	White mustard	IC-394639	27	Spice, condiments
Brassica rugosa (syn. *B. juncea*)	Cabbage leaf mustard	IC-340850	28	Vegetable
Perilla	Perilla	IC-557289	29	Biofuel
		IC-422911	30	
		IC-557307	31	
Brassica tournefortii (T)	Asian/African mustard	IC-493737	32	Cooking oil
		IC-493934	33	
Brassica nigra (B)	Black mustard	IC-426381	34	Spice, cooking oil

on 3% metaphor agarose gel at constant 80 V for 3 h. After completion of electrophoresis, the ethidium bromide stained gels were visualized under UV light in gel documentation system (Alpha-imager, USA). The molecular weight determination of amplified products was obtained with reference to standard molecular weight marker (Generuler, Fermantas). The data analysis was performed using Genealex 6.41(Peakall and Smouse, 4) and dendrogram was developed using PAST 2.71 (Hammer *et al*., 2).

Initially, we checked the transferability of genic-SSR markers towards all the *Brassica* species (Table 1). From analyses of 50 genic-SSR markers, 12 were able to exhibit consistently faithful amplification across the accessions of *Brassica* sp. undertaken in this study. Such result indicated ~25% of transferability

of markers across a spectrum of diverse *Brassica* sp. Although transferability rate as high as ~50% were also obtained but that was confined to only few, not all *Brassica* sp. included in the present study (data not shown). Since, we were interested in only those markers which could amplify products across all species, we pursued our study in that direction only. From such 12 markers, most of them failed to provide useful differences across all the species. However, three markers showed discernible differences among varieties and accessions of selected *Brassica* species. It is interesting to note that *Perilla*, which belongs to taxonomically different family, its three accessions faithfully amplified products as well. Such results reiterate the fact that genes remain under constant selection pressure and thus tend to maintain its functional integrity by not allowing changes to happen. The SSR markers used in the present study was developed from sequence information of expressed genes of *B. napus* and *B. rapa* (An *et al*., 1). Moreover, in contrast to SSR markers developed from non-genic regions, the SSR markers designed from sequence information of expressed gene also provides information with regards to functional diversity in allele, if any, in addition to its usefulness in DNA profiling, diversity analyses and candidate gene determination. Analyses of amplified products profile from three genic SSR markers (PUT-154, PUT-169 and PUT-256) (Fig. 1) readily provided useful information with regards to difference among most accessions/ varieties of each *Brassica* sp. and also across most of species.

It is very difficult to identify a single marker that could discriminate most of the varieties/ accessions of *Brassica* sp. However, the combination of markers, as revealed from Fig. 1 did provide unambiguous differences among the accessions of *Brassica* species. For example, the results obtained with three

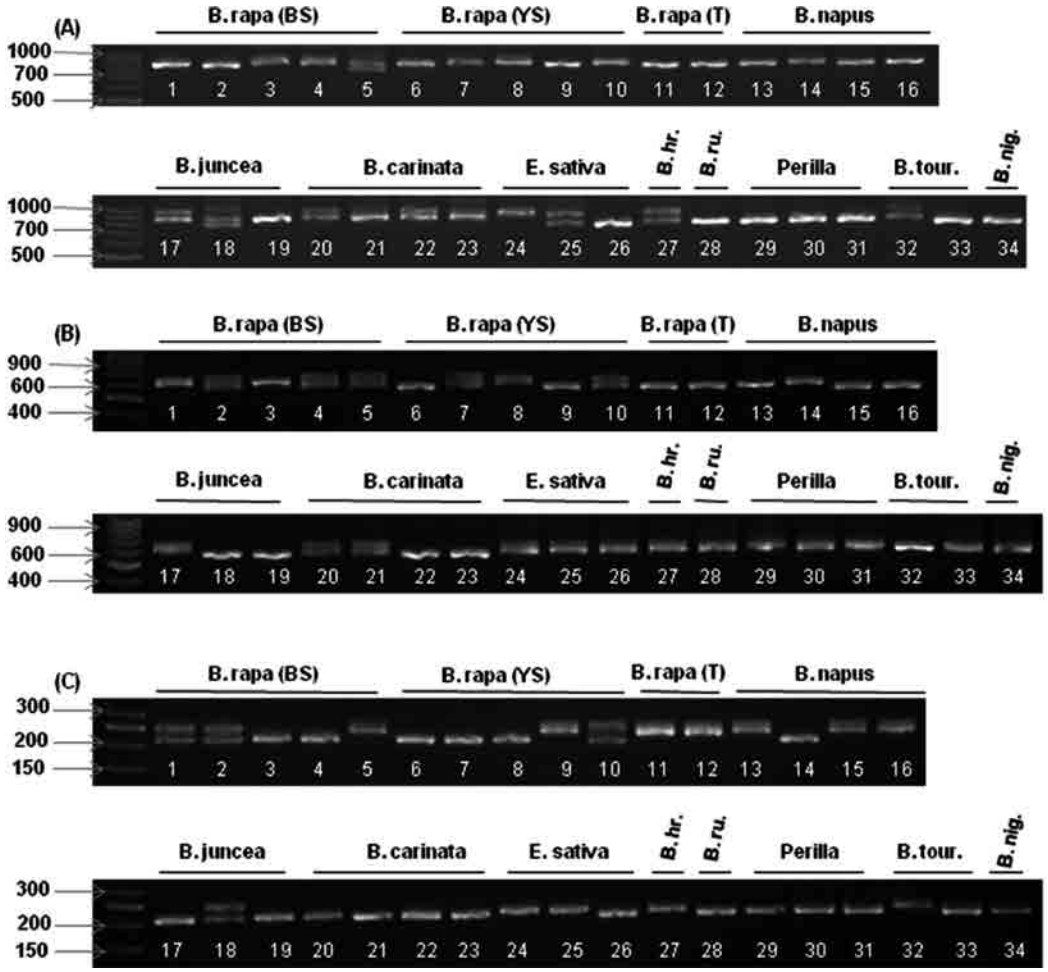

Fig. 1. DNA profile generated in accessions/ varieties of different *Brassica* and related species using genic-SSR markers (A= PUT154; B= PUT169 and C= PUT256) from *B. rapa*. (B. hr.= *Brassica hirta*; B. tour. = *B. tournefortii*; B. ru. = *B. rugosa*; B. nig. = *B. nigra*; BS = Brown sarson; YS = Yellow sarson, T = Toria]

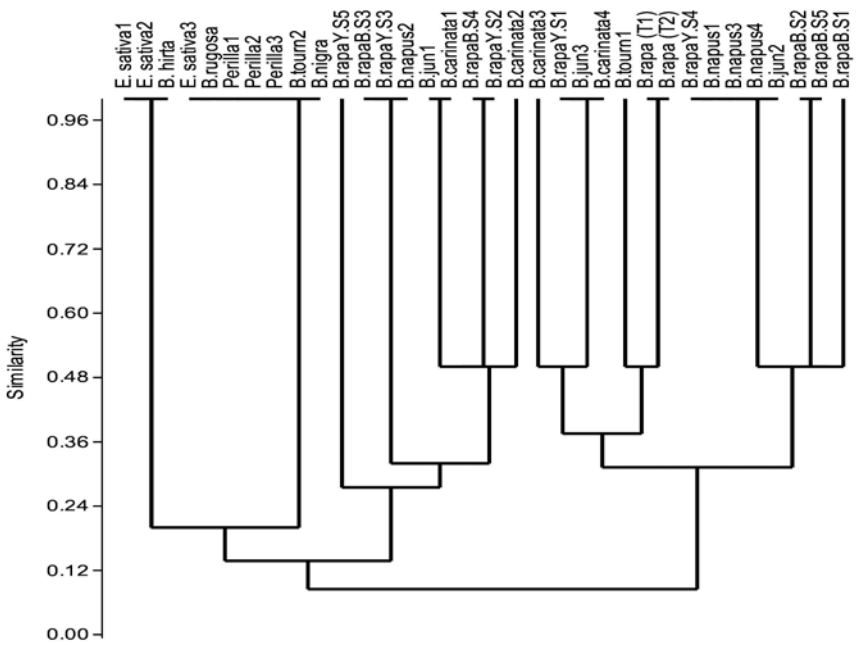

Fig. 2. Dendrogram of *Brassica* species generated using genic-SSR markers (No. in parentheses corresponds to those mentioned in Table 1).

markers combination could faithfully discriminate most of the varieties of *B. rapa* belonging to three different sub-groups, *viz.* Brown sarson, yellow sarson and toria (Fig. 2). Similarly, the accessions of *B. juncea*, *B. carinata* and *Eruca sativa* easily got discriminated based on its DNA profile.

One of the major advantages of using SSR markers is its ease of operation besides being less expensive and its co-dominant nature. Since the differences among accessions/ varieties were visually clear, we extended our study to study inter-relationship of vegetable *Brassica* (*Brassica rugosa*) with that of both related and distant *Brassica* species. The dendrogram was developed from the genetic similarity indices using Jaccard's coefficient. In one cluster, accession of *Eruca* and *Perilla* were grouped along with *B. hirta* (*Sinapis alba*) and *B. nigra*. However, in another cluster most of the cultivated varieties of several *Brassica* species were grouped. The dendrogram outgrouped the vegetable *Brassica* (*B. rugosa*) with remaining oilseed *Brassica* species but showed close association with distant oilseed species. The vegetable *Brassica* (*B. rugosa*) supposed to be closely associated with *B. juncea* and *B. napus*, however, the efficiency of identified genic-SSR markers could differentiate the vegetable *Brassica* with their close relatives. In fact, the dendrogram showed grouping of vegetable *Brassica* (*B. rugosa*) with *B. nigra* with which it shares one set of genome. Thus, the identified genic-SSR markers has potential for studying diversity in other vegetable *Brassica* species and also can be used in differentiating vegetable *Brassica* with oilseed *Brassica* species.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-NBPGR, New Delhi for facilities.

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Received : October, 2015; Revised : January, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00034.7

Indian J. Hort. 73(1), March 2016: 137-140

Short communication

Performance of amaranthus genotypes for growth and yield under different nitrogen levels

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ABSTRACT

Present investigation was undertaken to assess the stability of 20 amaranthus genotypes under varying levels of nitrogen. Nitrogen levels, *i.e.*, 75, 100 and 125 kg N / ha were kept in main plots, while the genotypes were evaluated in sub-plots. The stability of these genotypes was measured by three parameters, *viz*., mean performance over different nitrogen levels, the linear regression and deviation from regression. The linear increase in the total green yield of amaranthus was evident with every increase in dose of nitrogen, where maximum yield of 279.76 q/ha was recorded with 125 kg N/ ha against 174.06 q/ ha with the application of nitrogen @ 75 kg N/ ha. The application of nitrogen at the level up to 125 kg per hectare can be recommended to get high total green yield. The genotypes Local Thundukeerai and Selection-1 were high yielding and stable under varying N levels, whereas, the genotypes CO-5 and CO-2 were highly responsive to nitrogen application. Genotypes, namely, Local Thundukeerai, Selection-1, CO-5 and CO-2 can be exploited as potential genotypes for getting higher yield.

