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Genetic diversity of Algerian fig (*Ficus carica* L.) cultivars based on morphological and quality traits

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ABSTRACT

Assessment of the genetic diversity of 11 fig (*Ficus carica* L.) cultivars using 45 traits using international morphological descriptors was undertaken. The results show that 20 quantitative variables and 7 qualitative variables were useful for discrimination of cultivars. Skin thickness, length and fruit width were the more discriminating pomological variables. In terms of total soluble solids, the levels obtained (13.56-25.12%), constitute an index of high quality for all cultivars. The principal component analysis indicates that 61.38% of the total variability involve the first three components. Cluster analysis divided the 11 cultivars into 4 sub-groups characterized by a narrow genetic base. The colour of the fruit was not sufficient for their differentiation, while the cultivars Bakor Blanc and Bakor Noir present a case of homonymy. Some cultivars (Chetoui, Benacer, Taranimt, Toudjente, Bakor Blanc and Meroudji) are attractive to growers due to the good fruit quality traits. The use of morphological descriptors has proved a more suitable means to assess the genetic diversity in Algerian fig genotypes.

Key words: Clusters analysis, cultivars, descriptors, fig.

INTRODUCTION

The common fig (*Ficus carica* L., 2n = 26) is one of the oldest plants to be grown by man, even before wheat (Kislev *et al.*, 8). This species grows in a wide range of soils and adapts very well to Mediterranean basin. Fig is ubiquitous in Algeria, especially in the center of the country where it is found in abundance and in different cultivars. The names of the cultivars are often associated with the location of culture or to the fruit characteristics and some of them produce excellent quality fruits. Despite its importance, the cultivation of the tree is still considered as a secondary interest and local cultivars face recurrent problems of confusion in their names and genetic vulnerability. Currently, 1 caprifig, 6 local and 16 foreign cultivars are recorded, authorized in the market and grown in the country (ITAFV, 7). However, these figures are not sure as the identification of the genetic resources of the fig in Algeria and the exchange of data between the different operators are non-existent. Moreover, apart from some preliminary studies, the local germplasm of this species did not attract the interest of researchers and still remains unexplored. In this context, surveys and research are needed to identify and characterize the genetic resources of this fruit crop. Different methods of analysis exist, but in the lack of molecular tools, the use of phenotypic markers becomes an appropriate alternative. This research aims to analyze the genetic diversity of 11 local fig cultivars using international descriptors.

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MATERIALS AND METHODS

This study concerns an *ex situ* fig collection located at the agricultural farm of Hassiba Benbouali, University of Chlef, Algeria (altitude 109 m, latitude 36°10'N, longitude 01°14'E). The climate of Chlef is a typical Mediterranean climate, with relatively wet and cold winters and hot, dry summers. The average annual temperature is 19.3°C. Thermal amplitudes are 30.80°C in summer and 9.40°C in winter. The average annual rainfall is 552 mm and occurs mainly from November to April. The orchard floor is a clay-loam texture with a pH of 8.3.

Eleven cultivars (Taranimt, R'dani, Toudjente, Kadota, Chetoui, Benacer, Enk El H'mam, Bezoult Rhadem, Bakor blanc, Bakor noir, Meroudji), each one represented by a tree, have been studied over two years (2013 and 2014). Trees were planted in 2009 at a spacing of 6 m × 6 m. They were conducted in a free form and received the same cultural maintenance. For the analyses, we collected data from each tree 36 adults leaves and 36 mature fruits (2nd crop). There were three replicates each consisting of 12 fruits. Sampling was carried out at various sites on the tree periphery on 1-year-old shoots.

A set of 45 traits (25 quantitative and 20 qualitative) were measured or classified according to the fig descriptors of IPGRI and CIHEAM (6) including three additional traits related to leaf apex shape, beginning of defoliation and internal pulp thickness (Basheer-Salimia *et al*., 2). Quantitative characters analyzed include the leaf length, the fruit weight, the number of leaves and fruits per shoot and the ostiole width. Qualitative character such as tree vigour, beginning of maturity, harvest time, colour of the internal pulp, and fruit shape index were determined. Dimensional properties were performed with a scale sensitive to 0.01 g (Precisa XB 2200 C, UK) and with digital calipers (0-150 mm; BTS Tools, Malaysia). The total soluble solids were determined by a hand-held refractometer (NOW, 0-32% Brix). The titratable acidity, and TSS : acid ratio were calculated.

The analysis of variance (one-way ANOVA) was performed to assess the variations between the 11 cultivars. The mean values were separated by Duncan's multiple range test at a level of $p \leq 0.05$. To establish multivariate analyzes among cultivars a principal component analysis (PCA) was performed. The PCA factor was accepted when the means values were superior than 1. Recorded factors equal or greater than 0.7 were considered as a strong correlation between the principal component and the descriptive characters. To assess the similarity among cultivars, a hierarchical cluster analysis was performed by measuring the Euclidean distance using the Ward's method. The software used was SPSS 10.0.

RESULTS AND DISCUSSION

The Analysis of variance revealed significant differences at a threshold of 95% level among cultivars for most characters. The average values of characters related to trees, branches and leaves are shown in Table 1. Our results show that tree vigour, the relative degree of branching, the colour of the terminal bud and the beginning of fruits maturity depended on cultivar. The average values of pomological variables are summarized in Table 2. They show that the skin thickness, the length and the width of fruits were more discriminating among all the analyzed parameters.

The principal component analysis concerns the branches characters, buds, leaves and fruits (Table 3, Fig. 1). It indicates that the first three axes concern 61.38% of the total variability. The first axis (PC1) shows 3 game variables positively correlated (the tendency to form suckers; the leaf shape apex; the width, the shape and the fruit weight; the length and the width of the neck; the stalk width; the ease of peeling; the pulp internal thickness) and 3 negatives (the tree vigour; the length and the shape of the fruit).

Table 1. Mean values and significance degree of differences between eleven fig cultivars for morphological traits.

Genotype	PLL	SLB	RDB	LM	LC	BOD	DLL	NFS	TFS	LAS	TV	LLN	HP	TGH	PT (mm)	TBC	BFM	LCL (cm)	NLS	LW (cm)	LL (cm)	PL (mm)	LA (cm^2)
Taranimt	0,34a	2ab	2,5abc	2,5abc	3abc	3abc	3abc	3,355abc	3,5abc	3,5abc	3,5abc	3,54abc	4abc	5abc	5,25abc	5,5abc	5,5abc	8,38abcd	16,1bcd	17,105cd	19,485d	66,4e	338,81f
R'dani	0,355a	2,5a	4ab	3,5a	3,5a	2a	3,5a	3,135a	3,5a	4,5ab	3,5a	4,3ab	4ab	4,...cccc, 4; 5ab	5,645abc	3,5a	5ab	10,48bc	11,65c	18,35d	21,61d	77,005e	399,06f
Toudjente	0,3a	4ab	2,5ab	3,5ab	3,5ab	3ab	4,5ab	3,315ab	3,5ab	2,5ab	2ab	4,17ab	5b	4,5ab	5,96bc	2ab	3ab	9,36c	15,385d	18,075de	19,915e	59,49f	367,215f
Kadota	0,385a	2a	4a	3a	2,5a	3a	3a	5,46a	3,5a	3,5a	3,5a	2,765a	5a	5a	5,56a	3,5a	5,5a	8,385a	13,175a	18,53a	20,675a	80,79b	401,28c
Chetoui	0,405a	3ab	5abc	4,5abc	2,5ab	3ab	3ab	3,625ab	3,5ab	2a	3,5ab	4,715abc	3,5ab	4,5abc	4,775abc	5,5abc	4ab	9,87abc	11,58c	18,075d	19,35d	78,43e	355,95f
Benacer Enk El H'mam	0,345a	3,5a	2,5a	3,5a	3a	3a	5,5a	7,885a	2,5a	4a	4a	4,94a	4a	4,5a	4,875a	2a	2a	9,05a	16,065a	17,07a	17,935a	62,935b	315,195c
Bezoult Rhadem	0,33a	2,5a	4ab	2a	3a	3a	3a	3,25ab	3,5ab	3,5ab	3,5ab	3,455ab	4ab	5ab	5,305ab	3,5ab	3a	10,635ab	12,05ab	18,015ab	21,295b	70,315c	390,97d
Bakor noir	0,34a	3a	5a	4a	3,5a	2a	3,5a	2,175a	3,5a	4a	4,5a	4,715a	4a	4a	4,635a	3,5a	5a	9,685a	14,285a	17,045a	19,8a	68,15b	346,585c
Bakor blanc	0,375a	4ab	2,5ab	4ab	2a	3ab	4ab	3,37ab	2a	2,5ab	4ab	4,225ab	2a	3,5ab	5ab	2,5ab	2a	10,965ab	7,28ab	15,35ab	20,695b	79,59c	380,185c
Meroudji	0,325a	2abc	5bc	3abc	3abc	2abc	3abc	1,675ab	3,5abc	3,5abc	4,5bc	4,26bc	4abc	3abc	4,385bc	5,5c	5,5c	11,035d	5,05bc	16,975e	19,885e	65,735f	344,72g
F-Value	0,27a	3,5abc	4abc	4,5abc	3,5abc	3abc	4abc	0,27ab	3,5abc	4abc	3,5abc	3,57abc	3,5abc	4abc	4,305abc	4,5abc	4abc	8,59c	7,5bc	16,64d	18,08d	48,65e	304,65f
	6,496**	1,625NS	12,300**	1,053NS	1,100NS	0,436NS	0,800*	6,291**	0,655NS	1,230NS	0,7143*	2,703**	0,6714*	0,180NS	3,505*	8,644**	1,753*	4,679**	2,452*	0,873*	1,233NS	11,772**	0,842*

Means within column followed by the same letters are not significantly different at P<0.05

NS: not significant; * and ** are statistically significant at P<0.05 and P<0.01 respectively, according to Duncan's multiple range test.

PLL: Petiole length / Length of leaf; **SLB**: Shape of leaf base; **RDB**: Relative degree of branching; **LM**: Leaf margin; **LC**: Leaf colour; **BOD**: Beginning of defoliation; **DLL**: Degree of leaf lobation / incision; NFS: Number of fruits per shoot; **TFS**: Tendency to form suckers; **LAS**: Leaf apex shape; **TV**: Tree vigour; **LLN**: Leaf lobes number; **HP**: Harvesting period; **TGH**: Tree growth habit; **PT**: Petiole thickness; **TBC**: Terminal bud colour; **BFM**: Beginning of fruit maturation; **LCL**: Length of central lobe; **NLS**: Number of leaves per shoot; **LW**: Leaf width; **LL**: Leaf length; **PL**: Petiole length; **LA**: Leaf area.

Table 2. Mean values and significance degree of differences between eleven fig cultivars for pomologial traits.

Genotype	TAC (%)	FSI	FSCK	OW (mm)	FSF	FST (mm)	FS	EPE	FFC	FC	ASFT	FSC	FSW (mm)	FNL (mm)	FNW (mm)	FSL (mm)	TSS (%)	FWG (g)	FPT (mm)	FW (mm)	FL (mm)	TTST (%)
Taranimt	0,25a	0,92a	2a	2,15a	2,5a	2,78ab	3ab	3ab	3,5ab	3,5ab	4ab	4ab	4,685ab	5,575ab	6ab	9,19b	19,75c	26,84d	33,62e	36,4e	39,895e	79,065f
R'dani	0,255a	0,845ab	2abcd	2,57abcd	2,5abcd	3,005abcd	2,5abcd	2abc	4,5cd	4,5cd	3abcd	2abc	5,25d	4,985cd	5,17cd	4,005bcd	25,125e	28,74f	33,28g	36,285h	42,9i	98,54k
Toudjente	0,275a	0,91ab	3cde	2,15bc	3cde	2,875cde	2,5cd	4def	3cde	4,5efg	3,5cdef	5fgh	6,005gh	6,36h	7,96i	8,38i	13,745k	25,96l	33,205m	36,08n	39,755o	49,975p
Kadota	0,19a	0,9ab	2abc	3,01abcd	2,5abc	3,265abcd	3abcd	4bcde	5cde	4,5bcde	3abcd	5cde	5,475cde	3,935abcde	7,055e	6,744de	19,12f	30,965g	34,4h	37,665h	41,88i	100,985k
Chetoui	0,25a	1,01ab	2abc	2,025abc	2,5abc	3,13abc	2,5abc	3abc	2abc	4,5bcd	3abc	2abc	5,665cd	5,73cd	7,645d	8d	22,26e	33,22f	36,525f	39,655f	39,34f	89,04g
Benacer Enk El H'mam	0,24a	0,905a	2a	2,53a	4,5a	2,615a	3a	3a	5a	5,5a	2,5a	3a	5,23a	6,515a	6,58a	5,13a	23,75b	31,66c	36,485cd	39,1de	43,345e	99,085f
Bezoult Rhadem	0,23a	0,75a	2a	4,125a	2,5a	3,635a	3,5a	4,5a	3,5a	4,5a	4a	6a	7,22a	21,47b	10,945ab	6,61a	21,485b	43,345c	46,98c	51,065c	62,845d	94,845d
Bakor noir	0,195a	0,95a	2a	2,5a	2,5a	2,48a	2,5a	3a	5a	4,5a	4a	2a	5,185a	2,555a	5,75a	5,3a	21,5ab	25,345ab	33,71b	36,19b	38,1b	118,33c
Bakor blanc	0,17a	0,8a	3a	2,695a	3,5a	3,205a	3,5a	4,5a	4a	2a	2a	2a	6,555a	7,76a	9,345a	5,985a	13,56a	51,045b	39,415ab	44,755b	56,25b	82,77c
Meroudji	0,23a	0,84a	2ab	1,62ab	3,5abc	3,75abc	3,5abc	3abc	5abc	4,5abc	2ab	5abc	5,74abc	9,98c	8,71bc	4,87abc	20,5d	31,25e	34,29ef	45,38g	38,04f	88,505h
F-Value	0,29a	0,98ab	2abc	2,595abc	4,5abc	4,01abc	2,5abc	4abc	2abc	4,5abc	3abc	6bcd	6,295cd	4,405abc	6,325cd	9,435d	17e	44,025fg	39,98f	43,99fg	45,07g	58,26h
	3,22*	5,911**	1,800 NS	3,64*	2,014 NS	8,522***	0,525 NS	1,957NS	3,136*	1,877 NS	1,700 NS	5,527 NS	15,55**	9,134**	2,586*	2,968*	4,261*	6,71*	8,21**	8,973***	13,87***	1,857 NS

Means within column followed by the same letters are not significantly different at P<0.05
NS: not significant; *, ** and *** are statistically significant at P<0.05 and P<0.01 respectively, according to Duncan's multiple range test.
TAC: Titratable acidity; **FSI**: Fruit shape; index; **FSCK**: Fruit skin cracks; **OW**: Ostiole width; **FSF**: Fruit skin firmness; **FST**: Fruit skin thickness; FS: Fruit shape; **EPE**: Ease of peeling; **FFC**: Fruit flesh colour; **FC**: Fruit cavity; **ASFT**: Abscission of the stalk from the twig; **FSC**: Fruit skin colour; **FSW**: Fruit stalk width; **FNL**: Fruit neck length; **FNW**: Fruit neck width; **FSL**: fruit stalk length; **TSS**: Total soluble solids; **FWG**: Fruit weight; **FPT**: Fruit pulp thickness; **FW**: Fruit width; **FL**: Fruit length; **TTST**: TSS / Titrable acidity.

Table 3. Principal component analysis based on morphological traits in fig.