Key words: *Amaranthus*, stability, growth indices, nitrogen fertilizer, yield.

INTRODUCTION

Amaranthus, a leafy vegetable, belongs to genus *Amaranthus*, family Amaranthaceae. The main vegetable amaranthus (*Amaranthus tricolor* L.) is believed to be a native of India or Southern China region. This is a hardy, fast growing leafy vegetable and its leaves contain 17.5 to 38.3% dry matter as protein of which 5% is lysine (Oliveira and De Carvallo, 8). In comparison to spinach, amaranthus contains three times more vitamin C, calcium and niacin, while in comparison to lettuce; it contains 18 times more vitamin A, 13 times more vitamin C, 20 times more calcium and 7 times more iron (Guillet, 6). A large number of species and horticultural varieties of amaranthus are cultivated as vegetable (Devdas *et al.*, 3). Although a large variation exists among various cultivated types grown at various places yet no systematic work has been done to identify genotypes with high yield and better quality under different fertility conditions. To grow this crop efficiently, it is necessary to know the effect of nitrogen fertilization on its yield because it has been found to be the primary limiting factor for its production (Pospisil *et al.*, 9). With the increase of soil nitrogen content, low efficiency of nitrogen is used by amaranthus. Therefore, higher nitrogen rate may have an adverse effect on the harvest due to increased plant height, lodging and protracted seed ripening (Elbehri *et al.*, 5).

The present study comprised of 20 amaranthus genotypes maintained at the Vegetable Teaching Farm, Department of Vegetable Science, PAU, Ludhiana. The nursery of the crop was raised and transplanted 30 days thereafter on ridges maintaining a distance of 60 cm and 30 cm between rows and plants, respectively. The design of the experiment was split plot replicated thrice. The levels of nitrogen, *i.e.,* 75, 100 and 125 kg per hectare were in the main plot, while 20 genotypes were in sub-plots. Therefore, there were 180 experimental plots in the present research trial. Farmyard manure was applied at the rate of 20 tonnes per hectare during soil preparation. Half of the nitrogen was applied along with full dose of phosphorus and potassium @ 60 kg/ha each at the time of transplanting, while half dose of nitrogen in each plot was applied after 30 days of transplanting. Growth and yield contributing parameters like plant height (cm), number of branches per plant, leaf length, leaf width, number of leaves per plant and total green yield (q/ ha) were recorded and statistically analyzed according to the procedure outlined by Steel and Torrie (11). Genotypes were assessed for their stability performance over environments in accordance with method described by Eberhart and Russell (4).

Highly significant differences were also recorded for nitrogen levels for all the characters under study. Highly significant differences were also recorded for Genotype × Environment interaction for all the traits (Table 1). Highly significant mean squares for environment indicated the tremendous effect of nitrogen levels on the growth. It provides an ample opportunity for selecting suitable nitrogen levels with high mean for all the traits of interest.

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Table 1. Mean values (μ), regression coefficient (b_i) and variation due to deviation ($\delta^2 d_i$) for plant height, leaf length, leaf width and leaves/ plant.

Genotype	Plant height (cm)			Leaf length (cm)			Leaf width (cm)			Leaves/ plant		
	μ	b_i	$\delta^2 d_i$	μ	b_i	$\delta^2 d_i$	μ	b_i	$\delta^2 d_i$	μ	b_i	$\delta^2 d_i$
Selection-3	45.61	0.81 ± 0.162	8.470	5.96	0.92 ± 0.304	0.546**	3.71	0.72 ± 0.268	0.116**	184.0	1.31 ± 0.058	3.369**
A-1	62.54	1.30 ± 0.064	1.310	5.69	0.99 ± 0.390	0.902**	3.09	1.08 ± 0.442	0.315**	144.67	0.99 ± 0.025	6.154
CO-5	27.71	0.42 ± 0.061	1.197	9.41	2.36 ± 0.084	0.042**	5.82	2.75 ± 0.321	0.166**	204.0	0.94 ± 0.161	0.026**
Local Peruppukeerai	57.11	1.09 ± 0.040	0.523	8.14	1.27 ± 0.471	1.313**	3.79	0.76 ± 0.022	0.001	177.33	1.41 ± 0.166	0.276**
Arakeerai	60.44	1.17 ± 0.186	11.148*	4.61	0.57 ± 0.096	0.055**	2.28	0.55 ± 0.056	0.005	150.33	0.93 ± 0.149	0.222**
Selection-4	88.50	1.10 ± 0.054	0.956	9.32	1.63 ± 0.350	0.736**	3.77	1.31 ± 0.201	0.065**	202.67	1.34 ± 0.131	0.002**
A-18	67.76	1.13 ± 0.066	1.396	9.57	0.99 ± 0.033	0.007	5.49	0.83 ± 0.113	0.021**	169.78	1.11 ± 0.201	4.039
Thundukeerai	51.64	0.81 ± 0.026	0.226	7.66	0.92 ± 0.022	0.003	4.29	0.81 ± 0.334	0.179**	222.67	1.09 ± 0.222	0.494**
CO-2	58.22	1.15 ± 0.008	0.020	10.73	1.53 ± 0.315	0.589	4.93	1.52 ± 0.353	0.201**	199.78	1.14 ± 0.125	0.016**
Selection-2	61.94	1.26 ± 0.092	2.73	5.49	0.89 ± 0.096	0.055**	2.91	0.88 ± 0.101	0.016**	185.0	0.88 ± 0.139	0.195**
Selection-5	48.73	0.89 ± 0.108	3.750	3.98	0.55 ± 0.132	0.103**	2.10	0.44 ± 0.019	0.001	149.89	0.82 ± 0.176	0.311**
Local Arakeerai	60.69	0.99 ± 0.229	16.950**	6.16	0.82 ± 0.322	0.615**	4.11	0.96 ± 0.253	0.103**	178.11	1.04 ± 0.298	0.893**
IC-415250	45.14	0.81 ± 0.155	7.724	5.30	0.55 ± 0.059	0.021**	2.93	1.03 ± 0.248	0.099**	164.33	0.77 ± 0.186	0.346**
A-14	62.71	1.20 ± 0.020	0.124	5.44	0.63 ± 0.139	0.115**	2.78	0.88 ± 0.229	0.085**	194.89	0.93 ± 0.115	0.132**
Selection-6	59.35	1.26 ± 0.146	6.831	5.89	1.24 ± 0.292	0.506**	3.70	0.92 ± 0.039	0.002	162.67	0.88 ± 0.078	6.141
Selection-7	45.98	0.99 ± 0.150	7.266	3.73	0.42 ± 0.11	0.072**	1.78	0.60 ± 0.144	0.034**	192.67	0.70 ± 0.221	4.889
Selection-8	59.98	0.96 ± 0.093	2.759	6.17	0.58 ± 0.0425	0.011	3.77	0.92 ± 0.189	0.058**	174.0	0.86 ± 0.163	2.657
A-19 Chulai Lal	44.47	0.88 ± 0.120	4.658	4.40	0.55 ± 0.093	0.051**	2.33	0.65 ± 0.121	0.024**	124.78	0.76 ± 0.089	0.796**
Selection-1	58.72	0.78 ± 0.106	3.619	10.83	1.23 ± 0.315	0.589**	6.21	0.79 ± 0.025	0.001	192.78	1.09 ± 0.244	0.059
Local Thundukeerai	58.61	0.95 ± 0.160	8.255	12.16	1.34 ± 0.195	0.225**	6.81	1.59 ± 0.027	0.001	256.11	1.01 ± 0.202	0.004
CD at 5%												
Genotype		7.37			0.26			0.18			11.27	
Environment		1.58			0.07			0.05			2.10	
G × E		7.08			0.33			0.21			9.39	

*, ** Significant at 5 and 1% levels

The data recorded wide range of variation among the genotypes for plant height. Among 20 genotypes, 13 genotypes excelled the mean performance. The genotype Selection-4 was the tallest followed by A-18 and A-14. The genotypes, Arakeerai and Local Arakeerai also had significantly more mean plant height than overall mean with regression coefficient near to one and significant deviations from regression line. This indicated that plant height varied with nitrogen application. The genotypes CO-2, Local Peruppukeerai and A-14 had mean plant height of 58.22, 57.11 and 62.71 cm, respectively being higher than the overall mean height and had regression coefficient close to one, *i.e.* 1.15, 1.09 and 1.20, respectively and recorded non-significant deviation from regression line. It suggested that these genotypes were stable under varying nitrogen application. The plant height increased with increase in N-fertilizer. Similar results were recorded by Chakhatrakan (2) and Olaniyi *et al.* (7).

Leaf length ranged from 3.73 to 12.16 cm. Among the genotypes, only seven genotypes had significantly higher leaf length than overall mean. The genotype Local Thundukeerai had the maximum leaf length, followed by Selection-1 and CO-2. The genotypes Local Thundukeerai, Selection-1, CO-5, Local Peruppukeerai and Selection-4 had below average stability. Thus, their performance cannot be predicted under different N levels. The genotype, Thundukeerai and A-18 did show average stability and can perform well under different levels of nitrogen. The leaf length of amaranthus increased gradually in different stages of growth, which was found to be important for yield contributing characters of amaranthus. Similar results were obtained by Prakash *et al.* (10).

Seven genotypes had significantly higher mean leaf width than overall mean. The genotypes Local Thundukeerai had higher leaf width followed by Selection-1, CO-5, A-18, CO-2, Thundukeerai and Local Arakeerai, *i.e*., 6.81, 6.21, 5.82, 5.49, 4.93, 4.29 and 4.11, respectively. The genotypes CO-5 and CO-2 had higher mean leaf width than overall mean recorded regression coefficient more than one, *i.e*., 2.75 and 1.52, respectively and significant deviation from regression line, indicating that these genotypes had below average stability and did show unpredictable growth under different levels of nitrogen. The genotypes A-18, Thundukeerai and Local Arakeerai elicited average stability but their performance cannot be predicted under different levels of nitrogen for this trait. The genotype Selection-1 showed better stability for leaf width under different levels of nitrogen. The leaf width of amaranthus increased gradually at different stages of growth, which was found to be important for yield contributing characters of amaranthus. Similar results were obtained by Prakash *et al*. (10).

Only five genotypes had significantly higher mean number of leaves per plant than overall mean. Local Thundukeerai had the highest number of leaves per plant followed by CO-5, Selection-4, CO-2 and A-14 with average number of 256.11, 204.00, 202.67, 199.78, and 194.89, respectively. The genotypes, Selection-4 and CO-2 showed significant deviation from regression line, therefore, indicated below average stability and performance of these genotypes cannot be predicted with accuracy under different nitrogen levels. The genotype CO-5 and A-14 reflected average stability and unpredictable growth under different levels of nitrogen. The genotype Local Thundukeerai had mean number of leaves per plant more than overall mean with regression coefficient close to one (1.01) and non-significant deviation from regression line. This genotype can be considered stable under varying levels of nitrogen. It has been revealed that the number of leaves per plant of amaranthus increased as the nitrogen fertilizer rate increased. Similar results were reported by Chakhatrakan (2), and Olaniyi *et al.* (7).

The average total green yield ranged from 142.13 to 360.3 q/ha (data not shown). Eight genotypes exceeded over all mean by a significant margin. The highest mean yield was recorded in genotype Local Thundukeerai followed by Selection-1, CO-5, Selection-4 and CO-2 with yield record of 360.30, 327.84, 315.02, 290.68 and 273.41 q/ ha, respectively. The genotypes CO-5 and Selection-4 were responsive to the application of nitrogen, while genotypes Local Thundukerrai and Selection-1 showed better stability for total green yield at optimum levels of nitrogen. A linear increase in yield was recorded with every dose of nitrogen levels. An average yield of 174.06 q/ha was recorded with the application of nitrogen at the rate of 75 kg N/ha, which increased with nitrogen application. A perusal of the data also revealed significant increase in the yield of amaranthus with the application of nitrogen upto 125 kg N/ha, where the yield was 279.76 q/ha. This interaction of yield with nitrogen application showed that nitrogen being the component of protoplast increased the growth of the plant in terms of leaf number and leaf size. The total green yield exhibited highly significantly and positive correlation with plant height, number of branches per plant, leaf length, leaf width, number of leaves and leaf area index both at genotypic and phenotypic levels. Similar results were obtained by Campbell and Abbott (1).

From the present study, it is revealed that the genotypes Local Thundukeerai and Selection-1 were found to be high yielding and stable under

varying levels of nitrogen, whereas, the genotypes CO-5 and CO-2 are highly responsive to nitrogen application.

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Received : June, 2014; Revised : December, 2015; Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00035.9

Indian J. Hort. 73(1), March 2016: 141-144

Short communication

Induction of variability through *in vivo* mutagenesis in chrysanthemum (*Chrysanthemum morifolium* Ramat.) var. Jaya

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ABSTRACT

The present investigation was carried out in 2013-2014 on Induction of variability through *in vivo* mutagenesis in chrysanthemum (*Chrysanthemum morifolium* Ramat.) var. Jaya. The experiment comprised application of two chemical mutagens, *viz.*, EMS and DES (0.02, 0.03 and 0.04%), one physical mutagen, *i.e.* Gamma rays (5, 10, 15, 20 and 25 Gray) including control and replicated thrice in Randomized Block Design. Lower doses of chemical and physical mutagens, *viz.*, 0.02 per cent EMS and DES and 5, 10 and 15 Gy gamma rays showed positive effect on growth related attributes as compared to higher doses. Lower doses of gamma rays (5, 10 and 15 Gy) altered the flowering behaviour from late to early. Distinct flower mutants were isolated with desirable colours with different γ-ray doses, *viz.*, light pink (10 Gy), yellow (20 Gy) and brick red with yellowish disc florets (20 Gy). Gamma rays created more variation as compared to EMS and DES in chrysanthemum.

Key words: Chrysanthemum, di-ethyl sulphate, ethyl methane sulphonate, gamma rays, mutagenesis.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) belongs to family Asteraceae and is popular commercial flower crop grown for cut as well as loose flowers and as a pot mum in different parts of the world. The florist's chrysanthemum is a highly heterozygous, self-incompatible and polyploid in nature. It can be easily propagated through vegetative means, hence, most suitable for mutagenesis. Mutation breeding has now been widely recognized as a useful complimentary tool for the improvement of modern day chrysanthemum cultivars.

The induction of mutation in chrysanthemum has attracted considerable attention due to the fact that any change in the dominant genes is easily expressed in the first generation and thus, the selection of mutation of directly perceptible characters like flower colour, shape and size is generally very easy and can directly be put to commercial use. Recently, the interest in evolving novel mutants is growing worldwide with the possibility of patterning the new cultivars. The main advantage of mutagenesis in chrysanthemum is the ability to alter one or few characters of an outstanding cultivar without changing the rest of the genotype. A number of gamma rays induced mutants and other morphological mutants of chrysanthemum have been commercialized (Broertjes, 3). Induction of flower mutants in chrysanthemum variety Jaya, which is red colour through chemical (EMS and DES) and physical (γ-rays) mutagens was the main objective of the present investigation.

MATERIALS AND METHODS

The present investigation was conducted at Floriculture Research Farm, ASPEE College of Horticulture & Forestry, Navsari Agricultural University, Navsari during 2013-2014. Terminal cuttings of 6-7 cm were treated with 0.1 per cent carbendazim solution for 15 min. and then planted in the sheltered beds in pure sand after quick dipping of basal ends in 500 ppm IBA for better rooting. Cuttings were ready in 30 days. The uniform size 6-7 cm (8-10 leaves) of rooted cuttings of variety Jaya were treated with three concentrations each of ethyl methane sulphonate (EMS) and di-ethyl sulphate (DES) at 0.02, 0.03 and 0.04%, five doses of gamma rays (5, 10, 15, 20 and 25 Gy) using Gamma Cell-200 (Cobalt-60 source emitting 3600 rads per min.) at Bhabha Atomic Research Centre, Trombay, Mumbai during October, 2013. The cuttings were planted in field with untreated rooted cuttings as control in Randomized Block Design. All the standard cultural practices were followed, except pinching and disbudding operations. Heritable effects of various mutagens on vegetative growth, flowering and quality related aspects; vegetative and floral abnormalities and mutation spectrum were investigated and statistically analyzed as advocated by Gomez and Gomez (7). Desirable variants with change in plant morphology, flower colour and early flowering was recorded as mutation spectrum.

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RESULTS AND DISCUSSION

Table 1 indicates that all the morphological characteristics relating to plant survival and growth were significantly affected by various mutagenic treatments. The plants that survived the irradiation was highest (96.67%) when they were exposed to 5 and 10 Gy gamma rays (lower doses), whereas higher doses of γ-rays decreased the plant survival. Reduction in survival after exposure to gamma rays was explained due to inactivation and/or decreases in auxin content that affect cell division resulting in poor establishment and survival. Datta and Banerji (4) also observed similar outcome in chrysanthemum with lethal effect of gamma rays caused due to chromosomal aberration. EMS 0.04 per cent treated plants decreased survival up to 41.67 per cent in chrysanthemum. Dilta (5) also reported that higher concentrations of EMS reduced the plant survival per cent in chrysanthemum. The drastic reduction in plant survival was due to the formation of certain toxic substances during oxidative stress and biochemical process with hydroxyl radical with DNA bases, *viz*., cytosine glycol, 5-hydroxyuracil, alloxan, oxazolone and disrupts protein synthesis, affects hormone balance, water exchange and enzyme activity which cause death of the cells ultimately resulting in the death of plants (Lagoda, 8; Sax, 13).

Lower dose of gamma rays significantly influenced plant height, plant spread (E-W and N-S) and number of branches per plant, whereas reduction was observed with higher doses. Reduction in vegetative characters of gamma rays treated plants depends on the nature and extends of chromosomal damage or due to physiological, morphological and cytological disturbance caused by irradiation (Banerji and Datta, 1). While EMS and DES treated plants showed drastic reduction as compared to gamma irradiated plants.

Table 1. Effect of different mutagens on vegetative growth parameters of chrysanthemum variety Jaya.

Treatment	Survival (%)	Plant height (cm)	Plant spread (E-W) (cm)	Plant spread (N-S) (cm)	No. of branches per plant	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Leaf area (cm^2)	Vegetative abnormalities (%)*
EMS @ 0.02%	66.67 (54.89)*	40.90 ± 1.33	20.63 ± 0.64	20.98 ± 0.94	20.27 ± 2.25	4.38 ± 0.39	2.60 ± 0.08	0.83 ± 0.09	6.26 ± 0.23	11.67 (19.50)
EMS @ 0.03%	55.00 (47.91)	38.13 ± 0.74	19.42 ± 1.11	19.14 ± 1.20	19.60 ± 0.93	3.73 ± 0.23	2.51 ± 0.14	0.71 ± 0.02	5.08 ± 0.17	15.00 (22.49)
EMS @ 0.04%	41.67 (40.07)	36.18 ± 0.90	20.54 ± 0.75	20.53 ± 0.60	16.00 ± 1.81	3.32 ± 0.37	2.15 ± 0.23	0.63 ± 0.03	4.83 ± 0.40	22.50 (28.24)
DES @ 0.02%	64.17 (53.31)	39.87 ± 0.99	19.92 ± 0.55	19.94 ± 0.74	20.83 ± 0.84	4.15 ± 0.35	2.79 ± 0.26	0.84 ± 0.09	5.59 ± 0.37	15.83 (23.25)
DES @ 0.03%	51.67 (45.96)	38.25 ± 0.60	21.02 ± 0.99	20.56 ± 0.91	15.17 ± 0.61	3.86 ± 0.80	2.26 ± 0.23	0.68 ± 0.14	5.08 ± 0.55	19.17 (25.89)
DES @ 0.04%	48.33 (44.04)	36.32 ± 0.41	20.07 ± 0.99	19.71 ± 0.83	11.10 ± 0.96	2.97 ± 0.10	2.08 ± 0.36	0.66 ± 0.04	4.44 ± 0.30	21.67 (27.41)
Gamma rays - 5 Gy	96.67 (81.67)	58.71 ± 1.79	26.23 ± 0.93	26.44 ± 0.84	26.77 ± 0.95	4.05 ± 0.13	2.77 ± 0.09	0.75 ± 0.01	8.45 ± 0.04	49.17 (44.52)
Gamma rays - 10 Gy	96.67 (79.63)	54.62 ± 0.86	24.36 ± 0.16	24.09 ± 0.13	28.03 ± 0.73	3.13 ± 0.06	1.99 ± 0.02	0.88 ± 0.02	6.82 ± 0.34	63.33 (52.78)
Gamma rays - 15 Gy	90.00 (71.66)	53.70 ± 1.10	21.71 ± 1.75	22.30 ± 1.90	23.40 ± 0.46	2.74 ± 0.11	1.98 ± 0.12	0.74 ± 0.00	6.69 ± 0.29	67.50 (55.28)
Gamma rays - 20 Gy	90.83 (72.41)	45.65 ± 1.72	18.37 ± 1.43	18.63 ± 1.17	18.93 ± 0.37	2.67 ± 0.31	1.84 ± 0.12	0.55 ± 0.02	5.41 ± 0.59	55.00 (47.87)
Gamma rays - 25 Gy	78.33 (62.76)	43.96 ± 3.00	16.92 ± 1.96	14.42 ± 1.86	9.63 ± 0.72	2.57 ± 0.33	1.57 ± 0.09	0.53 ± 0.00	4.63 ± 0.39	49.17 (44.52)
Control	96.67 (79.63)	47.63 ± 1.02	22.36 ± 0.15	22.81 ± 0.42	24.47 ± 0.66	2.73 ± 0.06	1.74 ± 0.03	0.71 ± 0.01	5.74 ± 0.08	00.00 (5.02)
$CD_{0.05}$	9.38	3.67	3.05	3.06	3.20	0.94	0.52	0.16	1.07	6.25

*Transformed data

Different mutagenic agents increased the leaf length and leaf width over the control, except 20 and 25 Gy gamma rays. Zargar *et al.* (15) documented that γ-rays significantly reduced the leaf length and width at and above 20 Gy in chrysanthemum. Petiole length was found shorter with increasing dose of mutagenic agents. All the treatments of chemical mutagens reduced the leaf area over control, except 0.02 per cent EMS treated cuttings. Increase in leaf area was observed when the rooted cuttings of chrysanthemum var. Jaya were exposed to gamma radiation from 5 to 15 Gy, while higher doses reduced leaf area over control. Earlier, Mahure *et al.* (9) recorded that lower doses like 10 and 20 Gy increased leaf area but 30 Gy decreased leaf area over control. Vegetative abnormalities included changes in plant morphology and branching habit, leaf shape, size margin, apex fission and fusion. Vegetative abnormalities were increased significantly over the control due to effect of different mutagenic treatments in chrysanthemum. The frequency of abnormalities was increased with increase in doses of mutagens. Priya Misra *et al.* (11) have documented similar evidences in chrysanthemum.