Principal component	PC1	PC2	PC3
Variance (%)	30.080	16.810	14.492
Cumulative variance (%)	30.080	46.890	61.382
Eigen value	13.36	7.565	6.521
Traits			
TGH (Tree growth habit)	-0.133	0.435	-0.772
TV (Tree vigour)	-0.842	0.077	0.125
TFS (Tendency to form suckers)	0.710	-0.124	0.567
RDB (Relative degree of branching)	-0.593	0.107	-0.083
TBC (Terminal bud colour)	0.668	-0.335	-0.033
NFS (No. of fruits per shoot)	-0.291	-0.120	0.033
BFM (Beginning of fruit maturation)	0.138	-0.711	0.138
HPD (Harvesting period)	0.512	0.328	0.015
NLS (No. of leaves per shoot)	-0.576	-0.062	0.517
LLN (Leaf lobes No.)	-0.159	0.238	-0.544
DLL (Degree of leaf lobation / incision)	0.325	0.433	-0.712
SLB (Shape of leaf base)	0.619	-0.388	0.155
LC (Leaf colour)	0.168	0.525	-0.359
LL (Leaf length)	0.338	0.528	0.518
LW (Leaf width)	-0.298	-0.007	0.593
LA (Leaf area)	0.278	0.594	0.532
LCL (Length of central lobe)	0.633	0.515	-0.158
LAS (Leaf apex shape)	0.725	0.327	-0.325
PLL (Petiole length/Length of leaf)	-0.078	0.338	-0.093
LM (Leaf margin)	0.138	-0.344	-0.723
PL (Petiole length)	0.082	0.763	0.134
PT (Petiole thickness)	-0.093	0.173	0.721
BOD (Beginning of defoliation)	0.199	-0.551	0.065
FW (Fruit width)	0.886	-0.273	0.129
FL (Fruit length)	-0.947	0.017	0.115
FS (Fruit shape)	0.806	0.372	-0.189
FSI (Fruit shape index)	-0.815	-0.360	-0.254
FWG (Fruit weight)	0.904	-0.175	0.043
FNL (Fruit neck length)	0.811	0.013	0.357
FNW (Fruit neck width)	0.885	0.279	0.155
FSL (Fruit stalk length)	-0.141	-0.533	0.074
FSW (Fruit stalk width)	0.936	-0.120	0.015
ASFT (Abscission of the stalk from the twig)	-0.090	-0.406	0.217
OW (Ostiole width)	0.578	-0.186	0.521

Contd...

Table 1 Contd...

Principal component	PC1	PC2	PC3
EPE (Ease of peeling)	0.777	-0.529	0.041
FSCK (Fruit skin cracks)	0.418	0.314	-0.578
FST (Fruit skin thickness)	0.595	-0.305	0.004
FSF (Fruit skin firmness)	0.073	-0.385	-0.751
FPT (Fruit pulp thickness)	0.875	-0.253	0.128
FSC (Fruit skin colour)	0.304	-0.432	0.075
FFC (Fruit flesh colour)	-0.445	0.441	-0.201
FC (Fruit cavity)	-0.546	-0.088	0.557
TSS (Total soluble solids)	-0.290	0.024	0.607
TAC (Titratable acidity)	-0.134	-0.726	-0.103
TSST (TSS: acid ratio)	-0.181	0.482	0.528

The second axis (PC2) corresponds to the length of the stalk which is negatively correlated with 2 other variables (the beginning of fruit ripening and the titratable acidity). The third axis (PC3) represents the petiole thickness which is negatively correlated with 4 variables (the tree growth habit; the degree of leaf lobation; the leaf margin and the fruit skin firmness).

Cluster analysis, based on the Euclidean distance separated the cultivars into 2 groups, *i.e.* I and II, at the level of 40% similarity (Fig. 2). The first group (I) contains 4 cultivars, which are separated into 2 sub-groups, I.I and I.II. The first subgroup (I.I) includes 2 cultivars Enk El H'mam and Bakor Noir (d=27.7), which are mainly characterized by high vigour, fruit shape oblong and high fruit weight. The second sub-group (I.II) consists of R'dani and Kadota (d = 16), the fruits of which have small length and neck's width. The second group (II) comprises 7 cultivars and is divided into 2 sub-groups, II.I and II.II. The first sub-group (II.I) is represented by Meroudji, which has a long harvest period, a globular fruit shape, a heavy weight, a thick pulp and is rich in sugars. The second sub-group (II. II) is broader and includes Chetoui, Benacer, Bezoult Rhadem, Toudjente, Bakor blanc and Taranimt. This second sub-group is mainly characterized by a long harvest period and very high sugar content.

The present study revealed that the variability of the tree vigour, the degree of leaf lobation, the relative degree of branching, the terminal bud colour, the beginning of fruit maturity and the harvest period, was in agreement with those of Kuden *et al.* (9) but can change with the environmental conditions (Gaaliche *et al*., 5). Therefore, we could speculate that the vegetative characters of the relatively young trees and their architectural forms do not remain constant and could evolve over time.

The values of leaf area recorded in this study were lower than those obtained by Abo-El-Ez *et al*.

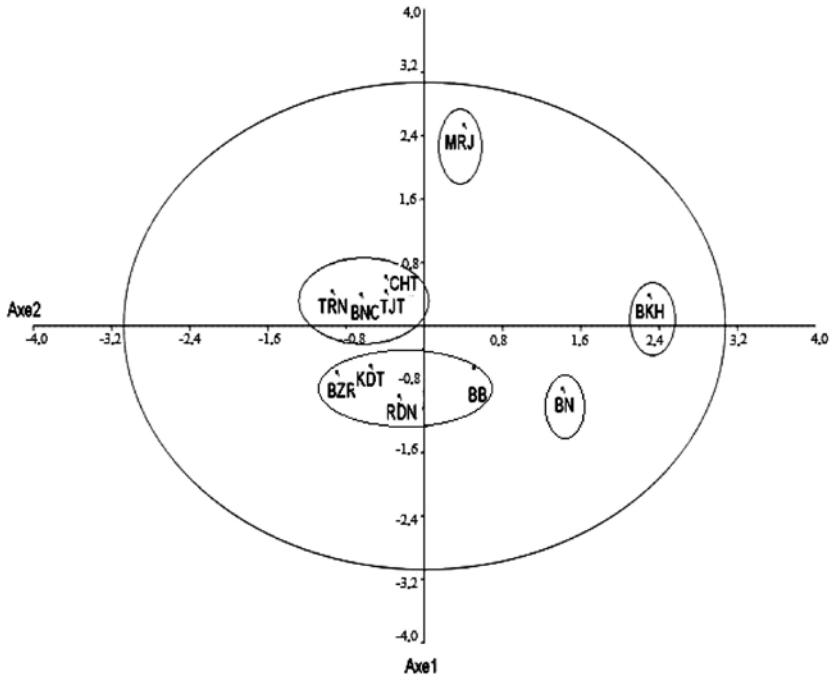

Fig. 1. Plot of the first and the second principal components resulting from a PCA using phenotypic traits in fig. MRJ = Meroudji; BZR = Bezoult Rhadem; RDN = R'dani; EKH = Enk El H'mam; TRN = Taranimt; BNC = Benacer; CHT = Chetoui; TJT = Toudjente; KDT = Kadota; BB = Bakor Blanc; BN = Bakor Noir.

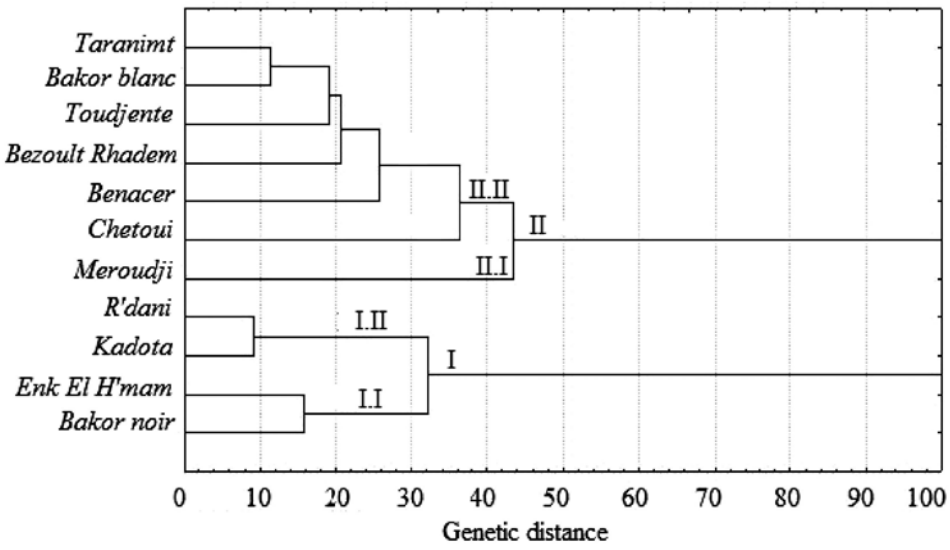

Fig. 2. Dendrogramm of the 11 fig cultivars based on phenotypic traits using Ward's method.

(1), whereas in terms of leaves number our results were higher than those reported by Simsek (11) who attributed the variability of the leaf surface and the number of leaves to genetic characteristics and environmental conditions. Furthermore, since the leaves number and the leaf size are expected to evolve with the trees growth, we suggest that it is better to evaluate these vegetative characteristics beyond 6 or 7-year-old age. The harvesting period recorded in this study coincides with that observed by Simsek and Yildirim (12), however it could last longer. The cultivars Chetoui, Benacer and Enk El H'mam are interesting in terms of fruit ripening (very late) and can be used in a programme to improve the duration of fruit ripening.

The shape and the index of the fruits are very important for their trade. Practically, it is assumed that the globose shape is the most suitable especially for packaging and fruits transportation. Our data show that 7 cultivars (Taranimt, Toudjente, Kadota, Chetoui, Bezoult Rhadem, Benacer and Meroudji) fulfil this commercial criterion. The others cultivars have an oblong shape and are more appropriate for confectionery or jam preparation. The fruit size is considered as an important qualitative trait for the consumption of fresh figs. A good fruit size is also a quality index that reflects the proper maintenance of the tree (Tamboli *et al*., 13). However, this character is known to be negatively influenced by the fruit load on the trees (Radivojevic *et al*., 10). It is noteworthy that besides the genetic effect, fruit weight depends also on the growing location as well as the interaction between the genotype and the maturity stage.

This study revealed that the maximum length of the neck and the stalk is different from that reported by Vrhovnik *et al*. (15). Simsek and Yildirim (12) consider that, contrary to a long stalk, a short neck is undesirable because it makes the picking difficult and is damaging to the fruits. Moreover, the fruits with a too long neck or with a large ostiole opening are a major problem to the fresh fig industry. In this study, the cultivars R'dani, Kadota, Bezoult Rhadem and Meroudji have the shortest fruit necks and therefore are less attractive to fig producers. On the other hand, the figs of the cultivar Enk El H'mam, which have the longest neck and a large ostiole opening, is also not very well appreciated by the industry. The results of chemical properties of the fruits show significant variability of soluble solids among cultivars and confirm previous reports (Crisosto *et al.*, 4; Trad *et al.*, 14). In Algeria, fresh figs with globular shape and rich in sugars are well sought by consumers. Most of these sugars are in the form of soluble solids and are involved with organic acids, particularly citric acid, in the fruit flavour. In this context, Basheer-Salimia *et al.* (2) consider that high quality table figs must have a solid soluble extract ranging from 13.0 to 25.1%. In our study, levels varying between 13.56% (Bakor Noir) and 25.12% (R'dani) constitute an index of high quality for all cultivars in terms of total soluble solids.

The results of the principal component analysis show that the total variability is expressed by the first three components, *i.e.* 30.08, 16.81 and 14.49%, respectively. The Euclidean distances indicate that Kadota has the closest similarity with R'dani and

the furthest with Meroudji. On the other side Bakor Blanc and Bakor Noir are significantly dissimilar, even though they have the same name, which implies a case of homonymy. The inclusion of cultivars with different fruit colours in the same group demonstrate that this character is not sufficiently discriminatory. The presence of 4 cultivars (Enk El H'mam, Bakor Noir, R'dani and Kadota) in group I and 7 cultivars (Meroudji, Chetoui, Benacer, Bezoult Rhadem, Toudjente, Bakor Blanc and Taranimt) in group II, reveals also that they are phenotypically similar. This similarity is probably due to the same mode of propagation.

According to Caliskan and Polat (3), the random selection from natural populations decreases the genetic diversity. However, due to vegetative multiplication, which probably uses the same propagation material, the fig has a narrow genetic base. This work demonstrated that the assessment of genetic diversity by morphological descriptors is an appropriate tool, and showed the richness of the fig genetic resources and their characteristics. It also revealed some promising cultivars that can offer new opportunities to growers. Their conservation can be used for future research works as well as for the constitution of a fig database.

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Comparative *in vitro* propagation of stress tolerant grape (*Vitis* spp.) rootstocks and assessment of clonal fidelity of plantlets

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ABSTACT

Micropropagation in nine *Vitis* rootstocks using nodal segments was studied. Optimum *in vitro* culture establishment was highest on Murashige and Skoog (1962) medium (MS) with 3.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA. Rootstock 110 R gave the earliest bud sprouting (4.03 days), whereas, 1616 C and 110 R gave the highest culture establishment (71.11 & 69.67%). The shoot proliferation was most efficient on MS medium + 4.0 mgl^{-1} IBA + 0.5 mgl^{-1} BAP. Dogridge showed the maximum multiplication rate/ sub-culture (10.07), while 1613 had the minimum (5.07). Half-strength MS medium supplemented with 4.0 mgl^{-1} IBA gave good rooting parameters, while half-strength MS medium with 1.5 mgl^{-1} IBA + 1.5 mgl^{-1} NAA induced more number of roots. Dogridge and Salt Creek had the higher rooting (77.58 & 74.24%) compared to other genotypes. High *ex vitro* plantlet survival (82.75%) was noted in 1103 P in glass jars, while 1616 C plantlets took the shortest time (44.40 days) for transfer to glasshouse. Application of two marker (RAPD & ISSR) systems further confirmed the genetic stability of micropropagated plantlets. Based on the overall performance of rootstocks for *in vitro* multiplication they could be ranged as Dogridge > Salt Creek > *V. parviflora* > St. George >1616C > 1103P > 140Ru > 110R > 1613C.

Key words: Clonal fidelity, comparative multiplication, grape rootstocks, *in vitro* propagation.

INTRODUCTION

Grapevine is one of the most important fruit crops grown in India occupying an area of 1,10,000 ha with production of 1.7 MT (NHB, 11). With the erratic weather patterns and extreme abiotic stress conditions have led to reduction in productivity. The use of biotic and abiotic stress tolerant rootstocks offers a feasible option for sustainable grape production. Dogridge is considered as one of the most important rootstock adopted commercially for establishing vineyards in western India. Over dependence on this rootstock necessitated the growers to adopt other rootstocks to combat multiple edaphic problems.

Grape rootstocks are propagated by cuttings, which are slow, labour intensive and largely influenced by weather and edaphic factors. Micropropagation is an alternative method that produces genetically identical, physiologically uniform and pathogen-free planting material. Successful *in vitro* clonal propagation methods have been reported in various *Vitis* sp. and genotypes (Zhang *et al.*, 15; Alizadeh *et al.*, 2). Though success in *in vitro* propagation has been reported earlier, however, it largely dependent upon the interaction between genotype, explant source and culture medium that necessitate developing specific regeneration protocols for individual genotype (Kurmi *et al.*, 8). Further, it is important to check the genetic stability of *in vitro* regenerated plantlets. Several molecular markers were used to check the clonal fidelity in many perennial crops such as grape rootstocks (Alizadeh and Singh, 2), apple rootstocks (Harshita and Vibha, 5). Hence, in the present study, we examined the *in vitro* multiplication behaviour of nine *Vitis* rootstocks and also checked the clonal fidelity of hardened plantlets.