Lower dose of gamma rays, *viz.*, 10, 5 and 15 Gy positively influenced early flowering (57.50, 57.67 and 60.20 days, respectively) as compared to control (60.60 days), while chemical mutagens (EMS and DES) delayed the flowering (64.03 and 63.30 days, respectively). Flowering duration significantly reduced by all the mutagenic treatments over control. Days to flowering and flowering duration may be affected as a result of irradiation or mutagenic treatments because many biosynthetic pathways are believed to be altered, which are directly and indirectly associated with the flowering physiology (Mahure *et al.*, 9). Significant reduction in flower head diameter, number of disc and ray florets was found with increasing levels of physical and chemical mutagens but increase in these parameters was noted with 5 and 10 Gy gamma rays over control. Earlier, Mahure *et al.* (9) recorded similar observations in cultivar Red Gold. Whereas, flower size was compared between gamma irradiated and chemically treated plants, smaller size flowers were found with EMS and DES treatments.

A significant reduction in the number of flower head was observed over the control after mutagenic treatments, except 10 Gy γ-rays. This result is in conformity with Banerji and Datta (2). Maximum peduncle length (14.91 cm) was noted with 10 Gy dose over the control, which was followed by 0.02 and 0.03% EMS and DES. Similar results were observed by Zargar *et al.* (15) in chrysanthemum cv. Satish Modi.

The different doses of gamma rays (5, 10, 15, 20 and 25 Gy) induced yellow colour chimeras in flowers and some promising mutants with changed colour (light pink, yellow and brick red with yellowish disc florets) and induced early flowering. Stewart and Dermen (14) also observed certain flower colour changes due to true gene mutations, whereas the change from absence to presence of yellow chloroplasts might be caused by the loss (of part) of a chromosome, which carries a dominant suppressor for yellow pigment formation. The results are supported by the findings of Banerji and Datta (1, 2), Mandal *et al.* (10), Dilta *et al.* (6) and Priya Misra *et al.* (12) in chrysanthemum.

ACKNOWLEDGEMENTS

Authors are thankful to Bhabha Atomic Research Institute, Trombay, Mumbai for providing facilities of gamma irradiation. We also acknowledge the help provided by scientists of Department of Floriculture and Landscape Architecture, ACHF, NAU, Navsari.

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Table 2. Effect of different mutagens on flowering and quality parameters of chrysanthemum variety 'Jaya'.

Treatment	Days to flowering	Flowering duration (days)	Flower head dia. (E-W) (cm)	Flower head dia. (N-S) (cm)	No. of disc florets per head	No. of ray florets per head	No. of flowers per plant	Flower wt. (g)	Peduncle length (cm)	Floral abnormalities (%)
E M S @ 0.02%	63.77 ± 0.81	35.37 ± 0.62	4.03 ± 0.06	4.00 ± 0.08	28.30 ± 1.13	124.33 ± 6.53	57.47 ± 1.97	3.38 ± 0.08	13.46 ± 0.42	7.50 (15.75)*
E M S @ 0.03%	63.50 ± 0.60	34.20 ± 0.21	3.56 ± 0.07	3.58 ± 0.10	27.00 ± 0.70	121.13 ± 4.63	54.37 ± 2.18	3.09 ± 0.29	13.06 ± 0.33	9.17 (17.50)
E M S @ 0.04%	64.03 ± 0.41	33.70 ± 0.56	3.29 ± 0.10	3.24 ± 0.09	18.93 ± 0.84	110.93 ± 2.29	53.67 ± 0.29	2.92 ± 0.14	12.10 ± 0.30	11.67 (19.59)
D E S @ 0.02%	61.10 ± 0.74	34.13 ± 0.42	3.94 ± 0.14	3.94 ± 0.12	31.07 ± 0.70	122.33 ± 3.65	53.83 ± 0.52	3.00 ± 0.17	13.14 ± 0.32	8.33 (16.74)
D E S @ 0.03%	63.13 ± 0.32	33.10 ± 0.64	3.47 ± 0.04	3.45 ± 0.03	19.73 ± 0.85	112.13 ± 3.87	53.13 ± 0.47	2.97 ± 0.06	13.25 ± 0.35	7.50 (15.75)
D E S @ 0.04%	63.30 ± 0.67	32.80 ± 0.21	3.13 ± 0.14	3.14 ± 0.14	17.20 ± 0.40	105.53 ± 1.22	53.60 ± 0.76	3.22 ± 0.06	12.82 ± 0.17	8.33 (16.60)
Gamma rays - 5 Gy	57.67 ± 0.76	36.17 ± 0.41	5.11 ± 0.14	5.12 ± 0.14	35.30 ± 0.67	133.10 ± 2.65	76.40 ± 0.10	3.40 ± 0.05	12.92 ± 0.16	16.67 (23.94)
Gamma rays - 10 Gy	57.50 ± 0.55	37.30 ± 0.44	5.10 ± 0.23	5.08 ± 0.28	36.80 ± 1.18	137.80 ± 2.95	78.43 ± 0.79	3.54 ± 0.12	14.91 ± 2.20	29.17 (32.52)
Gamma rays - 15 Gy	60.20 ± 0.54	35.80 ± 0.36	3.72 ± 0.13	3.70 ± 0.10	30.47 ± 2.01	123.77 ± 2.47	76.70 ± 0.42	3.30 ± 0.07	12.53 ±0.19	25.00 (29.88)
Gamma rays - 20 Gy	63.17 ± 0.54	34.87 ± 0.58	3.05 ± 0.16	2.87 ± 0.24	19.00 ± 0.40	102.67 ± 5.75	65.33 ± 2.17	3.11 ± 0.08	12.34 ± 0.38	28.33 (32.09)
Gamma rays - 25 Gy	63.17 ± 0.96	34.50 ± 0.49	2.93 ± 0.18	2.86 ± 0.24	16.60 ± 0.61	98.73 ± 1.80	55.90 ± 2.72	2.71 ± 0.24	12.65 ± 0.20	23.33 (28.88)
Control	60.60 ± 0.32	39.07 ± 0.38	4.08 ± 0.08	4.07 ± 0.06	34.90 ± 0.26	131.00 ± 1.77	77.43 ± 0.37	3.43 ± 0.06	13.05 ± 0.11	0.83 (3.03)
$CD_{0.05}$	1.88	1.38	0.38	0.43	2.80	11.12	4.14	0.41	1.99	5.69

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Received : November, 2014; Revised : September, 2015; Accepted : December, 2015

DOI : 10.5958/0974-0112.2016.00036.0

Indian J. Hort. 73(1), March 2016: 145-148

Short communication

Effect of packaging and storage temperature on shelf-life of okra pods

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ABSTRACT

The experiment comprised of packing okra pods in rigid plastic crates and LDPE bags of 25, 50, 75 and 100 micron thickness with subsequent storage at 12 and 16°C. Packaged okra pods in LDPE bags of 50 micron thickness and stored at 12°C emerged as the best post harvest treatment combination. Pods subjected to this treatment combination had the best visual appearance, lower chilling injury, lower physiological loss in weight and optimal marketability. This treatment combination suppressed quality deterioration and extended the shelf-life of okra by up to 12 days.

Key words: Okra, packaging, storage temperature, shelf life, quality

INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is an annual vegetable crop grown extensively in the tropical and sub-tropical parts of the world. Okra is a rich source of dietary fibre, minerals (calcium and potassium), vitamin C and folic acid. It is valued for its tender and green pods which are cooked as a vegetable. Pods can also be canned green or dried for off season use. The root and stem are used for clarification of cane juice in the preparation of *gur*. Plant fibre is utilized by jute textiles and paper industries. Ripened seeds are roasted and ground to form a caffeine free substitute of coffee. India ranks first among the okra producing countries of the world accounting for about 73 per cent of the total production. Gujarat is the third largest producer of okra in the country. In Gujarat, it occupies about 0.65 lakh hectares with a total production of 7.59 lakh MT and an average productivity of 11.5 MT/ ha (Anon, 1).

Freshly harvested okra pods have an extremely short shelf life, owing to high rates of respiration and water loss (Tamura and Minamide, 11). Although, water is a major component of fresh fruit and vegetables, even a loss of 5 to 10 per cent can lead to severe reduction in quality. In the case of okra, consumers identify this loss in quality with general yellowing, shrivelling and blackening of ridges. Modified atmosphere packaging coupled with low temperature storage is a promising and inexpensive method to improve the shelf life of perishable produce by minimizing quality deterioration. Limited attempts were made to prolong the shelf life okra pods by opting for packaging and storage at low temperatures. The present investigation was therefore designed to assess the shelf life and quality of okra pods under the influence of packaging and low temperature storage.

MATERIALS AND METHODS

This trial was carried out at Post Harvest Technology Laboratory, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari during 2010-11. Okra cv. VNR Green was procured from a farmer residing in Changa village of Gandevi Taluka under Navsari district. 'VNR Green' pods are five lobed, dark green in colour with five ridges, 12-15 cm in length and tolerant to Yellow Vein Mosaic Virus (YVMV). Pods were harvested early in the morning and transported to the laboratory within 30 min. Rigid plastic crates and LDPE bags of varying thickness, *viz.*, 25, 50, 75 and 100 micron thickness were selected for experimentation. The size of each LDPE bag was 30 cm × 25 cm and that of rigid plastic crates was 540 × 355 × 290 mm. Five hundred gram pods were placed in each crate and bag. The mouth of LDPE bags was tied tightly by a rubber band. Packed okra pods were stored at two different temperatures, *viz*. 12° and 16°C with 95% RH.

All observations except Overall Visual Quality (OVQ) were recorded on the 4th, 8th and 12th day of storage. OVQ was measured on the 8th day of storage. Physiological loss in weight (PLW) was calculated by weighing okra pods at the beginning of the experiment using an electronic balance and then after every four days interval during storage. Percentage changes in weight were determined. Chilling injury was calculated by counting the number of pods showing symptoms as a percentage of the total number of pods per bag. Symptoms considered for chilling injury were brownish discolouraton, pitting

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and water soaked lesions. Respiration rate was measured every fourth day by weighing selected fruits and placing them in a known volume of polythene bag. The initial CO_2 concentration was recorded before sealing the polythene bag. After one hour, the increase in CO_2 concentration was recorded using an infra-red gas analyzer and expressed as mg CO_2/kg/h. For determining marketable pods, visibly sound and healthy pods were counted and expressed as percentage over the total number of pods. Overall Visual Quality (OVQ) in terms of pod colour, shriveling, blackening of ridges and general appearance was measured from a sample size of ten pods by a panel of five judges using the following gradation for each character: Excellent (++++), Good (+++), Acceptable (++) and Not acceptable (+). The experiment comprising of eleven treatment combinations and four repetitions was laid out in Completely Randomized Design based on Factorial Concept (FCRD). The recorded observations were subjected to statistical analysis.

RESULTS AND DISCUSSION

There was a significant effect of packaging and storage temperature on physiological loss in weight of okra pods (Table 1). Physiological loss in weight increased progressively with extended storage, irrespective of the packaging treatment and storage temperature. Between the two storage temperatures, PLW was lower at 12°C as compared to 16°C. Okra pods packed in LDPE bags of 50 micron thickness and stored at 12°C showed the lowest PLW (12.86%) on the 12th day. For the same interval, highest PLW (18.63%) was recorded in okra pods kept in LDPE bags of 100 micron thickness and stored at 16°C. This could be attributed to temperature effects on vapour pressure deficit and increased water retention. In addition, high temperature accelerated the rate of respiration and other metabolic processes which caused depletion of substrates like sugars and proteins resulting in further weight loss (Buescher, 4).

Storage temperature had a significant effect on chilling injury on the 12th day. Okra pods stored at 16°C did not exhibit any symptom of chilling injury. Chilling injury symptoms observed at 12°C could be a result of dissociation of enzymes and other proteins into structural sub-units and hence a change in the kinetics of enzyme activity and structural proteins (Graham and Patterson, 6; Morris, 11; Wang, 21). At 12°C, okra pods packed in packed in LDPE bags of 50 micron thickness showed the minimum chilling injury (2.97%). The reduction in chilling injury is probably due to the prevention of water loss from the pods or to the maintenance of high relative humidity inside the bags. These results are in close conformity with the findings of Ngure *et al.* (7) in okra.

There was a significant increase in respiration rate with the passage of time in all treatments (Table 2). Storage at 12°C resulted in lower rate of respiration on the 12th day of experimentation (54.22 mg CO_2/kg/h). Of the different LDPE bags, respiration rate was the lowest in bags of 50 micron thickness. Curtailment of O_2 supply could have drastically reduced the respiration rate of LDPE packed okra pods. A gradual reduction in marketability was noticed from the first day of experimentation till the 12th day of storage. Okra pods packed in LDPE bags of 50 micron thickness and stored at 12°C resulted in the highest marketable pods (99.91%). This may be due to the creation of a modified atmosphere in the package, which slowed down respiration. This modified atmosphere helped in reducing moisture loss, degradation of green colour, blackening of ridges and shriveling. All of these collectively resulted in higher marketable fruits. Okra pods stored under the above conditions retained their colour and showed minimum shriveling with dark green ridges. The OVQ score for these pods was also high, which

Table 1. Effect of packaging and storage temperature on physiological loss in weight and chilling injury of okra pods cv. VNR Green.

Treatment	Physiological loss in weight (%)						Chilling injury (%)	
	4th day		8th day		12th day		12th day	
	12°C	16°C	12°C	16°C	12°C	16°C	12°C	16°C
Pl. crate	10.21	13.54	19.37	22.37	NA	NA	NA	NA
25 µ bag	1.13	3.98	7.86	10.72	13.57	17.14	3.0	0.00
50 µ bag	0.57	3.23	7.04	10.01	12.86	16.24	2.97	0.00
75 µ bag	1.87	4.62	8.63	11.80	14.46	18.00	6.00	0.00
100 µ bag	2.65	5.52	9.38	12.80	15.39	18.63	8.00	0.00
Mean	3.28	6.18	10.45	13.54	14.07	17.50	4.99	0.00

Initial value for physiological loss in weight = 0%

Table 2. Effect of packaging and storage temperature on respiration rate and marketability of okra pods cv. VNR Green.

Treatment	Respiration rate (mg CO_2/kg/h)						Marketable pods (%)					
	4th day		8th day		12th day		4th day		8th day		12th day	
	12°C	16°C	12°C	16°C	12°C	16°C	12°C	16°C	12°C	16°C	12°C	16°C
Pl. crate	46.62	46.96	51.97	52.75	NA	NA	90.00	89.33	79.14	76.25	NA	NA
25µ bag	44.25	45.48	49.25	50.54	54.02	55.22	99.73	97.23	90.00	85.38	77.50	73.00
50µ bag	44.11	45.24	48.98	50.27	53.91	54.95	99.91	97.50	90.50	86.50	77.95	73.38
75µ bag	44.67	45.99	49.48	50.96	54.35	55.34	99.50	96.00	89.00	84.00	76.50	72.54
100µ bag	44.85	46.28	49.97	51.27	54.61	55.78	98.50	95.00	88.00	82.50	74.25	71.25
Mean	44.90	45.99	49.93	51.16	54.22	55.32	97.53	95.01	87.33	82.93	76.55	72.54

Initial value for respiration rate = 43.50 mg CO_2/kg/h; Initial value for marketable pods = 100%

could have played a role in enhancing marketability. Reduced moisture loss may also have influenced the visual appearance and quality of okra. The lowest marketable pods (89.33%) were recorded in rigid plastic crates stored at 16°C.

Pods were visually examined for qualitative parameters. OVQ scores for pod colour, shrivelling, blackening on ridges and general appearance are displayed in Table 3. Maximum retention of dark green colour was observed in okra pods packed in LDPE bags of 25, 50 and 75 micron stored at 12°C temperature. This may due to the generation of a modified atmosphere in the package, which retarded the activity of chlorophyllase, an enzyme believed to be responsible for chlorophyll degradation (Ardao and Vennesland, 2). Slight yellowing was noticed in okra pods kept in rigid plastic crates, irrespective of temperature. Pods packed in LDPE bags and kept at 12°C were very succulent. However, complete shriveling was observed in pods placed in rigid plastic crates, regardless of storage temperature. As water evaporates from the tissue, turgor pressure decreases and the cell begins to shrink and collapse thus leading to a loss in freshness. These finding are in conformity with those of Saimbhi and Randhawa (8) in okra.

Table 3. Effect of packaging and storage temperature on Overall Visual Quality (OVQ) of okra fruits cv. VNR Green on the 8th day of storage.

Treatment	Colour	Pod shrivelling	Blackening on ridges	General appearance
P_0S_1	++	+	+	++
P_0S_2	++	+	+	++
P_1S_1	++++	++++	++++	++++
P_1S_2	+++	+++	+++	+++
P_2S_1	++++	++++	++++	++++
P_2S_2	+++	+++	+++	+++
P_3S_1	++++	++++	++++	++++
P_3S_2	+++	+++	++	+++
P_4S_1	+++	++++	+++	+++
P_4S_2	+++	+++	++	+++

Scale	Colour	Pod shrivelling	Blackening on ridges	General appearance
++++	Dark green	Very succulent	Dark green ridges	Excellent
+++	Light green	Succulent	Light green ridges	Good
++	Slightly yellow	Slightly shrivelled	Slightly blackening on ridges	Acceptable
+	Completely yellow	Completely shrivelled	Completely black ridges	Not acceptable

P_0 = Rigid plastic crates, P_1 = LDPE bags of 25 µ thickness, P_2 = LDPE bags of 50 µ thickness, P = LDPE bags of 75 µ thickness; P_4 = LDPE bags of 100 µ thickness; S_1 = Storage at a temperature of 12°C; S_2 = Storage at a temperature of 16°C

Dark green ridges were observed in pods packed in LDPE bags of 25, 50 and 75 micron thickness stored at 12°C temperature. Irrespective of temperature, pods packed in rigid plastic crates had completely black ridges. Ridge blackening is a black coloration that normally occurs on bruised or damaged pods of okra starting at the ridges or ribs and later spreads to other parts of the pod (Katende and Ssonkko, 5). Physical damage (*i.e*., surface injuries, impact bruising and vibration bruising) is a major contributor to deterioration in fresh produce. It leads to browning or blackening of damaged tissue, accelerates water loss, provides site for microbial infections and stimulates carbon dioxide and ethylene production by the commodity. Singh *et al.* (10) observed blackening of ridges in unpackaged okra pods and attributed it to bruising and secondary microbial infection. Moisture loss further accelerates this blackening process. Okra pods packed in LDPE bags (25, 50 and 75 micron) and stored at 12°C had excellent appearance. On the other hand, pods packed in rigid plastic crates and kept at 12 and 16°C were found to be of acceptable quality.

Based on the above investigation it can thus be concluded that packing okra pods cv. VNR Green in LDPE bags of 50 micron thickness and storing them at a temperature of 12°C was the most effective treatment for extending shelf-life (12 days). It also resulted in maximum marketable pods of the best visual appearance.

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Received : January, 2014; Revised : January, 2016; Accepted : February, 2016

DOI : 10.5958/0974-0112.2016.00037.2

Indian J. Hort. 73(1), March 2016: 149-152

Short communication

Studies on standardization of recipe for sapota nectar and its storage

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ABSTRACT

Nectar from sapota cv. Kalipatti with varying juice levels (20, 25 and 30%), TSS (15 and 18°Brix) and acidity (0.3%) was prepared and evaluated for changes in chemical and sensory qualities at 0, 3 and 6 months of storage at room temperature. The increase in TSS, reducing sugars, total sugars and pH with decrease in titratable acidity were noticed during storage irrespective of treatments. As the juice content increased to 30 per cent in the nectar with 15 or 18°Brix TSS, the nectar showed higher values of reducing sugars. The nectar prepared with higher TSS and juice content exhibited higher level of total sugars than those with low TSS and juice level. The nectar prepared with 25 per cent juice and 18°Brix TSS had the highest overall acceptability and remained at par with the nectar having 20 per cent juice and 18°Brix TSS. A decline in acceptability of nectar in terms of colour, flavour, texture and taste was observed during entire period of storage.

Key words: Recipe, sapota nectar, storage.

Sapota [*Manilkara achras* (Mill) Fosberg] is one of the important tropical fruits grown in west coastal region of India. At present, India is the largest producer of Sapota, followed by Mexico, Guatemala and Venezuela. Sapota fruit is a rich source of sugar which ranges from 12 to 14 per cent (Sulladmath and Reddy, 6). Sapota fruits are used for the preparation of several value-added products. Sapota fruits can be used for the preparation of beverage like nectar. The acceptability of fruit beverages is very much dependant on the juice content as well as brix-acid ratio in beverage. Hence, a study was undertaken to evaluate different recipes for the preparation of sapota nectar and observe quality during storage under ambient conditions.