MATERIALS AND METHODS

Experiment was conducted at the Central Tissue Culture Laboratory, Division of Fruits and Horticultural Technology, LBS Centre, ICAR-IARI, New Delhi during 2013-2015. Nine grape rootstocks, *viz.*, Dogridge, Salt Creek, 110 Ritcher, 1103 Paulsen, 1616 Couderc, 1613 Couderc, 140 Ruggeri, St. George and *Vitis parviflora* were selected for the present study. The protocol for initiating aseptic cultures developed by Alizadeh *et al.* (3) was followed. The nodal segments were inoculated individually in test tubes on solid Murashige and Skoog (10) medium supplemented with benzyl-aminopurine (BAP) either singly or in combination with low concentration of NAA (α-naphthalene acetic acid) and then incubated at 25 ± 1°C with 16/8 h light and dark photoperiod.

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The data on growth parameters, *viz.*, days taken for bud sprouting and culture establishment were recorded. After four weeks of culture establishment, two-node micro-shoot cuttings were excised and cultured on shoot proliferation-cum rooting medium comprising full-strength MS medium supplemented with IBA (indole-3-butyric acid) or NAA alone or combinations. Multiplication parameters, *i.e.*, shoot length (cm), number of shoots explant^{-1}, number of micro-cuttings explant^{-1}, multiplication rate, multiplication cycle, number of roots explant^{-1} and root length (cm), were determined upon each sub-culture. The rooted plantlets were hardened in the glass jars with polypropylene (PP) caps or plastic pots with polythene cover containing sterilized hardening medium (peat: vermiculite: perlite; 2:1:1) moistened with half-strength MS inorganic salts. Hardened plantlets were transferred to plastic pots filled with sterilized sand, soil and FYM (farm yard manure) (2:1:1) in a glasshouse during 6-8th week of hardening depending up on the genotype. The observations on plantlet survival and days taken to *ex vitro* transfer were recorded.

For clonal fidelity analysis, total DNA was extracted from young leaves of plantlets of mother vines and randomly selected tissue cultured plantlets using acetyl trimethyl ammonium bromide (CTAB) method (Simon *et al.*, 14). The samples were diluted to a concentration of 50 ng l$^{-1.}$ A total of 20 primers each (Macrogen®, USA) were used for RAPD and ISSR analyses out of which 10 were selected based on reproducibility of the bands. The PCR reactions were carried out with 20 µl reaction mix. The PCR amplifications were performed by using following thermal profile for each marker. Amplification was confirmed and alleles were separated by running on 1.5% agrarose gel and electrophoresed in 1.0X TAE at 120 volts for 2 h for both RAPD and ISSR analyses. In the present study, 45 samples were analyzed using RAPD and ISSR primers each set including one mother plant (raised in germplasm block) of individual along with four randomly selected in *vitro* raised plantlets. The data was analysed using SAS Ver 9.3 and the mean differences were separated using Duncan's Multiple Range Test (DMRT). Cluster analysis was carried out using the SHAN module (NTSYS pc 2.02) software package (Rohlf, 12). An unweighted pair group method of arithmetic mean (UPGMA) dendrogram was generated from Jaccard's similarity values individually for RAPD and ISSR markers.

RESULTS AND DISCUSSION

The comparative *in vitro* multiplication of nine grape rootstock genotypes was found to be strongly influenced by genotype and concentration of the growth regulators used (Table 1). Significant differences were observed in the treatment combinations of BAP and NAA (3.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA and 4.0 mgl^{-1} BAP + 0.25 mgl^{-1} NAA), which showed better response than BAP alone for time taken to initial bud sprout (5.78 and 5.77 days) and culture establishment (71.50 and 67.70%). The interaction effect of growth regulators enhanced culture establishment and minimized the time to bud sprouting in grapevines (Alizadeh *et al.*, 3; Abido *et al.*, 1; Itoo *et al.*, 6), which corroborated our findings. This difference might be due to the varying balance between endogenous and exogenous plant growth regulators. All the rootstock genotypes exhibited statistically significant variation for time taken for initial bud sprout and culture establishment. Rootstock 110 R took the minimum time (4.94 days), while it was more delayed in Dogridge (8.58 days) and *V. parviflora* (8.34 days). The rootstock 1616 C showed the highest culture establishment (77.11%), while it was lowest (54.44%) in Dogridge. These results are in tune with the findings of Alizadeh *et al.* (3) and Kurmi *et al.* (8), who also suggested that the level and combination of plant growth regulator(s) effective for a particular genotype may not be effective for another genotype or species.

It was clearly noticed from Table 2 that the longest shoots (10.61 cm) were recorded in MS medium supplemented with 4.0 mgl^{-1} IBA + 0.5 mgl^{-1} BAP, which also resulted in production of significantly higher shoots (1.62) compared to PGR-free control (0.04) (Fig. 2B & 2C. 1). Though the application of IBA alone induced shoot proliferation in grape rootstocks, the addition of BAP in the culture media enhanced the shoot growth and also increased the number of shoots explant^{-1}. The highest shoot length was recorded in *Vitis parviflora* (8.92 cm) and Dogridge (8.52 cm), while it was lowest in 1613C (5.61 cm). These findings were confirmed with the earlier results of Mukherjee *et al*. (9), who also found that addition of BAP in MS medium gave better shoot proliferation in rootstock DeGrassette and higher average number of proliferated shoots explant^{-1} in grape rootstocks (El-Agamy *et al.*, 4). The addition of auxin increased the enzyme activity that could breakdown starch and thus increased shoot proliferation of organogenesis.

The micro-cutting multiplication rate and multiplication cycle were monitored upto eight successive sub-cultures in the established cultures. Irrespective of the genotype, lower multiplication rate was observed till 3rd sub-culture, which thereafter gradually increased (Fig. 1A). In general, the multiplication rate in term of number of micro-cuttings explant^{-1} increased with the increase in number

Table 1. Effect of different plant growth regulators on time to shoot bud sprout and culture establishment in grape rootstock genotypes.

Treatment (mgl^{-1})	Days to bud sprouting						Mean	Culture establishment (%)						Mean
Genotype	2.0 BAP	3.0 BAP	4.0 BAP	2.0 BAP + 0.25 NAA	3.0 BAP+ 0.25 NAA	4.0 BAP+ 0.25 NAA		2.0 BAP	3.0 BAP	4.0 BAP	2.0 BAP + 0.25 NAA	3.0 BAP + 0.25 NAA	4.0 BAP + 0.25 NAA	
Dogridge	9.76	8.80	8.83	8.37	7.73	7.97	8.58[a]	46.67 (43.04)*	48.90 (44.37)	53.33 (46.89)	62.22 (52.14)	62.22 (52.14)	53.34 (46.92)	54.44[e] (47.58)
Salt Creek	8.23	7.10	6.67	6.40	5.90	5.53	6.64[c]	55.56 (48.23)	56.90 (48.97)	57.78 (49.55)	61.34 (51.60)	68.90 (56.16)	66.67 (54.83)	61.34[cd] (51.56)
110 Richter	5.90	4.87	5.23	5.20	4.43	4.03	4.94[f]	53.34 (47.75)	66.67 (54.83)	73.34 (59.15)	66.67 (55.37)	75.57 (60.45)	77.78 (61.94)	69.67[ab] (56.56)
1103 Paulsen	5.63	5.83	5.53	4.93	4.70	4.93	5.26[ef]	55.56 (48.18)	62.23 (52.20)	63.34 (52.86)	64.45 (53.70)	66.67 (55.17)	73.34 (59.05)	64.77[bc] (53.55)
1616 Couderc	6.20	5.53	5.00	4.97	4.60	4.53	5.14[ef]	57.78 (49.51)	66.67 (54.83)	75.56 (60.45)	71.11 (57.55)	80.00 (63.44)	73.33 (59.05)	71.11[a] (57.48)
1613 Couderc	8.63	8.03	7.30	7.10	6.80	6.90	7.46[b]	55.55 (48.22)	64.44 (53.44)	71.11 (57.55)	68.90 (56.16)	75.56 (60.45)	67.78 (55.49)	62.50[abc] (55.24)
140 Ruggeri	5.93	6.17	6.00	5.33	5.00	5.07	5.58[e]	56.89 (48.97)	65.56 (54.35)	67.00 (55.03)	71.34 (57.69)	73.55 (59.19)	72.22 (58.39)	68.10[ab] (55.61)
St. George	7.43	6.33	6.17	5.87	5.53	5.03	6.06[d]	61.12 (51.51)	58.34 (50.35)	66.67 (54.85)	61.11 (51.94)	75.00 (60.21)	63.88 (53.19)	64.35[bc] (53.67)
V. parviflora	9.76	8.90	8.00	8.13	7.33	7.90	8.34[a]	50.00 (45.00)	50.00 (45.08)	55.67 (48.26)	60.00 (50.81)	62.22 (52.14)	57.89 (49.62)	56.70[de] (48.45)
Mean	7.50[a]	6.84[b]	6.53[cb]	6.26[c]	5.78[d]	5.77[d]		54.90[d] (47.81)	60.33[c] (50.94)	65.20[b] (53.85)	66.20[b] (54.45)	71.50[a] (57.73)	67.70[ab] (55.37)	
LSD ($p \leq 0.05$)														
Treatment (T)							0.35							2.77
Genotype (G)							0.42							3.40
T × G							0.82							5.10

*Mean values of multiplication parameters within each column followed by the same letter(s) are not significantly different according to the Duncan's multiple range test ($p \leq 0.05$); Data in parentheses are transformed values

of sub-cultures, which was completely genotype dependent. The mean number of two-node micro-cuttings sub-culture^{-1} ranged from minimum of 5.07 to maximum of 10.7 in the selected genotypes. Irrespective of the PGR treatments, Dogridge recorded the maximum mean number of micro-cuttings explant^{-1} (10.07) followed by Salt Creek (8.67) and *V. parviflora* (8.16), while 1613C recorded the minimum (5.07). The multiplication cycle ranged from earliest (45 days) to most delayed (57 days) for different rootstocks. The rootstocks 1103 P and 110 R had shorter multiplication cycle (45.86 and 46.20 days), whereas it was most delayed in *V. parviflora* (57.02 days) followed by 1613 C (56.70 days).

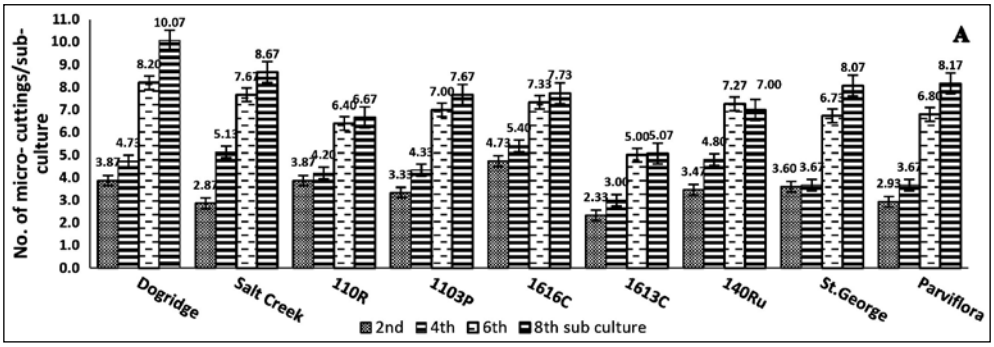
Rootstocks Dogridge, Salt Creek and *V. parviflora* were more responsive with regard to shoot length, number of shoots explant^{-1} and multiplication rate under *in vitro* conditions. Earlier also it was proposed that *in vitro* shoot proliferation of grape rootstock genotypes is largely due to the interaction of cytokinin and genotype (Alizadeh *et al.*, 3; El-Agamy *et al.*, 4).

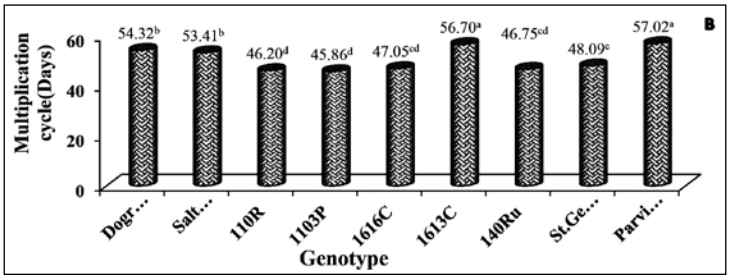
Rooting of micro-shoots in grape rootstocks was comparatively better in the medium supplemented with IBA alone (2.0 or 4.0 mgl^{-1}) or in combination with BAP (0.5 mgl^{-1}), while low success and delayed rooting was observed in control (MS without PGRs) (Table 2). The media supplemented with higher concentrations of IBA and NAA recorded more number of roots but it also led to callus formation (Fig. 2C.2). It was clearly observed from the data and Fig. 2B that maximum root length (9.57 cm) and rooting (84.10%) were recorded in MS medium + 4.0 mgl^{-1} IBA but maximum number of roots per shootlet (10.6) was noticed on MS medium + 1.5 mgl^{-1} IBA + NAA (1.5 mgl^{-1}) + BAP (1.0

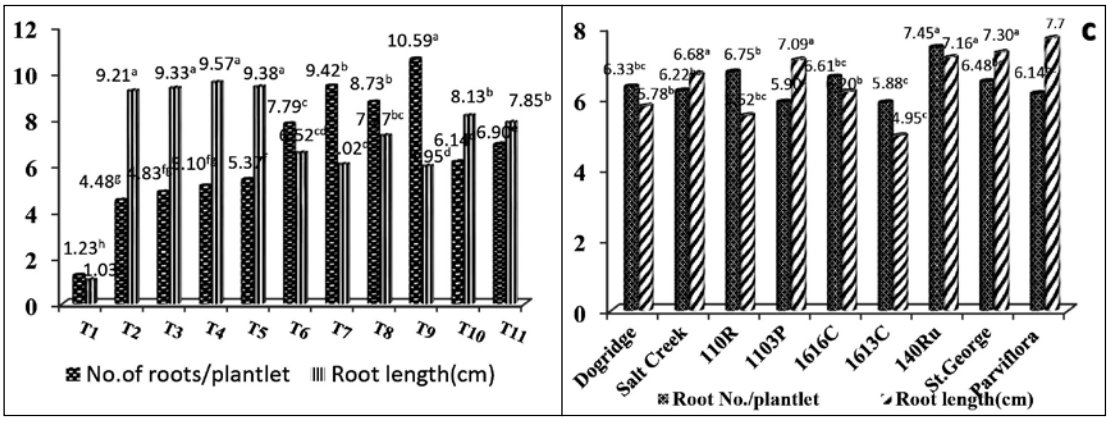
Table 2. Effect of different plant growth regulators on shoot length (cm) and rooting (%) in grape rootstock genotypes.