The fully matured sapota fruits cv. Kalipatti were procured from the orchard of Regional Horticultural Research Centre, ASPEE College of Horticulture and Forestry, NAU, Navsari, Gujarat. The well ripened fruits were peeled and sliced with stainless steel knife. The seeds as well as the central white core were removed and fruit pieces were chopped to obtain pulp. Later on, juice was extracted by squeezing the pulp through two-layer of muslin cloth. Sapota nectar was prepared as per the following treatments: T1 = 20% juice + 15°Brix TSS + 0.3% acidity, T2 = 20% juice + 18°Brix TSS + 0.3% acidity, T3 = 25% juice + 15°Brix TSS + 0.3% acidity, T4 = 25% juice + 18°Brix TSS + 0.3% acidity, T5 = 30% juice + 15°Brix TSS + 0.3% acidity and T6 = 30% juice + 18°Brix TSS + 0.3% acidity. The nectar was heated gently up to 80ºC and preserved with potassium meta bisulphite (140 mg/ kg) in pre-sterilized glass bottles (200 ml) and stored under ambient temperature conditions for 6 months. Total soluble solids, acidity, pH and sugars were estimated using the standard. The sensory evaluation of the product was done initially and at three months interval up to six months on hedonic scale by a panel of five judges. The data collected on changes in chemical composition of products and sensory qualities during storage were statistically analyzed by the standard procedure.

The changes in chemical constituents during storage are presented in Table 1. The total soluble solids in sapota nectar were significantly influenced by the various recipes. However, the differences were due to the initial level of TSS maintained in the sapota nectar. In the present investigation, the recipes with 18°Brix TSS (T2, T4, and T6) had higher level of mean TSS than those with 15°Brix TSS maintained at the time of preparation of the product. The recipes having same TSS levels, but with varying levels of juice content remained at par with each other. This evinced that the increasing the levels of the juice content in the product had no effect on the TSS content of the nectar. The TSS level in the nectar increased significantly during the storage period of six months. This could be due to the conversion of polysaccharides into simple sugars. Shere *et al.* (5) also reported gradual increase in TSS of *amla* RTS due to the conversion of polysaccharides present in the pulp into sugar during process of hydrolysis.

The titratable acidity of sapota nectar did not exhibit any variation. This has also resulted into the non-significant effect of the recipes on the pH level of the sapota nectar. However, the decreasing trend in acidity with corresponding increase in pH level in the sapota nectar was noticed during storage. The

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Table 1. Changes in chemical composition of sapota nectar during storage.

Treatment	Storage period (month)					CD @ 5%
	0	3	6	Mean		
			TSS (°Brix)			
T1	15.33	15.43	15.80	15.52	Tr.	0.38
T2	18.25	18.30	18.50	18.35	Period	0.27
T3	15.33	16.03	16.23	15.86	T × P	NS
T4	18.35	18.55	18.79	18.56		
T5	15.23	15.93	16.33	15.83		
T6	18.44	18.48	18.93	18.61		
Mean	16.82	17.12	17.43			
			Titratable acidity (%)			
T1	0.295	0.271	0.275	0.280	Tr.	NS
T2	0.300	0.280	0.279	0.286	Period	0.007
T3	0.295	0.278	0.270	0.281	T × P	NS
T4	0.295	0.274	0.280	0.283		
T5	0.295	0.273	0.274	0.280		
T6	0.299	0.281	0.275	0.285		
Mean	0.296	0.276	0.275			
			pH			
T1	3.23	3.39	3.46	3.36	Tr.	NS
T2	3.19	3.33	3.35	3.29	Period	0.045
T3	3.21	3.41	3.44	3.35	T × P	NS
T4	3.15	3.40	3.45	3.33		
T5	3.15	3.41	3.45	3.34		
T6	3.18	3.40	3.45	3.34		
Mean	3.18	3.39	3.43			
			Reducing sugars (%)			
T1	8.44	11.24	11.95	10.54	Tr.	0.11
T2	9.06	12.84	13.24	11.71	Period	0.08
T3	8.68	11.49	12.22	10.79	T × P	0.20
T4	9.16	12.92	13.38	11.82		
T5	8.87	11.65	12.41	10.97		
T6	9.54	12.85	13.77	12.05		
Mean	8.96	12.16	12.83			
			Total sugars (%)			
T1	14.30	14.22	14.54	14.35	Tr.	0.10
T2	16.91	17.28	17.41	17.20	Period	0.07
T3	14.40	14.49	14.97	14.62	T × P	0.18
T4	17.13	17.53	17.76	17.47		
T5	14.51	14.69	15.23	14.81		
T6	17.47	17.69	17.91	17.69		
Mean	15.79	15.98	16.30			

T1 = 20% juice + 15°Brix TSS, T2 = 20°Brix juice + 18°Brix TSS, T3 = 25% juice + 15°Brix TSS, T4 = 25% juice + 18°Brix TSS, T5 = 30% juice + 15°Brix TSS, T6 = 30% juice + 18°Brix TSS

Table 2. Changes in sensory qualities of sapota nectar during storage.

Treatment	Storage period (month)					CD @ 5%
	0	3	6	Mean		
			Colour			
T1	7.70	7.35	6.80	7.28	Tr.	0.12
T2	7.70	7.25	6.95	7.30	Period	0.09
T3	7.60	7.30	6.85	7.25	T × P	0.21
T4	7.55	7.30	6.85	7.23		
T5	7.30	7.30	6.30	6.97		
T6	7.65	7.00	6.20	6.95		
Mean	7.58	7.25	6.66			
			Flavour			
T1	7.20	6.90	6.60	6.90	Tr.	0.12
T2	7.50	7.35	6.75	7.20	Period	0.08
T3	7.35	7.20	6.85	7.13	T × P	0.21
T4	8.30	7.75	7.40	7.82		
T5	7.40	7.30	6.80	7.17		
T6	7.75	7.40	7.00	7.38		
Mean	7.58	7.32	6.90			
			Texture			
T1	7.60	7.55	7.30	7.48	Tr.	0.14
T2	7.65	7.30	7.10	7.35	Period	0.10
T3	7.15	6.85	7.00	7.00	T × P	0.24
T4	7.10	7.25	6.90	7.08		
T5	6.80	6.75	6.65	6.73		
T6	7.00	6.70	6.70	6.80		
Mean	7.22	7.07	6.94			
			Taste			
T1	7.35	6.90	6.60	6.95	Tr.	0.11
T2	7.75	7.45	7.10	7.43	Period	0.08
T3	7.20	7.10	6.75	7.02	T × P	0.19
T4	8.15	7.70	7.25	7.70		
T5	7.35	7.10	6.90	7.12		
T6	7.75	7.20	7.10	7.35		
Mean	7.59	7.24	6.95			
			Overall acceptability			
T1	7.36	7.18	6.83	7.12	Tr.	0.07
T2	7.65	7.34	6.98	7.32	Period	0.05
T3	7.33	7.11	6.86	7.10	T × P	0.12
T4	7.78	7.50	7.10	7.46		
T5	7.22	7.12	6.66	7.00		
T6	7.54	7.08	6.75	7.12		
Mean	7.48	7.22	6.86			

T1 = 20% juice + 15°Brix TSS, T2 = 20°Brix juice + 18°Brix TSS, T3 = 25% juice + 15°Brix TSS, T4 = 25% juice + 18°Brix TSS, T5 = 30% juice + 15°Brix TSS, T6 = 30% juice + 18°Brix TSS

decrease in the acidity could be due to utilization of acids in the conversion of polysaccharides into hexose sugars. Similar results were reported by Tandon *et al.* (7) in the blended *bael* beverages.

The variation in the reducing as well as total sugars in the sapota nectar was mainly due to the initial levels of TSS adjusted at the time of the preparation of sapota nectar and the juice content in the nectar as well. In general, reducing sugars in the nectar increased significantly with the advancement of storage period. Highest conversion into reducing sugars was observed in the recipe having higher level of TSS (18°Brix) and juice (30%). When the juice content increased to 30 per cent in the nectar with either 15 or 18°Brix TSS, the higher levels of reducing sugars in the nectar was noticed (T6 and T5). This might be attributed to more availability of polysaccharides and non-reducing sugars in these treatments for conversion into reducing sugars. The nectar recipes with higher TSS and juice content showed higher levels of total sugars than those with low TSS and juice level. Improvement in the total sugars up to six months could be due to the conversion of polysaccharides into simple sugars and degradation of the pectic substances. Nectar with 20 per cent pulp, 15°Brix TSS and 0.5% acidity prepared from Dashehari mango by Altaf *et al*. (1) exhibited the increasing trend in reducing as well as total sugars content during storage period of five months.

It was observed that the sensory parameters (Table 2) except colour were significantly influenced by different nectar recipes, whereas other than texture, all other organoleptic parameters exhibited variation due to the storage period. The colour acceptability score did not vary according to the recipes, but it declined significantly during entire period of storage irrespective of the recipe treatments. The sapota nectar with 25 per cent juice and 18°Brix TSS (T4) was most acceptable recipe with respect to flavour owing to the optimum level of juice and TSS in the product. Even though the recipe T6 that had higher (30%) juice level than the recipe T4, the product secured lower score than the recipe T4 due to the intense flavour of the nectar. The flavour acceptability of the sapota nectar could be improved by increasing the juice level to the tune of 25 to 30 per cent as evinced by the flavour scores of the treatments T3 and T5, which were at par with T2 and T6 having high TSS. In the present study, a significant decline in flavour acceptability score has been observed with the advancement of storage period. This decline in flavour might be due to loss of typical aroma owing to the reactions of acids with other constituents especially the polyphenols. The recipes with low (20%) juice content, *i.e.* T1 and T2 exhibited considerably high sensory score for the texture as compared to those with either 25 or 30 per cent juice level in the product. Increasing the juice level in the nectar caused more sedimentation of particles affecting the texture acceptability of the product. The texture acceptability score, however, was not affected by the storage period. The sensory score for taste of the nectar was greatly influenced by the TSS content as evinced by the higher taste acceptability score secured by T4, T2 and T6 with 18°B TSS. This has been due to optimum level of brix-acid blend in these recipes that enhanced the taste of the nectar. This clearly indicates that the taste of the product could be improved by maintaining the TSS of 18°B with either 20 or 25 per cent juice level in the nectar. The present findings are supported by Chaudhari (2). Taste acceptability score of the sapota nectar declined significantly during storage. It could be due to loss of delicate flavour of sapota and the reduction in acid level in the nectar.

As regards overall acceptability score, the sapota nectar with 18°Brix TSS was extremely liked by the panelist except T6 with 30% juice + 18°Brix TSS with higher overall acceptability scores.