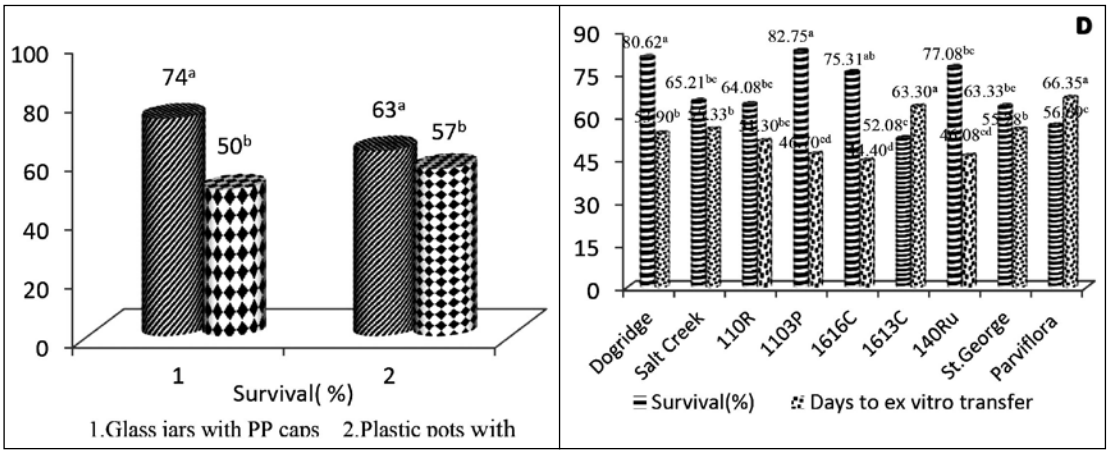
Treatment (mgl^{-1})	Shoot length (cm)									Mean	Rooting* (%)									Mean
Genotype	Dogridge	Salt Creek	110 R	1103 P	1616 C	1613 C	140 Ru	St. George	*V. parviflora*		Dogridge	Salt Creek	110 R	1103 P	1616 C	1613 C	140 Ru	St . George	*V. parviflora*	
0.0 (control)	3.77	3.84	3.03	2.28	1.80	2.13	2.43	3.07	2.37	2.74^{d}	23.34	26.67	20.00	20.00	30.00	20.00	20.00	23.34	23.34	22.97f
2.0 IBA	9.65	8.56	6.82	13.47	8.16	6.95	12.01	11.16	12.68	9.94^{a}	93.34	93.34	80.00	90.00	90.00	73.34	83.34	83.34	70.00	84.07^{a}
4.0 IBA	9.89	9.54	7.61	11.63	9.52	7.15	11.07	11.39	12.81	10.07^{a}	86.67	86.67	76.67	86.67	86.67	76.67	83.34	83.34	73.34	82.22^{a}
2.0 IBA + 0.5 BAP	10.81	9.69	9.52	11.12	11.13	7.71	10.94	12.93	10.45	10.47^{a}	96.67	90.00	76.67	80.00	86.67	80.00	80.00	83.34	83.34	84.10^{a}
4.0 IBA + 0.5 BAP	11.76	9.92	10.03	11.47	11.18	8.44	10.91	10.07	11.66	10.61^{a}	93.34	83.34	80.00	80.00	83.34	66.67	70.00	76.67	80.00	79.25^{a}
1.0 IBA + 1.0 NAA	8.66	9.19	7.73	4.20	4.94	5.01	4.51	4.95	7.95	6.35^{c}	80.00	70.00	60.00	66.67	73.34	56.67	63.34	60.00	60.00	65.56^{cd}
1.5 IBA + 1.5 NAA	6.97	4.55	5.06	6.02	5.83	4.05	5.91	6.12	7.65	5.79^{c}	83.34	73.34	66.67	63.34	63.34	46.67	53.34	56.67	56.67	62.60^{de}
1.0 IBA + 1.0 NAA + 1.0 BAP	7.57	6.13	5.56	4.77	8.66	4.63	4.74	4.52	8.00	6.06^{c}	73.34	70.00	76.67	73.34	73.34	63.33	70.00	70.00	70.00	71.11^{bc}
1.5 IBA + 1.5 NAA + 1.0 BAP	7.12	6.82	4.64	4.09	7.58	4.30	4.71	6.09	6.94	5.81^{c}	63.34	63.34	63.34	60.00	60.00	50.00	50.00	56.67	53.34	57.78^{e}
2.0 IBA + 0.25 NAA + 0.5 BAP	8.73	8.85	7.07	8.47	6.73	6.03	9.51	8.90	9.53	8.20^{b}	83.34	80.00	73.34	73.34	80.00	70.00	76.67	80.00	73.34	76.67^{b}
4.0 IBA + 0.5 NAA + 0.5 BAP	8.81	7.14	6.20	7.73	7.21	5.35	8.86	8.30	8.10	7.49^{b}	76.67	80.00	76.67	73.34	76.67	63.34	70.00	73.34	56.67	71.85^{b}
Mean	8.52^{ab}	7.67^{c}	6.67^{d}	7.75^{bc}	7.52^{c}	5.61^{e}	7.78^{bc}	8.00^{bc}	8.92^{a}		77.58^{a}	74.24^{ab}	68.18^{d}	69.70^{cd}	73.03^{bc}	60.61^{e}	65.45^{de}	67.88^{d}	63.64^{de}	
LSD ($p \leq 0.05$)																				
Treatment (T)										1.02										3.70
Genotype (G)										1.42										3.35
T × G										2.08										7.15

Mean values of parameters within each column and row followed by the same letter(s) are not significantly different according to the DMRT ($P \leq 0.05$); *transformed data









*Mean values of parameters followed by the same letter(s) are not significantly different according to the DMRT ($p \leq 0.05$)

Fig. 1. A. Comparison of rate of multiplication rate; B. multiplication cycle of grape rootstocks; C. Effect of plant growth regulators on *in vitro* rooting; D. Effect of hardening strategies on plantlet survival and days taken for *ex vitro* transfer in grape rootstocks.

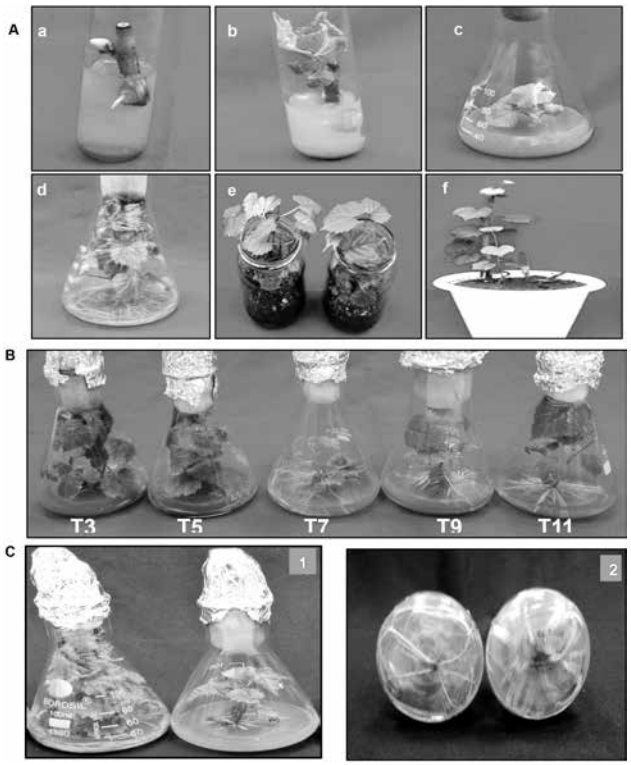


Fig. 2. A. Protocol for *in vitro* propagation of grape rootstocks. (a, b, c). Culture initiation stage, (d) Shoot-cum-root multiplication, (e). Hardening stage, and (f) Hardened plantlet; B. Performance of grape rootstock Dogridge on various treatment combinations. [T_3 = IBA (4.0 mgl^{-1}), T_5 = IBA (4.0 mgl^{-1}) + BAP (0.5 mgl^{-1}), T_7 = IBA (1.5 mgl^{-1}) + NAA (1.5 mgl^{-1}), T_9 = IBA (1.5 mgl^{-1}) + NAA (1.5 mgl^{-1}) + BAP (1.0 mgl^{-1}), T_{11} = IBA (4.0 mgl^{-1}) + NAA (0.5 mgl^{-1}) + BAP (0.5 mgl^{-1}); C. 1. Shoot growth on best treatment with lowest; 2. Root No. on medium supplemented with IBA alone and in combination with NAA.

mgl^{-1}). These results were in conformity with those of Abido *et al.* (1). The duration for root initiation (Table 3, Fig. 2) revealed that though Dogridge (16.28 days) and Salt Creek (15.50 days) responded late but had higher rooting success (77.58 and 74.24%). The highest number of roots micro-shoot^{-1} was recorded in 140 Ru (7.45), while it was least in 1613 C (5.88). Maximum root length was observed in the species *V. parviflora* (7.71 cm) and St. George (7.29 cm), while it was minimum in 1613 C (4.95 cm). These difference are expected as the result of genotypes and to some extent influence of PGR for *in vitro* rhizogenesis.

There was significant difference among the plantlet hardening strategies. The earliest time taken for *ex vitro* transfer and maximum plantlet survival was noted in glass jars with PP caps (Fig. 1D) as there was low desiccation and minimal microbial infection. Highest hardening success was achieved with the rootstock 1103 P (82.75%) cultured in glass jars. Rootstock 1616C plantlets were earliest to harden (44.40 days). Similar strategy has earlier been reported by Singh *et al.* (13) for *V. vinifera* cultivars and Alizadeh *et al.* (3) in some grape rootstocks. It was observed that in glass jars, the elongation of plantlet was better, which had positive influence on acclimatization.

Clonal fidelity or genetic uniformity of micropropagated plantlets, showed 1,655 bands in RAPD analysis out of which 1,341 bands showed sufficient polymorphism (Table 3). The scorable bands for each primer ranged from 4 (U13, U20) to 12 (J07) with band size ranging from 200 to 1500 bp. Maximum number of 310 bands were amplified with primer OPA15 with band size of 200 to 1500 bp, while minimum number (110 bands) were obtained with primers OPG 14 & OPU 20 within the size range of 300 to 550 bp and 210 to 550 bp, respectively. Monomorphic pattern of the bands indicated that there was no genetic variation in the *in vitro* regenerated plantlets compared to mother plants. In ISSR analysis, a total of 1600 amplified bands were produced with 10 ISSR primers with a band size ranging from 200 to 1500 bp. About 1209 bands showed sufficient polymorphism (Table 4). Primer UBC 824 amplified the maximum number of 290 bands with band size 300-2000 bp, while primer UBC 873 amplified the lowest number of 69 bands (300-1100 bp). According to pooled data analysis of two marker systems, 138 distinct and scorable bands were generated ranging from 200 to 2000 bp (Table 5). A total of 3,255 bands were generated with the both the markers and all were found to be monomorphic, corroborating high degree of clonal fidelity of micropropagated

Table 3. Details of oligo-nucleotide decamer primers used for assessing clonal fidelity of grape rootstock plantlets.

Primer No.	Sequence (5′-3′)	Total amplified bands	Polymorphic bands	Band size (bp)
OPA15	TTCCGAACCC	310	130	200-1500
OPJ01	CCCGGCATAA	250	240	200-1100
OPJ07	CCTCTCGACA	140	130	300-1500
OPG14	GGATGAGACC	110	110	300-550
OPH19	CTGACCAGCC	148	120	300-1000
OPP02	TCGGCACGCA	121	121	300-1500
OPP09	AGGTGACCGT	186	150	250-1200
OPU13	GGCTGGTTCC	160	110	400-1000
OPU16	CTGCGCTGGA	120	120	200-500
OPU20	ACAGCCCCCA	110	110	210-550

Table 4. List of different ISSR primers used for detecting clonal stability in tissue cultured grape rootstock plantlets.

UBC Primer No.	Primer sequence (5′-3′)	Total amplified bands	Polymorphic bands	Band size (bp)
UBC 807	AGA GAG AGA GAG AGA GT	71	25	300-850
UBC 809	AGA GAG AGA GAG AGA GG	190	100	300-1500
UBC 824	TCT CTC TCT CTC TCT CG	290	200	300-2000
UBC 858	TGT GTG TGT GTG TGT GRT	125	125	300-1000
UBC 859	TGT GTG TGT GTG TGT GRC	225	135	200-850
UBC 860	TGT GTG TGT GTG TGT GRA	130	130	380-1000
UBC 861	ACC ACC ACC ACC ACC ACC	145	145	300-1500
UBC 862	AGC AGC AGC AGC AGC AGC	100	100	300-1150
UBC 868	GAA GAA GAA GAA GAA GAA	255	180	300-1000
UBC 873	GAC AGA CAG ACA GAC A	69	69	300-1100

Table 5. Comparative data obtained by RAPD, ISSR and pooled analyses of *in vitro* grape plantlets for evaluation of clonal fidelity.

Particulars	RAPD	ISSR	Pooled analysis
No. of primers used	10	10	20
Scorable band classes per prime	4-12	3-11	3-11
Total No. of bands obtained	1655	1600	3255
Av. No. of bands per primer	7.0	6.7	6.9
Band size (bp)	200-1500	200-1500	200-1500
In vitro induced variation	Nil	Nil	Nil

grape rootstocks in the present study. The UPGMA dendrogram generated for ISSR (Fig. 3A) and RAPD (Fig. 3B) further confirmed the true-to-type nature of *in vitro* derived plantlets with their respective mother plants. Similarity matrix based on Jaccard's coefficient revealed that the pairwise value between the mother plant and the plantlets was 1, indicating 100 per cent similarity. This finding was in conformity with those reported earlier (Khawale *et al.*, 7; Alizadeh and Singh, 2; Zhang *et al*., 15) in grape rootstocks, and Harshita and Dhawan (5) in apple rootstock.

The protocols standardized for *in vitro* multiplication of the above grape rootstock genotypes (Fig. 2A) can be used commercially with minimum possibility of any *in vitro* induced variability.

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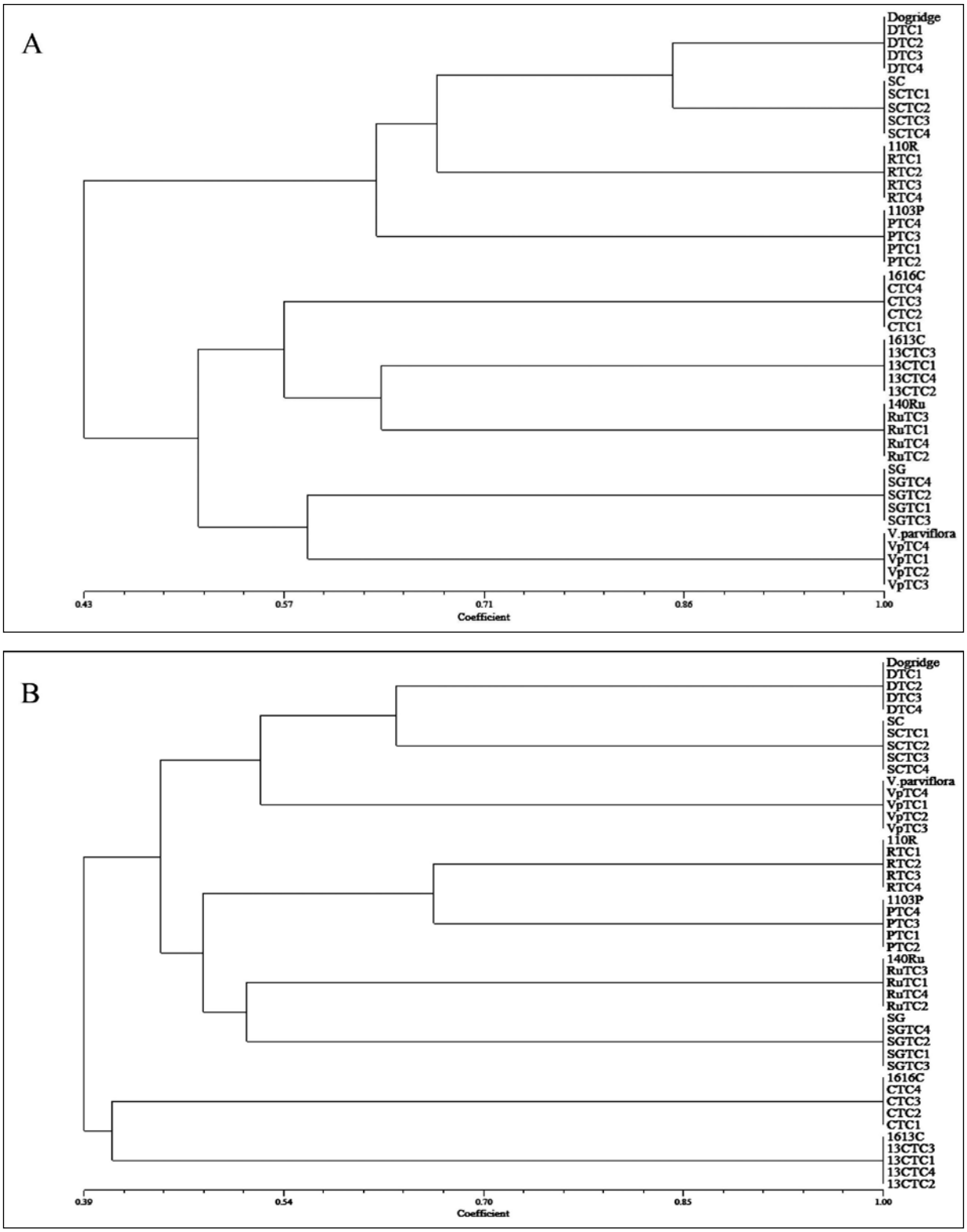

Fig. 3. Dendrogram obtained based on data A. ISSR and B. RAPD corroborating high levels of similarity among in mother plants and their respective *in vitro* raised plantlets (Dogridge (DTC1 to DTC4), Salt Creek (SCTC- SCTC4), 110 Richter (RTC1-RTC4), 1103 Paulsen (PCT1 –PCT4), 1616 Couderc (CTC1-CTC4), 1613 Couderc (13CTC1-13CTC4), 140 Ruggeri (RuTC1-RuTC4), St. George (SGTC1-SGTC4), and *V. parviflora* (PvTC1- PvTC4).