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Received : May, 2015; Revised : December, 2015;
Accepted : January, 2016

DOI : 10.5958/0974-0112.2016.00038.4

Indian J. Hort. 73(1), March 2016: 153-156

Short communication

Quality evaluation and storage stability of *jamun*-mango blended squash

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ABSTRACT

Utilization of *jamun* fruit for preparing *jamun*-mango blended squash and their effects on physico-chemical changes and sensory qualities was investigated for six months of storage. *Jamun* and mango juices were blended in the ratio of 100% *jamun* juice as control, 90:10:: *jamun*:mango, 80:20:: *jamun*:mango, 70:30:: *jamun*: mango and 60:40:: *jamun*: mango. During six months of storage, total soluble solids increased whereas acidity decreased slightly. Treatment T_4 (70:30:: *jamun*-mango) blend was having maximum anthocyanin contents which decreased from the initial levels of 46.10 to 42.80 mg/100 g during six months of storage. Iron contents decreased throughout the storage period. The result shows that squash prepared from the treatment T_4 (70:30:: *jamun*:mango) was adjudged the best on the basis of sensory attributes which scored 7.62, 7.84, 7.96 and 7.80 points, as per hedonic scale for colour, aroma, body and overall acceptability. No microbial count was detected in different blended squashes upto four months of storage. After six months of storage, microbial count of 2×10^6 CFU per ml was observed in treatments T_1 (100:0:: *jamun*:mango) and T_2 (90:10:: *jamun*:mango) and a count of 1×10^6 CFU per ml was observed in treatments T_3 (80:20:: *jamun*:mango) and T_5 (60:40:: *jamun*:mango) and the count was considered to be in safe zone.

Key words: Jamun, mango, squash, blend, storage.

India has emerged as the 2nd largest producer of fruits and vegetables in world. In fruit production, it ranks next to China and produced 88,977 MT of fruits and 1,62,897 MT of vegetables (Anon, 2). Unfortunately, a big chunk (20-30%) of this hard earned valuable produce goes waste due to inadequate post harvest infrastructure and poor utilization (1.8%) by processing industries (Verma and Joshi, 15). Though with the efforts of Ministry of Food Processing Industry, New Delhi, the growth of this sector is accelerated, however, there is need to discuss and sort out various related issues amongst people of various categories to increase level of value-addition and improve the quality of value added food products for domestic market as well as for export (Anon, 3).

Jamun (*Syzygium cumini* L.) fruit can be eaten raw and processed for making syrup, nectar, ready-to-serve, squashes, sauces and jam. *Jamun* fruit is a good source of iron, vitamin C and E. Its fruit is delicious and is of wider interest for its medicinal application than for its edible fruit. Similarly, mango fruit is reported to be rich in minerals, vitamins (Gopalan *et al*., 8). Due to their perishable nature of both *jamun* and mango, they require immediate processing to avoid post-harvest losses. As *jamun* juice is acidic as well as astringent and therefore not generally preferred, hence blending of two or more juices and preparation of beverages is thought to be a convenient alternative for utilizing them in developing some value-added fruit drinks, which will be of high quality in respect to both sensory and nutritional aspects.

Secondly, it is reported that blending of fruit juices helps in improving nutrient elements, reducing cost of production by using cheaper fruits in the blends and also leads to new product development (Kalra *et al*., 11). Moreover, there is always a demand from the consumers all over the world for new products, which should be nutritious and delicately flavoured. Keeping in view the above, the present investigation has been undertaken with the objectives to develop value added products from *jamun*-mango blends and to study storability of the finished product.

The study was conducted at pilot plant of the Division of Post Harvest Technology, Sher-e-Kashmir University of Agricultural Sciences and Technology, Udheywalla, Jammu during the year 2010-11 and 2011-12. Mature fruits of *jamun* (*Syzygium cumini* L.) were obtained from the avenue trees of R.S. Pura orchard and mangoes (cv. Dashehari) were purchased from Fruit Trans-Shipment Centre (FTC), Narwal, Jammu. Both *jamun* and mango fruits were transported to the pilot plant of the Division of Post Harvest Technology, for further processing. The *jamun* fruits were washed in water, crushed for the extraction of juice and on the other hand mango fruit it was washed, peeled and passed through the pulper for obtaining the pulp. The pulp so obtained was

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passed through stainless steel strainer, homogenized followed by heating at 85°C for 30 sec.

Squash was prepared as per the procedure given by Jain *et al.* (10). Fruit juices of *jamun* and mango were mixed in different ratios for squash preparation such as T_1: (100:00:: *jamun*-mango blend), T_2: (90:10:: *jamun*-mango blend), T_3: (80:20:: *jamun*-mango blend), T_4: (70:30:: *jamun*-mango blend) and T_5: (60:40:: *jamun*-mango blend). Desired quantity of sugar and citric acid were dissolved properly in warm water, strained through cheese muslin cloth and mixed properly with *jamun*-mango blends so as to maintain total soluble solids as 48°Brix and acidity as 1.3% and preserved with 600 ppm of sodium benzoate. The squash prepared was filled in pre-sterilized glass bottle crown corked, processed for 30 min. in boiling water, cooled immediately, labelled and stored at room temperature. The squash was analyzed at various interval of 0, 2, 4 and 6 months for physico-chemical, organoleptic and micro-biological parameters as per the method given by Harrigan and McCance (9).

Total soluble solids of fresh fruits and processed products were determined using hand refractometer and reading expressed as degree Brix (°Brix) at 20°C using reference table (Ranganna, 12). Titratable acidity was determined by titrating a known quantity of sample (10 g) against standardized solution of 0.1N sodium hydroxide to a faint pink colour using phenolphthalein as indicator. The result was expressed as percent citric acid (AOAC, 4).

Anthocyanins content were estimated according to the method given by Swain and Hillis (14). Ten ml of sample was centrifuged for 15-20 min. A measured aliquot of 0.2 ml of sample was taken in 10 ml test tubes to which 3.8 ml of methanolic hydrochloride acid and 1 ml of anthocyanin reagent was added. A blank sample was made in the same manner without using sample. The extracted samples were kept undisturbed for 15 min. and OD was recorded at 525 nm wavelength using UV-spectrophotometer.

A known quantity (5 ml) of pre-digested aliquot was taken, to which 0.5 ml conc. H_2SO_4, 1.0 ml potassium per sulphate and potassium thiocyanate 2 ml were added and volume was made to 15 ml with double-distilled water. After 20-30 min. the absorbance was recorded at 480 nm. The iron content was calculated by plotting against the standard curve obtained by taking known amounts of potassium iron solution. Results were express as mg/100 g (AOAC, 4). The samples were evaluated on the basis of colour, body, flavour/aroma and overall acceptability by semi-trained taste panels of 6-7 judges using 9 point hedonic scale assigning scores 9–like extremely to 1-dislike extremely. A score of 5.5 and above was considered acceptable (Amerine *et al.*, 1).

The data obtained was analyzed statistically using Completely Randomized Design (CRD) and CRD factorial for interpretation of results though analysis of variance. Five treatments and three replications were used for the present data of jamun: mango blended squash.

The results showed that during 6 months of storage total soluble solids (TSS) of *jamun*-mango blended squash increased gradually from the initial levels of 48.00 to 50.39°Brix, which might be due to partial hydrolysis of complex carbohydrates (Chakrabraborty *et al*., 6). Similar results were also reported by Dayal and Srivastava (7) in cape gooseberry squash. During six months of storage mean titratable acidity declined significantly from the initial levels of 1.3 to 1.26 per cent (Table 1). The

Table 1. Effect of treatments and storage period on total soluble solids and titratable acidity of *jamun*-mango blended squash.

Treatment	Total soluble solids (°Brix)				Mean	Titratable acidity (%)				Mean
	Storage period (months)					Storage period (months)				
	0	2	4	6		0	2	4	6	
T_1 (100:0:: *Jamun*:mango)	48.00	48.56	49.51	50.15	49.06	1.3	1.29	1.26	1.24	1.27
T_2 (90:10:: *Jamun*:mango)	48.00	48.69	49.68	50.34	49.19	1.3	1.30	1.27	1.26	1.28
T_3 (80:20:: *Jamun*:mango)	48.00	48.75	49.82	50.36	49.25	1.3	1.30	1.27	1.26	1.28
T_4 (70:30:: *Jamun*:mango)	48.00	48.67	49.57	50.26	49.14	1.3	1.29	1.28	1.27	1.29
T_5 (60:40:: *Jamun*:mango)	48.00	48.93	49.96	50.82	49.44	1.3	1.30	1.27	1.25	1.28
Mean	48.00	48.72	49.71	50.39		1.3	1.29	1.27	1.26	
CD (P = 0.05)										
Treatment		0.03		NS						
Storage		0.02		0.01						
Treatment × Storage		0.05		NS						

Table 2. Effect of treatments and storage period on anthocyanins and Iron contents of *jamun*-mango blended squash.

Treatment	Anthocyanins (mg/100 g)					Iron (mg/100 g)				
	Storage period (months)					Storage period (months)				
	0	2	4	6	Mean	0	2	4	6	Mean
T_1 (100:0:: *Jamun*:mango)	51.40	49.80	46.50	42.50	47.55	0.31	0.30	0.29	0.27	0.29
T_2 (90:10:: *Jamun*:mango)	49.30	46.70	42.70	38.90	44.40	0.29	0.28	0.27	0.26	0.28
T_3 (80:20:: *Jamun*:mango)	47.80	46.20	43.40	41.30	44.68	0.27	0.27	0.26	0.24	0.26
T_4 (70:30:: *Jamun*:mango)	46.10	44.70	43.90	42.80	44.38	0.24	0.24	0.23	0.23	0.24
T_5 (60:40:: *Jamun*:mango)	45.70	43.70	41.70	36.90	42.00	0.22	0.21	0.20	0.18	0.20
Mean	48.06	46.22	43.64	40.48		0.27	0.26	0.25	0.24	

Effect	CD (P = 0.05)	CD (P = 0.05)
Treatment	1.65	0.02
Storage	1.48	0.02
Treatment × Storage	N.S.	N.S.

decline in acidity might be due to conversion of some amount of acids to sugars (Babsky *et al.*, 5). Similar findings have been reported by Chakrabraborty *et al.* (6) in litchi beverage, respectively. During storage (6 months), the anthocyanins content decreased in all the treatments because the pigment is heat liable and it decreased from the initial mean levels of 48.06 to 40.48 mg/100 g, which might be due to hydrolysis of protective 3-glycoside linkage to give unsuitable anthocyanins. These findings are in agreement with the findings of Waskar and Khurdiya (16) in *phalsa* beverage. The maximum iron contents of 0.31 mg/100 g were recorded in treatments T_1(100:0:: *jamun*-mango. The data in Table 2 revealed that with the advancement of storage period iron contents of *jamun*-mango blended squash decreased from the initial levels of 0.27 to 0.24 mg/100 g, respectively. The possible reason for the decrease might be due to its heat sensitiveness even at the ambient temperature, which causes the destruction of minerals during storage. Similar results have been reported by Sharma (13) in guava-papaya RTS beverage.