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Morphological and genetic diversity in citrus genotypes to substantiate rootstock breeding for root rot resistance

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ABSTRACT

Investigations were conducted on six citrus rootstocks, *viz*., rough lemon (RL), trifoliate orange (TO), Swingle citrumelo (SC), X 639 (X), Rangpur lime (RA) and Gou Tou (GT), to assess the morphological and genetic variability; and reaction against *Phytophthora*. Smooth trunk surface was recorded in all the genotypes. Three genotypes had erect growth habit, while it was spreading in Gou Tou (GT), Rangpur lime (RA) and rough lemon (RL). The leaves of GT, RA and RL were of unifoliate, while trifoliate orange (TO) had trifoliate leaf and Swingle citrumelo (SC) and X 639 (X) had multifoliate leaf division. No variation was recorded with respect to flowering season in all citrus rootstocks, *i.e.* mid February to last week of March. Fifty-five SSR markers were used for evaluation of genetic diversity amongst the six rootstocks. Twenty markers exhibited high polymorphism and showed wide allelic diversity. Capacity of each SSR to show polymorphic loci, varied from 0.29 (F98) to 0.83 (CCSME46 and CCSMEc4) with an average PIC value of 0.61. The resolving power (Rp) was highest for the primer CCSME43 (8.33) and was lowest for F90 (1.33). Significant differences were observed in the value of MI and were found to be the highest for primer F40 (9.25), while minimum MI was recorded for F98 (0.38). The PIC values of a marker vary with the crop and the set of the genotypes used. The reduction in the number of sporangia and lesion size in trifoliate orange and Swingle citrumelo indicated their tolerance against *Phytophthora nicotianae* var. *parasitica*. The number of sporangia counted after 48 h of incubation showed that all leaf baits of each rootstock were attacked by large number of sporangia. The number of sporangia on each leaf disc of rootstocks decreased after 48 h as the sporangia germinated into mycelium on the edges of leaf discs.

Key words: Citrus, *Phytophthora*, SSR markers, variability.

INTRODUCTION

Citrus occupies an important place in the horticultural wealth of India by covering around 0.95 million ha area with an annual production of 11.66 million tonnes (Anon, 2). The average productivity of citrus in India is 10.44 MT/ha, which is far behind the highest productivity in world. This is mainly attributed to *Phythophthora* root rot, citrus decline, fruit drop, poor nutrition and non-availability of quality planting material. Most of these factors are influenced by rootstock, which is the major contributor to tree performance and longevity as it determines tolerance to various biotic and abiotic stresses. In order to understand the genetic background and the breeding value of the available germplasm, systematic study related to characterization and evaluation of germplasm is of great importance for current and future breeding and genetic improvement of the citrus. A large number of citrus species/ progenitors of commercial citrus fruits are believed to have originated in India.

Molecular markers based on DNA sequence proved to be an ideal means for the identification and estimation of relatedness among genotypes of different origin. Due to the limited number of morphological and biochemical markers, molecular markers have been reported to be powerful tools for elucidating genetic diversity, determining parentage and revealing phylogenetic relationships among various *Citrus* species. Several molecular markers have been used for practical application in citrus (Aleza *et al*., 1).

The rough lemon rootstock has superiority for tree vigour, high yield, resistance against tristeza virus and suitability to high pH soils, thus, it occupied most prominent place among citrus rootstocks in India. However, it is vigorous in nature, which limits its use in high density planting and imparts poor quality to fruits of scion varieties. Hence, there is an urgent need to develop *Phytophthora* tolerant rootstock of citrus suitable for Kinnow mandarin under Punjab conditions. Sour orange, several hybrids of trifoliate orange, *viz*. X 639, citranges and Swingle citrumelo and Rangpur lime are also tolerant to *Phytophthora,* which are widely being used as rootstocks in the citrus growing regions (Castle, 4). These rootstocks can be used to develop

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new rootstocks, which are suitable under Indian conditions. A more precise system for identification of genotypes and for assessing the genetic variation in the existing genetic resources is a fundamental requirement for establishing breeding programmes and the registration of new cultivars in citrus. Hence, the present study was conducted to assess the morphological and genetic variability; and reaction against *Phytophthora* of six citrus rootstocks.

MATERIALS AND METHODS

Six citrus rootstocks, *viz.*, rough lemon (RL), trifoliate orange (TO), Swingle citrumelo (SC), X 639 (X), Rangpur lime (RA) and Gou Tou (GT) planted in the College Orchard, Department of Fruit Science, PAU, Ludhiana, is located at 29.3° N latitude and 76.5° E longitude, 270 m amsl were evaluated during the years 2015-2016. Twenty quantitative and 27 qualitative characters based on Descriptors for Citrus (Anon, 3) were studied for each genotype. The rootstocks were screened against *Phytophthora nicotianae* var. *parasitica* by using leaf bait method as described by Dhakad *et al*. (6) for quick detection of resistance or susceptibility. Pathogen was isolated from root zone soil of infected plant on selective PARPH media (Naqvi, 12) by using soil plating method. Multiplication of pathogen was done on sorghum seeds as described by Kaur *et al*. (9). Spore suspension was made as described by Naqvi (13).

DNA was isolated from young leaf using the modified CTAB with some modifications (Cheng *et al*., 5). The purified DNA, approximately 50 ng, was used for amplification with SSR primers in polymerase chain reaction (PCR). Amplification was carried out in a 10 µl reaction mixture (2.5 mM *Taq* buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM primer and 1.0 U of *Taq* DNA polymerase) in a gradient thermo-cycler (Veriti 96-Well Thermal Cycler, Thermo Fisher Scientific). PCR cycling conditions for amplification of SSR fragments consisted of an initial denaturation at 94°C for 4 min. followed by 35 SSR cycles consisting of 1 min. denaturation at 94°C, 1 min. annealing at 52-56°C (primer-specific) and 1½ min. elongation at 72°C, and finally single extension cycle of 7 min. at 72°C. The amplified product was resolved on 6.0% polyacrylamide gel electrophoresis (PAGE) at 300 V for 1½ h. The gels stained with 100 µl of ethidium bromide (Promega, USA) were visualized under UV gel documentation (Syngene, G: Box, USA).

Fifty five SSR markers were used for evaluation of genetic diversity of the six citrus rootstocks. The SSR amplicons were recorded as described by Kulhari *et al.* (11). Marker index (MI) for SSR markers was calculated according to Powell *et al*. (15). Diversity index/ genetic diversity are the expected heterozygosity and were calculated according to Weir (21). Polymorphism information content (PIC) values and resolving power (Rp) of the primers were calculated as per the formulae of Roldan-Ruiz *et al*. (17) and Prevost and Wilkinson (16), respectively. The morphological data was analyzed as per randomized block design. For *Phytophthora* screening test the observations were recorded for three leaf baits per replication and with three replications per treatment. Data were analysed by using completely randomized block design, or *t*-test statistics using SAS (9.4 version) computer software.

RESULTS AND DISCUSSION

Smooth trunk surface, high density of branches and glabrous shoot tip surface was recorded in all the citrus genotypes (Table 1). Three rootstocks had ellipsoid tree shape, while GT and RA trees have obloid shape but RL has spheroid. Variability was studied for growth habit (erect and spreading) among different genotypes. The three genotypes had erect growth habit, while it was spreading in GT, RA and RL. The variation was recorded in branch angle of all rootstocks. The spine shape did not vary among different genotypes and was straight in all rootstocks except TO, which had curved shape. However, variability was observed in the colour of shoot tip of various rootstocks. The shoot tip colour was green in X and GT and yellow green in TO. Study on tree behaviour of these genotypes was made to describe their vegetative life cycle under Punjab conditions due to their differential ability to tolerate the low temperature and high temperature, which prevailed in the region during winter and summer season, respectively. Genotypes TO, SC and X were deciduous; whereas RA, GT and RL were evergreen. Similarly, differences in growth habit and tree shape were also observed by Singh (19) among different strains and species of citrus. In a similar study Kaur *et al.* (10) recorded glabrous shoot tip surface in rough lemon and trifoliate orange, while it was uneven among different strains of Rangpur lime.

The leaves of GT, RA and RL were of unifoliate type but, TO had trifoliate leaf and SC and X had multifoliate leaf division (Table 2). Leaves were unifoliate in Box orange, Cleopatra mandarin, *pectinifera*, Rangpur lime, rough lemon and trifoliate in case of Carrizo and Troyer citrange. Earlier, Singh (19) also found unifoliate leaves in Schaub rough lemon and Brazilian Rangpur lime; and trifoliate leaves in Flying Dragon. The variation in intensity of green colour of leaf blade was observed in citrus rootstocks varied from light green to dark green colour. Three rootstocks had dark green colour

Table 1. Qualitative tree traits in different citrus rootstocks used in hybridization.

Trait \ Genotype	PT	SC	X	RA	GT	RL
Trunk surface	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Tree shape	Ellipsoid	Ellipsoid	Ellipsoid	Obloid	Obloid	Spheroid
Tree growth habit	Erect	Erect	Erect	Spreading	Spreading	Spreading
Density of branches	Dense	Dense	Dense	Dense	Dense	Dense
Branch angle	Narrow	Narrow	Narrow	Wide	Wide	Medium
Spine density	High	High	High	Low	Low	High
Spine shape	Curved	Straight	Straight	Straight	Straight	Straight
Shoot tip colour	Yellow green	Purple	Green	Purple	Green	Purple
Shoot tip surface	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
Vegetative life-cycle	Deciduous	Deciduous	Deciduous	Evergreen	Evergreen	Evergreen

Table 2. Qualitative leaf characteristics of different citrus rootstocks used in hybridization.

Trait \ Genotype	TO	SC	X	RA	GT	RL
Leaf division	Trifoliate	Trifoliate	Multifoliate	Simple	Simple	Simple
Intensity of leaf blade green colour	Medium green	Dark green	Dark green	Light	Dark green	Medium
Leaf colour variegation	Absent	Absent	Absent	Absent	Absent	Absent
Leaf lamina attachment	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate	Brevipetiolate
Leaf lamina shape	Ovate	Lanceolate	Ovate	Elliptic	Elliptic	Elliptic
Leaf lamina margin	Dentate	Dentate	Dentate	Dentate	Entire	Entire
Leaf apex	Obtuse	Obtuse	Obtuse	Acute	Acute	Acute
Presence/ absence of petiole wings	Present	Present	Present	Absent	Absent	Absent
Petiole wing width (mm)	Narrow	Narrow	Narrow	Absent	Absent	Absent
Petiole wing shape	Linear	Obdetate	Obovate	Absent	Absent	Absent
Junction between petiole and lamina	Articulate	Articulate	Articulate	Fused	Fused	Fused
Leaf emergence	22nd February	10th February	4th February	25th February	12th February	25th February

intensity, while it was light green colour in RA, but RL had the medium green colour intensity. The leaf colour variegation was absent among all the genotypes. Singh (19) reported medium and dark leaf colour intensity in trifoliate orange and red fleshed pummelo, respectively. All the rootstocks had brevipetiolate leaf lamina attachment. The variation was observed in leaf lamina shape of all four genotypes, which was observed lanceolate in SC, ovate in TO and X and elliptic in GT, RA and RL. Singh (19) also reported elliptic leaf lamina shape for various rough lemon strains. In all the citrus rootstocks, the variation was recorded in leaf lamina margin. Rootstocks RL and GT showed entire leaf margin, whereas in TO, SC, X and RA rootstocks dentate leaf margins were observed. Acute leaf apex was observed in RA, RL and GT, whereas, it was obtuse in TO, SC and X. Earlier, Singh (19) also recorded similar findings for leaf lamina margin in Rangpur lime, Swingle citrumelo and trifoliate orange. Presence or absence of petiolar wing was also studied in citrus rootstocks, which was found present in TO and its hybrids, but absent in RA, GT and RL. TO and its hybrids had narrow petiole wings as compared to absent in RA, GT and RL. Some scientists reported that the width of petiole wing is a morphological marker for screening of hybrids in citrus. Absence of petiole wing in RL and RA was also confirmed by Singh (19). The petiolar wing was

obdurate in SC and obovate in X and absent in RA, GT and RL. Variability was recorded for junction between petiole and lamina; it was articulate in TO and its hybrids. Fused junction was studied in RA, GT and RL. The leaf emergence started from 4th February in X and it was observed in RL on 25th February. The leaf emergence in all the rootstocks was recorded between 4th February (X 639) to 25th February (rough lemon). Leaf lamina length, width and their ratio varied significantly among parentage used in hybridization during the year of study (Table 3). Maximum leaf lamina length (72.75 mm) and width (43.83 mm) were recorded in RL, which was significantly higher than all the other rootstocks. The maximum leaf lamina length: width ratio (1.99 mm) was observed in SC, which was significantly higher than that of all other rootstock genotypes. Similar results were recorded by Kaur *et al*. (10) on citrus rootstocks. The leaf lamina length: width ratio was found highest (2.78) in SC. The maximum petiole wing length (15.52 mm) was recorded in GT. The maximum petiole wing width (2.28 mm) was recorded in SC, whereas, petiole was not reported in rough lemon. Leaf thickness was found non-significant for all the parents.

Data regarding qualitative flower characters, *viz.* flowering season, flowering month, length of anthers relative to stigma, flower type, colour of open flower, colour of anthers, petal colour, number of sepals and petals per flower were studied (Table 4). No variation was recorded with respect to flowering season in all citrus rootstocks. Mid of February to last week of March was observed as main flowering season in all the rootstocks. All the genotypes of citrus under study were found to bloom from mid of February to second week of April. Among all rootstocks X was earliest to flower (February 13), while RL was last (March 1). X was observed to be earliest with respect to mean full bloom with date (February 20-28) and the RL was last too attain full bloom (March 22-28). All the citrus rootstocks also differed with respect to end of flowering. The end bloom was studied late in RL (April 20). Similar variation with respect to start of flowering, full blooming period and end of flowering was obtained by Singh (19) in different citrus

Table 3. Morphological traits of different citrus rootstocks used in hybridization.

Trait / Genotype	Leaf lamina length (mm)	Leaf lamina width (mm)	Leaf lamina length: width ratio	Petiole wing length (mm)	Petiole wing thickness (mm)	Leaf thickness (mm)
TO	48.33d^{e}	27.67^{c}	1.75^{bc}	15.23^{ab}	0.58^{c}	0.41^{a}
SC	62.60b^{c}	31.47b^{c}	1.99^{a}	15.48^{ab}	0.62^{bc}	0.23^{d}
X	44.90^{e}	28.98^{c}	1.55^{c}	11.73^{c}	0.65^{c}	0.23^{d}
RA	55.23^{cd}	30.76^{bc}	1.80^{ab}	14.2^{bc}	0.55^{bc}	0.36^{b}
GT	65.49^{ab}	35.03^{b}	1.87^{ab}	15.52^{ab}	0.84^{a}	0.26^{d}
RL	72.75^{a}	43.83^{a}	1.66^{bc}	17.18^{a}	0.74^{ab}	0.31^{c}

Table 4. Qualitative inflorescence traits of different citrus rootstocks used in hybridization.

Genotype / Trait	TO	SC	X	RA	GT	RL
First bloom	4th March	20th February	13th February	17 March	17th February	1st March
Date of full bloom	8-12th March	1th-8th March	20th-28th February	22-26 March	25thFeb.-3rd March	22-28 March
Date of end bloom	17th March	20th March	12th March	20 April	17th March	20th April
Length of anthers relative to stigma	Shorter	Shorter	Shorter	Longer	Longer	Shorter
Flower type	Hermaphrodite and male	Hermaphrodite and male	Hermaphrodite and male	Staminate	Staminate	Hermaphrodite and male
Colour of open flower	White	White	White	Violet	Violet	Violet cream
Colour of anthers	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Pale yellow	Yellow
Petal olour	White	White	White	White	White	White
No. of sepals per flower	5/6	5	5	5	5	5
No. of petals per flower	5	5	5	5	5	5

rootstocks. Little variation was observed for flower type as both perfect and staminate flowers were observed on a single tree in all the citrus rootstocks. The variation was also observed in the length of anther in all the rootstocks. Length of anthers was longer relative to stigma in GT, when compared to all other rootstocks showed shorter length of anthers relative to stigma. Similarly, Singh (19) reported only hermaphrodite flowers were observed in rough lemon strains. The flower colour was white in TO and its hybrids but, violet in RA, RL and GT. Little variation was observed in anther colour. The colour of petal was white in all the citrus genotypes. The variability with respect to petal colour, flower type and anther colour in different genotypes might be due to their inherent varietal characteristics. The results of this investigation were similar with those of Singh (19) and Kaur *et al.* (10) regarding petal colour and anther colour and flower types, while white coloured flowers were noted in trifoliate, Rangpur lime and rough lemon. No variation was recorded in number of sepals and petals among different genotypes. The maximum mean flower diameter (45.12 mm) was recorded in TO (Table 5). Maximum flower length (26.40 mm) was recorded in TO, which was significantly higher than all other rootstocks. Kaur *et al*. (10) investigated the maximum flower length in rough lemon. Maximum pedicel diameter (2.89 mm), pedicel length (11.23 mm) was recorded in TO and calyx diameter (9.15 mm) was recorded in RL. Maximum petal length (25.45 mm) was observed in GT, which was significantly higher than all other rootstocks. The maximum petal width of 16.60 mm was observed in RL, which was significantly higher than all other rootstocks. The present findings are in accordance with the findings of Singh (19), who reported the higher petal length (21.2 mm) in TO as compared to RL and RA. Width is also observed higher in TO and minimum was recorded in RL, whereas, RA showed results in between. The maximum number of stamens (28.30) was recorded in RL, which were significantly higher than all the other rootstock genotypes under study. Similarly, Singh (19) and Kaur *et al*. (10) also recorded variability for number of stamens among different rootstocks. Style length was recorded maximum (7.94 mm) for GT. While minimum length (6.31 mm) was found in SC, which was significantly less than all other rootstock. This variability may be attributed to the differences in genetic make-up of different genotypes. However, Singh (19) observed minimum filament and style length in trifoliate orange and maximum in rough lemon and Rangpur lime.

Analysis of variance revealed significant variation in lesion size and number of sporangia among the genotypes. Tolerance was judged by the lesser lesion size and minimum number of sporangia.

Table 5. Quantitative floral traits of different citrus rootstocks used in hybridization.

Trait / Genotype	Flower dia. (mm)	Flower length (mm)	Pedicel dia. (mm)	Pedicel length (mm)	Calyx dia. (mm)	Petal length (mm)	Petal width (mm)	No. of stamens	Filament length (mm)
TO	45.12^{a}	26.4^{a}	2.89^{a}	11.23^{a}	8.34^{b}	25.45^{a}	14.27^{b}	27.56^{a}	16.34^{a}
SC	41.11^{ab}	19.18^{cd}	2.17^{b}	6.47^{d}	6.02^{c}	20.58^{cd}	12.24^{cd}	23.71b^{c}	14.56^{b}
X	28.20^{d}	18.31^{d}	1.77^{d}	5.9^{d}	6.18^{c}	18.64^{d}	10.45^{d}	21.67^{d}	11.46^{c}
RA	43.67^{a}	23.12^{abc}	1.99^{c}	9.67^{ab}	7.56^{b}	21.34^{cd}	13.12^{bc}	22.45^{cd}	13.78^{b}
GT	35.31^{bc}	21.89^{bcd}	2.28^{b}	7.5^{cd}	8.15^{b}	24.35^{ab}	13.38^{bc}	24.08^{b}	13.7^{b}
RL	32.66^{d}	23.93^{ab}	2.30^{b}	9.15^{bc}	9.60^{a}	22.23^{bc}	16.60^{a}	28.30^{a}	15.97^{a}

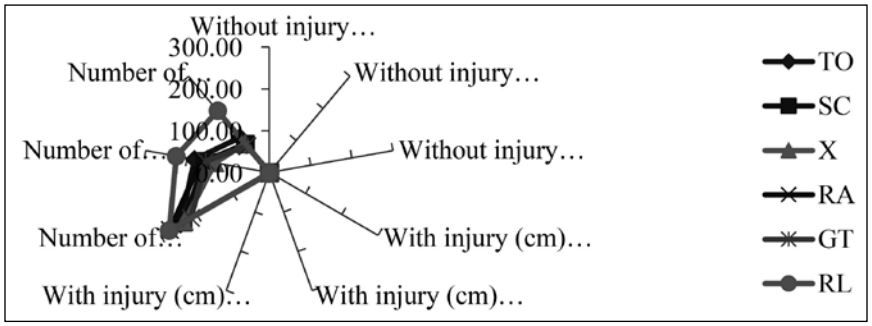

Fig. 1. Screening of citrus rootstocks by leaf bait technique against *Phytophthora* root rot.

After 48 h of incubation, smallest lesion size (0.41 cm) was observed in TO (Fig. 1), while, maximum lesion size was observed for RL (1.00 cm). After 96 and 120 h of incubation, TO show the minimum lesion size (1.12 and 1.67 cm, respectively) and RL showed the maximum lesion size (above 2.0 cm). Rough lemon leaf discs developed the largest lesions on the wounded leaf (2.42 cm) after 120 h of *P. parasitica* incubation. In the leaf discs with injury after 48 h of incubation, the minimum lesion size was recorded with TO (0.61 cm) and largest on RL (1.25 cm). The lesion size exceeded up to 1.34 cm after 72 h incubation on RL. The rootstock, TO showed consistently smallest lesions after 96 (1.22 cm) and 120 h (1.77 cm) of incubation. The number of sporangia counted after 48 h of incubation showed that all leaf baits of each rootstock were attacked by large number of sporangia. The minimum number of sporangia after 48 h of inoculation were observed on TO (216.33) and maximum on RL (276.67). The number of sporangia on each leaf disc of rootstocks decreased after 48 h as the sporangia germinated into mycelium on the edges of leaf discs. Sharma (18) used leaf baiting for screening against *Phytophthora* in potato. While, Harada and Kondo (8) utilized detached leaf method for evaluation of resistance for *Phytophthora* in beans who observed water soaked large spreading lesions as susceptible reaction. Dhakad *et al*. (6) also used leaf bait screening against *Phytophthora* in Kinnow mandarin. The reduction in the number of sporangia and lesion size in TO and SC indicates their tolerance against *Phytophthora nicotianae* var. *parasitica.*

A total of 655 alleles were amplified by 55 markers and the number of alleles ranged from 2 (CCSMEc8) to 11 (F40) with an average of 5.69 alleles per locus

Table 6. Primer sequence and other details of information generated by 24 SSR markers used in characterization of six citrus rootstocks.

Primer code	G + C content (%)	Anneal. temp. (°C)	PIC	Genetic diversity	Resolving power (Rp)	Marker index (MI)
CCSMEc3-F	50.00	58.00	0.69	0.88	5.00	7.03
CCSMEc4-F	60.00	60.00	0.83	0.97	2.00	5.83
CCSMEc10-F	50.00	58.00	0.63	0.83	3.67	4.14
CCSMEc12-F	50.00	58.00	0.63	0.83	3.00	3.31
CCSME5-F	50.00	58.00	0.75	0.92	2.67	5.53
CCSME23-F	50.00	58.00	0.67	0.88	2.67	3.50
CCSME27-F	45.00	56.00	0.67	0.84	6.00	7.56
CCSME33-F	55.00	58.00	0.60	0.79	6.33	6.31
CCSME42-F	45.00	58.00	0.63	0.81	3.67	4.03
CCSME46-F	55.00	60.00	0.83	0.97	2.00	5.83
CCSME49-F	47.62	58.00	0.63	0.84	3.00	3.36
TAA15-F	52.38	58.00	0.72	0.91	3.33	5.44
Ci03C08-F	52.94	53.00	0.71	0.90	4.00	6.33
F02-F	45.83	60.00	0.67	0.87	2.00	2.61
F13-F	41.67	60.00	0.63	0.78	3.00	3.14
F29-F	41.67	60.00	0.73	0.89	2.67	4.44
F40-F	43.48	60.00	0.65	0.84	7.67	9.25
F43-F	37.50	58.00	0.67	0.83	2.00	2.50
F50-F	45.83	60.00	0.76	0.94	3.33	6.56
F53-F	50.00	60.00	0.67	0.87	2.00	2.61
F77-F	45.83	58.00	0.71	0.87	2.33	3.47
F90-F	55.00	60.00	0.67	0.89	1.33	1.78
TAA1-F	50.00	60.00	0.62	0.83	7.67	8.25
CAC33-F	55.00	60.00	0.72	0.91	3.33	5.44

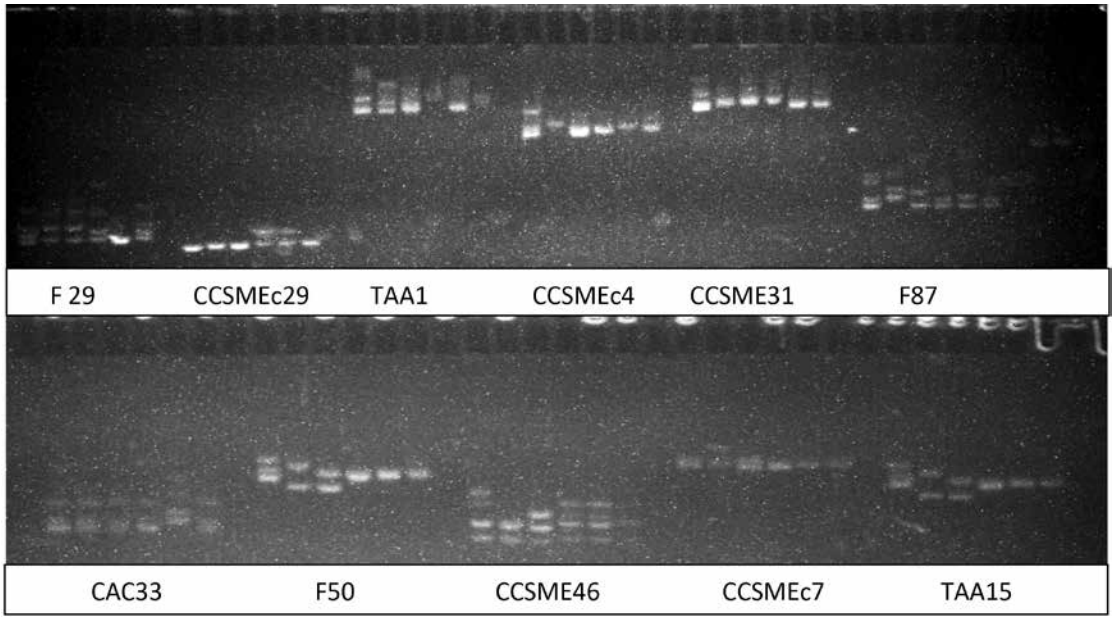

Fig. 2. PCR amplification of DNAs from six citrus genotypes. RL = rough lemon, SC = Swingle citrumelo, X = X639, TO = trifoliate orange; GT = Gou Tou, and RA= Rangpur lime.

(Table 6). All primers showed polymorphism among all genotypes, while six primers (F29, CCSMEc29, TAA1, CCSMEc4, CCSME31 and F87) showed polymorphism between all the genotypes RL, TO, RA, X, GT and SC (Fig. 2). The PIC value provides an estimate (Table 1) of the discrimination power of a marker by taking into account not only number of alleles at locus but also the relative frequencies of those alleles in the rootstocks and analyzed to characterized the capacity of each SSR to show polymorphic loci, varied from 0.29 (F98) to 0.83 (CCSME46 and CCSMEc4) with an average PIC value of 0.61. The Rp (resolving power) was the highest for the primer CCSME43 (8.33) and was lowest for F90 (1.33). Significant differences were observed in the value of MI (Marker Index) and were found to be the highest for primer F40 (9.25), while minimum MI was recorded for F98 (0.38). The PIC values of a marker vary with the crop and the set of the genotypes used. Froelicher *et al*. (7) reported that PIC value from 0.05 to 0.70 over the four loci in 77 genotypes. Whereas, in Rangpur lime it ranged from 0.32 (CCME8, F16, CCSMEc3, CCSME49) to 0.828 (CCSME29), with an average of 0.51 across all strains, Similarly, PIC values of 0.68, 0.63, 0.61, 0.64 and 0.41 were recorded in lemon, mandarin, grapefruit, natural hybrids and sweet orange, respectively (Novelli *et al*., 14). The data on number of alleles amplified and PIC values showed that higher the number of alleles amplified, the higher is the PIC value but, the trend was not followed consistently. The markers, which have two alleles, had a PIC value ranging from 0.37 to 0.5. However, the markers, which amplified four alleles had PIC value 0.40 to 0.72. The markers with 5 alleles had PIC value of 0.78 and 6 alleles of 0.78 to 0.81. Hence, the data depicted that the highest PIC value did not confirm with the higher number of alleles. The PIC value across all loci ranged between 0.09 to 0.71 with an average of 0.37 (Soriano *et al*., 20). The PIC values vary with markers, species and set of the genotypes used. The lower PIC value may be due to closely related genotype and higher PIC value may be due to the diverse genotypes. Marker loci with an average number of alleles running at equal frequencies will have the highest PIC value. The higher PIC values in the present studies could be due to the use of polyacrylamide gels in comparison to agrose gels.