The samples were evaluated organoleptic attributes for colour, taste, flavour and overall acceptability. Colour is important sensor character on which the consumer preferences dependent. The treatment T_4 (70:30:: *jamun*: mango) received the highest score of 8.30 for body, 8.23 for colour, 8.35 for aroma and 8.32 for overall acceptability. As for as the overall acceptability score of the squash was concerned it declined significantly from the initial mean level of 7.90 to 7.53 and to 7.30 after four and six months of storage period. In general, decrease in sensory scores of different characteristics of *jamun*-mango blended squash, irrespective of treatments during storage might be attributed to changes in their objective characteristics like loss of colour pigment, breakdown in soluble solids change in sugar-acid ratio. This probably might have affected the organoleptic score of the product. These findings are in conformity with the findings of Jain *et al*. (10) in litchi squash.

No microbial count has been detected in different blended squashes upto four months of storage. However, a count of 2×10^6 (CFU/ ml) was detected in treatments T_1: (100:0:: *jamun*: mango) and T_2 (90:10:: *jamun*: mango) and a count of 1×10^6 (CFU/ ml) was detected in treatments T_3 (80:20:: *jamun*: mango) and T_5 (60:40:: *jamun*: mango) after six months of storage and the count was considered to be in safe zone. Similar finding have also been noted by Chakrabraborty *et al.* (6) in litchi squash during storage at ambient temperature.

Studies conducted on different treatment combination of *jamun*-mango on quality and storability of squash shows that T_4 (70:30::*jamun*:mango) was adjudged the best by way of retaining the maximum anthocyanin content after 6 months of storage (Table 3). The same treatment scored maximum points for aroma, body, colour and overall acceptability after 6 months of storage.

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Table 3. Effect of treatments and storage period on mean score evaluation of overall acceptability of *jamun*-mango blended squash.

Treatment	Storage period (months)				Mean
	0	2	4	6	
T_1 (100:0:: *Jamun*:mango)	7.84	7.66	7.57	7.24	7.58
T_2 (90:10:: *Jamun*:mango)	7.66	7.46	7.26	7.16	7.39
T_3 (80:20:: *Jamun*:mango)	7.64	7.28	7.24	7.06	7.30
T_4 (70:30:: *Jamun*:mango)	8.66	8.54	8.26	7.80	8.32
T_5 (60:40:: *Jamun*:mango)	7.72	7.54	7.34	7.25	7.46
Mean	7.90	7.69	7.53	7.30	
CD (P = 0.05)					
Treatment	0.05				
Storage	0.05				
Treatment × Storage	0.10				

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Received : March, 2015; Revised : January, 2016; Accepted : February, 2016

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(Platinum Jubilee Celebrations of HSI Congress)

7th Indian Horticulture Congress-2016

(15-18th, November 2016)
New Delhi

Title: Shaping India's Horticulture Future

Background

The R&D efforts made in the last three decades have impacted Horticulture in the country in many ways. The importance of horticulture is being realized by one and all be it a small farmer, the corporate sector and policy makers at all levels. There is now a large R&D network in the country with several institutions and implementation of number of developmental programmes. Horticulture has come out of village confines to the urban areas & corporate sector as an organized enterprise.

Horticulture crop production surpassed food crop production in India for first time during 2013-14. The trend continued during 2014-15 with 283.5 MT. Share of horticulture output in agriculture has increased to more than 33%. There has been highest annual growth of 9.5% in fruit production (2013-14) and annual growth of 7% in vegetable production (1991-92 to 2014-15). Cut flower production has increased significantly. A virtual revolution has taken place in potato production too.

Indian horticulture has been gradually penetrating in the International market with significant increase in export of different horticulture commodities. Export of horticulture produce has risen significantly 536.42 times in quantity and 2007.80 times in value (1991-92 to 2012-13). The share of horticulture output in agriculture GDP is now more than 33 per cent. The impact of the above initiatives has become quite visible and their role in development of this sector has been recognized in our country. As a consequence the Hon'ble Minister of Finance, Govt. of India, during his speech on unraveling Economic Survey 2015-16 in the parliament made a special mention about the achievements made in this sector in the country.

Despite, ample increase in production of the horticultural crops, the low productivity and profitability in several crops is still a challenge and is required to be attended in order to doubled the income of small farmers and provide ample opportunities to youth. The role of Hi-tech horticulture technologies are thus required to be continuously updated and given to the famers leading to science-led development.

Earlier Congresses

To achieve the objectives, the Horticultural Society of India has been organizing biennially Indian Horticulture Congresses in different states of the country. To sensitize various stake-holders of the latest technologies and opportunities, these congresses are very well attended and found useful by the participants. The society has been organizing congresses biennially since 2004. The six earlier congresses on different topical issues were successfully organized at below:

- First Congress on ***Improving Production, Productivity, Quality and Trade of Horticulture Crops*** held at New Delhi in 2004
- Second Congress on ***Opportunities and Linkages for Horticulture Research and Development (Focus: North Eastern Region)*** held at Barapani, Meghalaya in 2007
- Third Congress on ***New R & D Initiatives in Horticulture for Accelerated Growth and Prosperity*** held at Bhubaneswar, Orissa in 2008
- Fourth Congress on ***Horticulture to Horti- Business*** held at New Delhi in 2010
- Fifth Congress on ***Horticulture for Food & Environment Security*** held at Ludhiana, Punjab in 2012
- Sixth Congress on ***Horticulture for Inclusive Growth*** held at Coimbatore, Tamil Nadu in 2014

Themes/ Topics

The following sessions & themes have been provisionally identified for the 7th Indian Horticulture Congress-2016:

I. **Biotechnological Intervention in Horticulture:** Genomics; Proteomics, Phenomics and Metablomics in Horticulture Crops; Molecular Breeding; Development of Transgenics; Regulatory issues

II. **Improving Productivity & Profitability:** Controlling Plant architecture/ Canopy Management; Flower regulation; Adaptation to Climate Change; Grafting in annual horticulture crops; Diagnostics for disease free planting material

III. **Precision Horticulture & Efficient Input Management:** Soil health management; Enhancing nutrient use efficiency; Micro nutrient management; More crop per drop; Fertigation; Production and availability of bio-fertilisers, bio-pesticides & Biologicals; Nanotechnology; GIS/ remote sensing in crop production; Mechanisation; Use of Solar energy (non-conventional energy sources) & Robotics

IV. **Pests, Diseases and Physiological Disorders of national importance:** Phytophthora; Fruitfly; Potato blight; Bacterial blight in pomegranate; Mango decline and wilt; Management of Viral diseases; Sucking pests; Borers & Bud rot: Pests management in green house;

V. **Diversifying Horticulture Production Systems :** Protected Horticulture; Urban and Peri-urban Horticulture; Hydroponics and Aeroponics; Organic Horticulture & Establishing Horticulture Estates

VI. **Post harvest Management and Utilisation of Horticulture Produce & products through value addition:** Dehydration of Horticulture Crops; Functional foods & Storage innovations and cold chain; Overcoming malnutrition (Nutrients- Starch, fat, Vitamins, Pectins and Lycopene)- Bio-fortification; Neutraceuticals for Health; Colour & Dyes from Horticulture plants; Oleoresins from spices; Essential oils from aromatic crops & Carbon sequestration (green energy)

VII. **Marketing and Trade:** Self Help Groups (SHG); Farmer Producer Companies (FPC); Agriculture Products Marketing Company (APMC); Role of Co-operatives; Contract farming; Price stabilization; Imports and Exports; Minimum support price & PHM Infrastructure

VIII. **Attracting youth to Horticulture (Emerging opportunities for youth):** Mushroom Production; Fruit Nursery production; Plug plant production in annual crops; Production of bioagents; Apiary and Honey production; ICT; Skill Development; Seed production of vegetables; Horti-tourism & other start-ups in Horticulture

IX. **Policy Issues:** Insurance Scheme; Food Safety Standards; GAP (EUREPGAP), CODEX, AGMARK etc; Organic standards and Ensuring finance & credits

Participants

The congress aims is open to all interested in horticulture R&D and related sectors, namely representatives from both public and private sectors, central and state government ministries, scientists and students from agricultural institutes and universities, farmers, representatives from international agencies, farmers' associations, agri-input associations (like Seeds, Fertilizers, Plant Protection chemicals, Grower's association, etc), NGOs, etc. The deliberation of the Congress shall be in English.

Presentation

The Congress will cover Lead, Oral and Poster Presentations.

Lead Papers: Lead papers on specific topics related each session will be invited by the Programme Committee on the basis of suggestions received. These will be allotted to eminent scientists. The extended abstracts (about 2 pages) of these papers are required to be submitted within a fortnight of request, while the full-length papers will be required to be submitted latest by 15 September, 2016 for publication of proceeding and release during the inaugural session.

Oral Presentations: Both members and non-members of HSI within India and abroad shall be considered for oral presentation subject to the relevance of the title and experience of the scientist related to the subject to the presentations shall be state of the art report.

Poster Papers: The researchers/students are invited to submit abstracts (max two as senior registered author) relating to broad theme areas of the congress, which would be peer reviewed and presented as poster papers in the four day event. Abstract(s) of only registered authors will be published.

The abstract should be prepared in MS word not exceeding 250 words. It must contain a clear title, name and affiliation of the authors. The name of the presenting author should be underlined and E-mail should be given at the end. There should not be any sub-headings, figures, tables or references in the abstract. The abstract may be submitted through email and/or by post along with a soft copy. The detailed specifications for preparing the poster paper (size $4^{1/2}$' x 3'), would be mailed to those authors whose abstracts are accepted for presentation.

The poster presentation will be considered under different theme areas as per the following schedule:

Important Dates

Last date for receiving abstract(s)	: August 31, 2016
Last date for sending acceptance letter	: September 15, 2016
Last date for sending registration fee (without late fee)	: September 30, 2016

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Details of Registration Fee for various categories of participants are as under:

Category	On time	With late fee
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Place of publication	–	New Delhi, India
Periodicity of publication	–	Quarterly
Printer's name	–	Shri Vinay Malhotra
Whether citizen of India	–	Yes
Address	–	B-6, DSIDC Packaging Complex, Kirti Nagar, New Delhi-110 015, India
Publisher's name	–	Dr K.L. Chadha
Nationality	–	Indian
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***Review/ Strategy paper*:** This article is received through invitation. It should be comprehensive, up-to-date and critical on a recent topic of importance. The maximum page limit is of **16 double-spaced typed pages** including tables and figures. It should cite latest literatures and identify some gaps for future. It should have a specific **Title** followed by the **Name(s) of the author(s)**, **Affiliation**, **Abstract, Key words,** main text with subheadings, **Acknowledgements** (wherever applicable) and **References**.

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Abstract: A concise summary (200 to 300 words) of the entire work done along with the highlights of the findings.

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Materials and methods: Describe the materials used in the experiments, year of experimentation, site etc. Describe the methods employed for collection of data in short.

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Published by Dr K.L. Chadha and edited by Dr S.K. Singh for the Horticultural Society of India, F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi 110 012, India and printed at Malhotra Publishing House, B-6, DSIDC Packaging Complex, Kirti Nagar, New Delhi 110 015, India.