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Efficacy of gene-based markers associated with sex expression in papaya

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ABSTRACT

The papaya (*Carica papaya* L.) is a polygamous plant, has three types of sex forms, *viz.* male, female and hermaphrodite. Efforts were made to identify sex expression and validation of gene-linked molecular markers associated with it. Molecular markers (ISSR and SCAR) were employed to distinguish male, female and hermaphrodite sex forms in seedlings and well differentiated adult plant types. The important markers which have been found linked with sex forms in papaya were used for validation in population of six papaya genotypes. Total 10 sex linked DNA markers (9 SCAR and 1 ISSR) were employed for validation of sexes in 6 papaya genotypes (dioceious and gynodioecious) . Out of 10 only five (T12, W11, SCAR SDSP, C09/20 and GACA4) were amplified for the sex expression. However, five SCAR (SCARp, SCARpm, T1, STS 807 and STS 831) marker did not get amplified with the all sex genotypes. Of the two marker systems tested, SCAR markers were most consistent. Markers, namely, T12, CFW+CRV and W11 were most informative to predict cent per cent sex forms. These markers can be used by the breeders and commercial papaya growers to identify desired sex types at seedling stage in nursery for establishing a productive plantation.

Key words: Papaya, sex form identifican, molecular markers.

INTRODUCTION

The papaya, *Carica papaya* L., is a native of Central and South America and belongs to the family Caricaceae. Papaya is a commercial fruit crop cultivated throughout the tropical and sub-tropical regions of the India and ranks fifth with regards to area and production among different fruit crops. Propagation of papaya by seed is still the most practical method of raising commercial crop. There are several reasons why a desirable sex type of papaya plant needs to be identified prior to planting. Generally, the number of male plants outnumbers the females in a plantation, which renders it unproductive.

The papaya generally flowers 75 to 150 days after transplanting and identification of the desirable plants at seedling stage would help in raising the orchard with appropriate design. In subtropical region the dioecious varieties like Pusa Nanha and Pusa Dwarf are preferred in over gynodioecious once due higher number of stable female plants in a population, besides their dwarf stature and high yields. Conversely in the tropical areas, gynodioecious varieties are preferred because of their high yield potential. Sex expression in papaya is controlled by a single gene with three alleles, which have a pleiotropic effect (Hofmeyr, 8; Storey, 15). With the advancement of the science and techniques new facts has been explored, *i.e.* physical mapping and sample sequencing of the non-recombination region led to the conclusion that sex determination is controlled by a pair of primitive sex chromosomes with a small male-specific region (MSY) of the Y chromosome (Ming *et al.*, 10).

The leaf extracts of a large number of sexually undifferentiated seedlings at nursery stage, with modified Almen reagent were analysed. A colorimetric test for total phenols can differentiate females (86%) from males (77%), but was unable to detect the bisexual plants (Jindal and Singh, 9). Paper chromatography also indicated that trans-cinnamic acid is dominantly expressed in the leaves of hermaphroditic seedlings, but females and males could not be differentiated (Poller, 13). In addition, isozymes have also been exploited to identify markers that could co-inherit with sex types in papaya using the banding patterns of cationic peroxidase, males could be differentiated from females, but females could not be distinguished from hermaphrodites (Sriprasertsak *et al.*, 14).

The failure of morphological tags, cytological evidences and isozyme markers to determine the sex types in papaya at the seedling stage has led to use of DNA markers for determining the sex differences. Hence, the present study was undertaken with an objective to validate the efficacy of gene based markers associated with sex expression in papaya.

MATERIALS AND METHODS

Seedling of six papaya genotyes (dioecious and gynodioecious), namely, Pusa Nanha, Red Lady, P-7-2, P-9-5, P-9-12 and P-7-2 x SAM were raised

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to examine the sex expression at the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi during 2014-2015. Genomic DNA extraction was done at seedling stage and same tagged plants were planted in the net house to validate the male, female and hermaphvrodite type of sex expression at the flowering. Polymorphism study was carried out using Inter Simple Sequence Repeats (ISSR) and Sequence Characterized Amplified Region (SCAR). Total 10 primers (Table 1) were employed to distinguish male, female and hermaphrodite sex forms in seedlings and well differentiated adult plants.

From tender leaves of papaya seedling, genomic DNA was extracted using the protocol and the steps involved in CTAB method of DNA isolation as given by Murray and Thomson (11). Two hundred milligram of young, succulent and healthy leaves were taken and grind in liquid nitrogen to a fine powder and transferred to a 1.5 ml Eppendorf tube. Quantification of DNA was done by analysing the purified DNA on 0.8% agarose gel with Hind III-cut λ DNA as standard. The concentration of DNA in individual sample was determined based on the intensity of the bands in the λ DNA ladder. Part of the DNA samples was diluted with appropriate amount of TE buffer to yield a working concentration of 20 ng/µl and stored at 4°C. The reaction (Touchdown PCR) with initial denaturation at 94^0C for 5 min., denaturation 94^0C for 1 min., primer annealing at 55^0C for 1 min. 35 cycles, primer extension at 72^0C for 1 min., final extension at 72^0C for 8 min., touch down of 0.5^oC for the first 20 cycles were carried out. The amplified fragments were resolved on 2 per cent agarose gel. Number of polymorphic bands generated by each primer was scored as 0 or 1 for absence or presence of band, respectively.

RESULTS AND DISCUSSION

Out of 10 only five (T12, W11, SCAR SDSP, C09/20 and $GACA_4$) were amplified for the sex expression, while rest five SCAR (SCARp, SCARpm, T1, STS 807 and STS 831) markers did not get amplified with the all the sex types. Among the amplified markers some of them did show ambiguity in the results particularly with the validation of hermaphrodite plants. Though, some of the markers were consistent with particular genotypes.

The Fig. 1 exhibited PCR amplification of SCAR T12 DNA markers showing segregation for male, female and hermaphrodite sexes of Pusa Nanha,

Table 1. List of 10 primers (9 SCAR and 1 ISSR) were used to validate male, female and hermaphrodite sex forms in six papaya genotypes.

Name	Primer sequence (5'……..3')	Reference
SCAR marker		
T12	T12F:GGGTGTGTAGGCACTCTCCTT T12R:GGGTGTGTAGCATGCATGATA	Deputy *et al.* (5)
W11	W11F:CTGATGCGTGTGTGGCTCTA W11R:CTGATGCGTGATCATCTACT	Deputy *et al.* (5)
SCAR SDSP	CFW:AAACTACCGTGCCATTATCA CRV:AGAGATGGGTTGTGTCACTG	Chaves-Bedoya and Nunez (2)
C09/20	FP:CTCACCGTCCATTTTAATTA RP:CTCACCGTCCGCGGCATCAATGTA	Niroshini *et al.* (12)
SCARp	SDP-1:GCACGATTTAGATTAGATGT SDP-2:GGATAGCTTGCCCAGGTCAC	Urasaki *et al.* (17)
SCARpm	SDP-2-F: GGATAGCTTGCCCAGGTCAC SDP-2-R: GGTAAGAGTTTTTCCCAAGC	Urasaki *et al.* (16)
T1	T1F:TGCTCTTGATATGCTCTCTG T1R:TACCTTCGCTCACCTCTGCA	Deputy *et al.* (5)
STS 807	STS 807-F ATTAGCCCCCAAAACAGAGC STS 807-R ATGGAGGGGGAGGACTCTAA	Conomikes *et al.* (3)
STS 831	STS 831- F ATA TAT ATA TAT ATA TYA STS 831-R ATA TAT ATA TAT ATA TYC	Conomikes *et al.* (3)
ISSR marker		
(GACA)4	(GACA)4:GACAGACAGACAGACA	Gangopadhyay *et al.* (7)

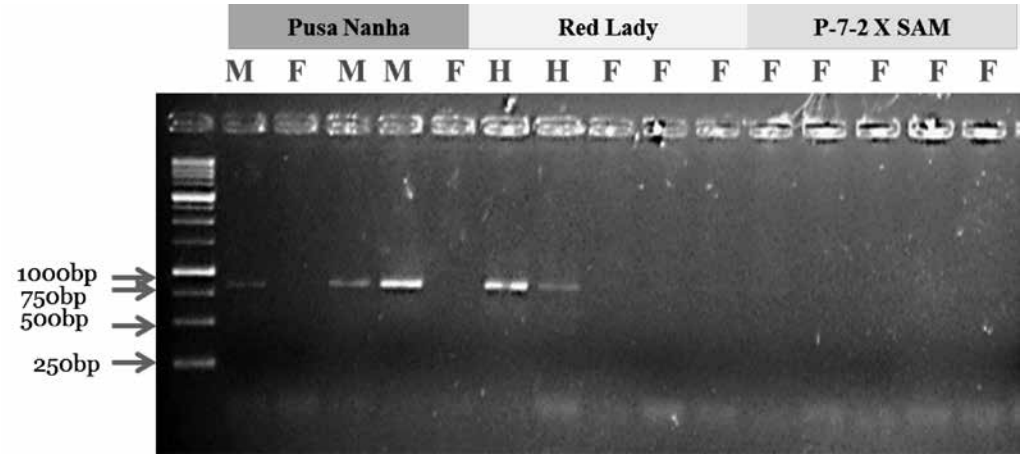

Fig. 1. PCR amplification showing segregation of SCAR T12 with papaya sex. Genotype names are depicted in coloured boxes, M = male, F = female, H = hermaphrodite, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples and their sexes.

Red Lady and P-7-2 × SAM genotypes. PCR was run on genomic DNA from papaya genotypes Pusa Nanha, Red Lady and P-7-2 × SAM using primers of SCART12 marker. The desired size of band (~800 bp) is present in male and hermaphrodite plants but not in female plants among the dioecious and gynodioecious genotypes. It was most effective DNA marker could be useful to identify to the desired sex across the genotypes the male and hermaphrodite. The similar results were reported by Deputy *et al.* (5) markers tightly linked to Sex1, the gene, which determines type of plant sex in papaya with a population of 182 F_2 plants from a SunUp × Kapoho.

PCR amplification and segregation of SCAR SDSP (CFW, CRV) with female and hermaphrodite types of papaya sexes (Fig. 2). PCR was run on genomic DNA from papaya genotypes Red Lady, P-9-12 and P-7-15 using CFW and CRV primers of SCAR SDSP marker. The desired size of band (~375bp SCAR SDSP) is present only in hermaphrodite plants but not in female plants among studied genotypes. PCR was run on genomic DNA from Red Lady, P-9-12 and P-7-15 using primers for C09/20 SCAR marker (Fig. 3). SCAR C09/20 with ~1000 bp band size is present only in hermaphrodite plants but not in female plants. SCAR C09/20 was able to validate hermaphrodite plants of the two gyndioecious genotypes indicate that it is very effective for P-9-12 and P-7-15 but presence of too faint band in female Red Lady indicates human errors. The findings of SCAR C09/ 20 are in accordance with the earlier report of Deputy *et al.* (5); Urasaki *et al.* (16) identified after screening of 25 arbitrary

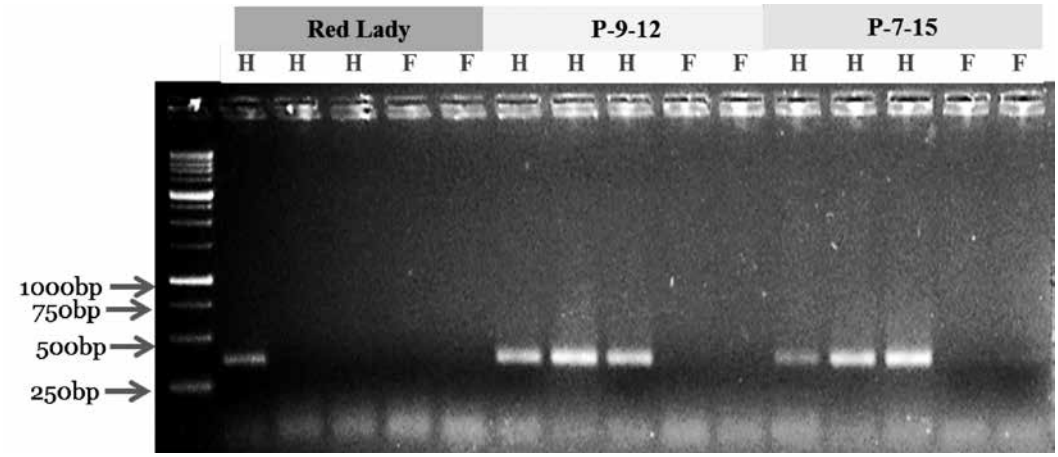

Fig. 2. PCR amplification showing segregation of SCAR SDSP (CFW, CRV) with papaya sex. Genotypes names are depicted in coloured boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples with their type of sexes.

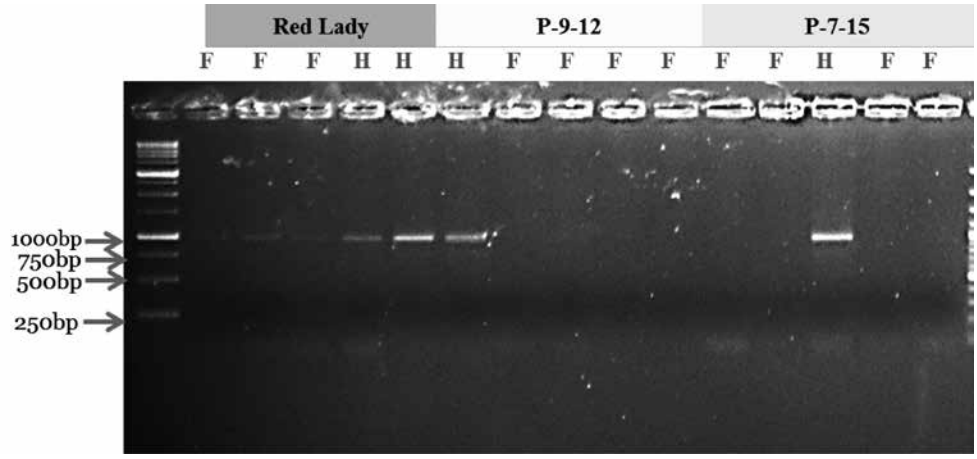

Fig. 3. PCR amplification showing segregation of SCAR C09/20 with papaya sex. Names of the genotypes are depicted in boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples.

primers in Sunrise Solo papaya and De Oliveira *et al*. (6), Niroshini *et al.* (12) from 100 arbitrary decamer primers in 10 plants of papaya from Sri Lanka for type of sexes and Chaturvedi *et al.* (1) with six sequences of SCAR from the 84 F_1 plants of papaya. DNA is extracted from the leaves of the papaya plants and is subjected to PCR amplification showing segregation of SCAR W11 with papaya sex (Fig. 4). PCR was run on genomic DNA from papaya varieties Red Lady, P-7-2 and P-9-5 using primers for W11 SCAR marker. SCAR W11 with a band size of ~825 bp is present only in hermaphrodite plants but not in female plants. The results are in accordance to Urasaki *et al.* (16) who had tested these primers on three papaya genotypes.

The Fig. 5 exhibits PCR amplification showing segregation of ISSR in male, female and hermaphrodite types. The PCR was run on genomic DNA from papaya varieties Pusa Nanha, Red Lady and P-7-2 × SAM using $(GACA)_4$ primers for ISSR marker. The P1 (~ 600 bp) amplicon was present in male and female plants but not in hermaphrodite plants. The P2 (~2 kb) amplicon is present only in female plants but not in male and hermaphrodite plants. The P3 (~ 3 kb) amplicon present only in P-7-2 × SAM genotype but not in others. The $(GACA)_4$ primer was also very effective to validate male, female and hermaphrodite plants among the diverse genotypes for sexes. The similar finding have been reported by Parasnis *et al*. (1999) identification of male sex specific markers in 8 dioecious papaya varieties of papaya using $(GATA)_4$ and $(GACA)_4$ simple sequence repeats, Gangopadhyay *et al.* (7) differentiated sex expression

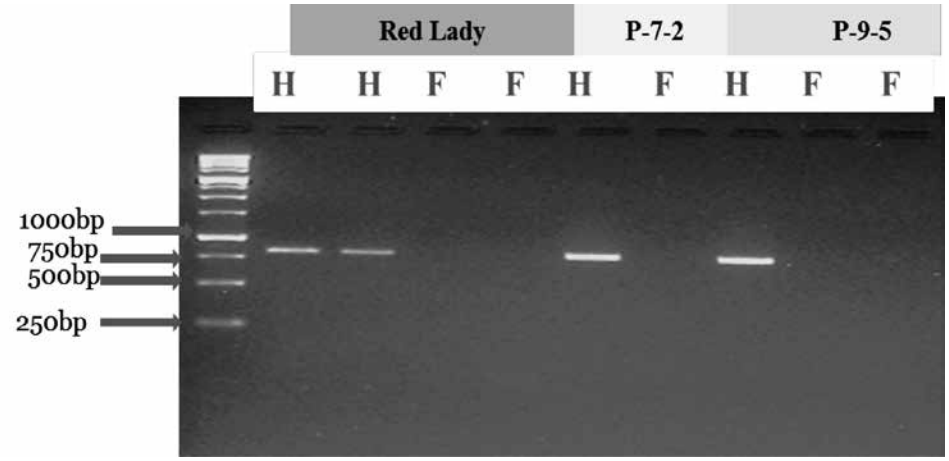

Fig. 4. PCR amplification showing segregation of SCAR W11 with papaya sex. Genotypes names are depicted in boxes, H = hermaphrodite, F = female, L = 1 kb DNA ladder and numeric over the agarose gel represent different samples.

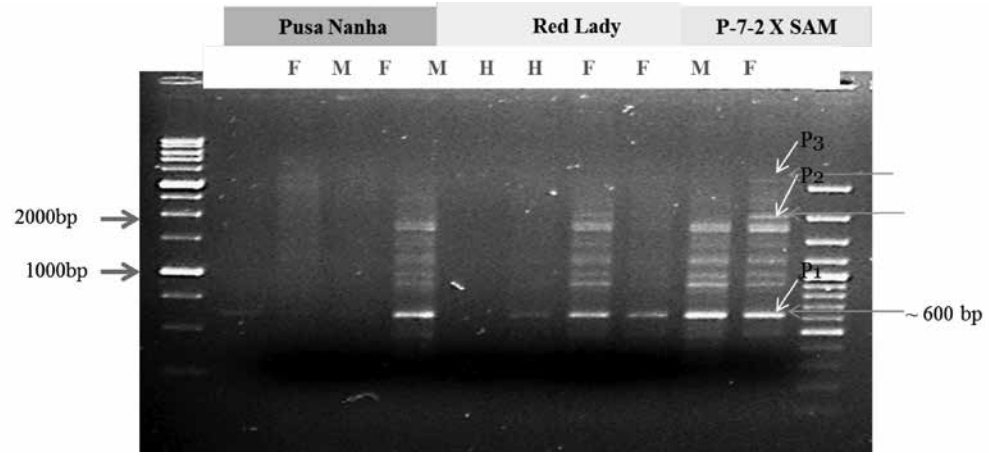

Fig. 5. PCR amplification showing segregation of ISSR with papaya sex. The genotypes names are depicted in coloured boxes, M = male, H = hermaphrodite, F = female, L = 1 kb DNA ladder, L2 = 100 bp DNA ladder and numeric over the agarose gel represent different samples of the papaya.

in *Carica papaya* and *Cycus ciranilis*. Costa *et al*. (4) established sex differentiation through ISSR marker at 500 bp in *Carica* and *Vasconcellea* spp. Among the five amplified sex-linked DNA markers some of them have shown the ambiguity in the results particularly with the validation of hermaphrodite plants. ISSR markers were consistent with P-7-2 × SAM genotypes. Of the two marker systems tested, SCAR markers were most consistent. Markers, namely, T12, CFW+CRV and W11 were most informative to predict 100% sex forms. These markers can be used by the breeders and commercial papaya growers to identify desired seedlings at early stage.

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Floral morphology of *Eleaegnus latifolia* L.

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ABSTRACT

***Eleaegnus latifolia* L. is one of the most important life sustaining underutilized fruit crops among the tribes of the eastern Himalaya region. The fruits have several uses and all parts of the fruits including seed are edible at all stages of fruit growth. Despite of great potential, little or no information is available on its flower characteristic, which is a pre-requisite for understanding the reproductive biology of the species. Five genotypes of *Eaeagnus latifolia* L. collected from different locations of Meghalaya were used to study its flowering and fruiting traits. Result revealed that inflorescence is a raceme, appears in clusters from the leaf axils, flowers are light yellow coloured, hermaphrodite, actinomorphic and gives strong aroma. Ovary is inferior surrounded by hypanthium. Significant variations were observed among the genotypes for all the flower characteristics such as dimension of ovary, stigmas and pollen length in the polar region. Maximum bud intensity was recorded in REC-3 (16.0) on current season shoot. Number of flower buds was higher in the current season shoots as compared to previous season. Regardless of genotypes, number of flowers was higher in the middle portion of the shoot. Total number of flowers per shoot was maximum in REC-4 (127.0). Flowering duration per inflorescence varied from 6.0 days (REC-1 and REC-2) to 8.67 days in REC-4. Initial fruit set was recorded maximum in REC-1 (37.95%) and minimum in REC-2 (14.15%).**

Key words: *Eleaegnus latifolia*, floral morphology, *Sohshang.*

INTRODUCTION

Eleaegnus latifolia L., the vernacular name is *Sohshang* in Khasi Hills and *Slangi* in Jaintia Hills of Meghalaya. The fruit has traditionally been used for centuries as one of the most potential underutilized fruit crops among the tribal habitat of North Eastern Himalayan region, India. Geographically, the region stretches between 21°50' and 29°34' N latitudes and 85°34' and 97°50' E longitudes, and altitude varies from near sea-level to over 7,000 m above msl. It is native to the North Eastern Region, India. It is a perennial and semi-deciduous multi-stem shrub, belonging to the family Elaeagnaceae. The family consists of three genera, *viz*., *Elaeagnus Hippophae* and *Shepherdia*. The genus *Elaeagnus* consist about 40 species of shrubs and trees, however, only 3 species are known for planting in other parts of the world, *viz*., Russian olive (*Elaeagnus angustifolia*), silverberry (*Elaeagnus commutate* Bernh. Ex Rydb) and autumn olive (*Elaeagnus umbellate* Thunb). Apart from the fruits, the seeds of most of the species including *Elaeagnus latifolia* are edible. Recently, the genus has become a critical underutilized fruit crops because the trees of the genus *Elaeagnus* have a symbiotic relationship with certain soil bacteria like the genus *Actinomycetes* responsible for producing root nodules and fix atmospheric nitrogen (Follstad Shah, 2). Because of its atmospheric nitrogen fixing abilities, an increase in fruit production up to 10% on intercropping with plum and nuts was reported. The species are quite resistant to high wind velocity and performed well even on nutrient poor acidic soil and soil moisture stress conditions. The fruits are also rich in vitamins, minerals and other bio-active compounds (Rymbai *et al*., 6). More importantly, the fruits are also capable of minimizing the incidence of cancer and reversing the growth of cancer cells (Matthews, 4).

Breeding to develop improved cultivars with high yield and quality is one of the approaches including recent efforts on standardization of agro-techniques (Deka and Rymbai, 1). Thus the development of methods for reliable and efficient breeding techniques in this crop is critical for higher success. However, to achieve success in breeding programme, knowledge of floral morphology and fruiting attributes become pre-requisite. Thus, understanding the reproductive and yield attributes of *Elaeagnus latifolia* is an important issue and indispensable to successful conservation efforts. Flower is an important reproductive organ of any flowering plants. The flower characteristics are unique, expressed consistently and are controlled genetically with very little or no influence of the environmental factors. Therefore, flower structures are very essential component for identification of plants and crop improvement. Till date, very little information is available on flower traits of

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other species of the genus *Eleaegnus,* such as Russian olive (*Elaeagnus angustifolia*), silverberry (*Elaeagnus commutate* Bernh. Ex Rydb), autumn olive (*Elaeagnus umbellata* Thunb) and *Elaeagnus commutate*. However, there is no report available on floral biology of *Elaeagnus latifolia*. Therefore, the present study on floral morphology of this species was undertaken to provide greater insights on floral characters and fruiting attributes, which would facilitate efficient breeding programme for higher crop productivity.

MATERIALS AND METHODS

The present study was conducted during 2013-15 on five genotypes of *Eleaegnus latifolia* L., *viz*., REC-1, -2, -3, -4, -5 collected from different locations of Meghalaya and maintained at the ICAR Research Complex for NEH Region, Umiam. *Elaeagnus latifolia* is a perennial semi-deciduous tree. Flowering appears during October-November. Three fully grown healthy and well maintained trees were selected from the orchards. All the trees received uniform recommended cultural management practices during the course of study. Qualitative flower characteristics were critically observed in the field. Characters observed were flower bearing habit, colour on sides, *i.e*., abaxial and adaxial, sex, symmetry and aroma. Number of lobe per flower, number of perianth per flower and nature of trichomes were examined under dissecting microscope (Leica EZ4D) at 8X magnification. Bud intensity was observed when the buds appeared on the shoot (30 cm shoot length) separately for current season and on shoots of previous season. Number of flower buds per inflorescence was noted by counting on tagged inflorescences (n = 10) in four directions of each tree. In order to avoid error, the counted flowers on each inflorescence were removed and fresh open flowers were counted on daily basis. Numbers of flowers at apical, middle and base portion of shoot was also recorded as and when they opened. Total number of flowers per shoot was recorded by addition of all the flowers recorded from the apical, middle and base portion of the shoot. Flowering duration per inflorescence was assessed by noting the date of first flowers visible to last flower appeared in an inflorescence on a daily basis. Initial fruit set per inflorescence was recorded at grain stage of fruit development in all four directions of the tree. During full bloom, the newly opened flowers were collected in the morning hours and were directly put in the fixative FAA for microscopic studies. The fixed flowers were mounted on the stage of microscope and flower parts were measured accordingly at different magnifications depending upon the floral organs. Observations on 50 flowers were taken from each genotype for all the parameters. Flower size, petal, ovary, style, anthers, filaments and stigma characters were observed with the help of dissecting microscope (Leica EZ4D) at specified magnification. Flower size and petal dimensions were measured at 8X magnification. Flower length was taken from the base of the ovary to the top of the flower, stamens or stigma. The flower width was observed at the maximum width in two directions of the flower. The average of these two directions was taken as flower width. Petal length was noted from tip to base and width was measured at the maximum portion of the petal. Ovary, stigma and style dimension were determined at 35X magnification. The length was taken from tip to base of the ovary. Ovary width was taken at three portions, *i.e*., apical, middle and at the base. The style length was recorded from tip to base of the style. The style width was recorded at three portions, *i.e.* tip, middle and top. Stigma length was taken from tip to base and diameter was measured at the maximum width portion (middle) of the stigma. Anther and filament dimensions were observed (n = 50) at 25X magnification. The anther length was recorded longitudinally and width was noted in the central portion where the maximum width was observed. The filament length was taken from tip to base, whereas, width was assessed at the base, middle and tip portion of the filament. Pollen dimension was assessed under the light microscope (Olympus BX53) at 40X. Flowers near anthesis period were collected in the morning hours (8:30 am to 9:30 am) and the anthers were dusted on the slide and covered with a cover slip after applying one to two drops of the staining agent fluorescein diacetate (FDA). The slides were then placed under the microscope to measure the length along the polar and the equatorial regions. The data on different parameters were analyzed using analysis of variance (ANOVA) based on randomised block design (RBD) using SPSS (version 18.0). Valid conclusions were drawn only on significant differences between the genotype mean at 0.05 level of probability.

RESULTS AND DISCUSSION

Reproductive structures are known to reveal evolutionary relationships more clearly among plants. The present finding divulged that flowers of all the genotypes appears in clusters in the leaf axils and are short (Table 1; Fig. 1a). Flowers are hermaphrodite, actinomorphic, four-lobed and having single perianth (Fig. 1b). It produces strong aroma, which might have been released from the conical papillae of the adaxial epidermis of the perianth. The presence of essential oils in the epidermal cells of the petals might have also been one of the reasons for this strong aroma. The presence of essential oils have also been reported in

Table 1. Qualitative flowering characteristics of different *Elaeagnus latifolia* L. genotypes.

Genotype	Bearing habit	Colour		Sex	Symmetry	Aroma	No. of lobe	No. of perianth	Nature of trichome
		Adaxial	Abaxial						
REC-1	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-2	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-3	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-4	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate
REC-5	Cluster in the leaf axils	Light yellow	Light green	Hermaphrodite	Actinomorphic	Strong	4	1	Peltate

many plant species (Stpiczynska, 8). Apart from the strong aroma, another attractive feature of the flowers is colouration which is light yellow from the adaxial side and light green in the abaxial side, exhibiting its ability to attract high number of pollinators. Attractive and shining peltate hairs of silvery star shape were found on adaxial surface of sepals. Silvery star shape hairs were observed at the apical portion of the perianth lobes and on the style (Fig. 1g). Peltate hairs are known for their function in reducing transpiration from organs. All these features together might be responsible for attracting high numbers of certain dipteran insects resulting in heavy fruit-set.

Significant differences were detected for flowers bud intensity, number and duration of flower among the genotypes (Table 2). Maximum bud intensity was recorded in REC-3 (16.00) for current season shoot and REC-2 (6.33) on previous season shoot. REC-4 showed the maximum number of flower buds per inflorescence in both the current season (21.67) and previous season shoot (14.33). Result suggests that irrespective of genotypes, bud intensity and number of flower bud per inflorescence were higher in the current season shoot as compared to the previous season shoot. Number of flowers at apical, middle and base portion of shoot revealed a significant variation among genotypes. REC-1 recorded the maximum number of flowers at apical portion (23.33), whereas, REC-5 recorded maximum at the middle (64.00) and base (40.67) portion of the shoot. This indicates that number of flowers varies with position at various length of the shoot. Regardless of genotypes, numbers of flower were higher in the middle portion of the shoot. Therefore, if any hybridization programme is to be carried out successfully, emphasis may be given to the current season and middle portion of the shoot for greater option on flowers availability.

Total number of flowers per shoot was maximum in REC-4 (127.00) and minimum in REC-2 (69.67). Duration of flowering per inflorescence varies from 6.00 (REC-1 and REC-2) to 8.67 days in REC-4. Result indicates that for efficient hybridization, operation must be taken within this period. Initial fruit set was recorded maximum in REC-1 (37.95%)

Table 2. Flower bud intensity, number and duration of flowers and fruitset characteristics of *E. latifolia* L.

Genotype	Bud intensity at 30 cm length		No. of flower buds per inflorescence		No. of flowers			No. of flowers per shoot	Flowering duration (days)	Fruit set (%)
	Current season shoot	Previous season shoot	Current season shoot	Previous season shoot	Apical portion of shoot	Middle portion of shoot	Base portion of shoot			
REC-1	8.33	1.33	11.33	9.33	23.33	40.67	24.67	88.67	6.00	37.95
REC-2	13.67	6.33	17.67	7.00	16.00	32.00	21.67	69.67	6.00	14.15
REC-3	16.00	1.33	19.33	1.00	15.33	58.00	39.67	113.00	7.00	35.18
REC-4	8.33	2.33	21.67	14.33	22.00	34.00	36.67	92.67	8.67	19.38
REC-5	14.00	2.33	19.66	6.33	22.33	64.00	40.67	127.00	7.67	28.48
$CD_{0.05}$	2.61	1.67	2.12	4.29	3.06	5.37	5.16	13.45	1.04	1.41

and minimum in REC-2 (14.15%). This suggests that there is variation among genotypes, which might be due to genetic constitution.

Flower size and density determine the food resources for pollinators, which in turn lead to the reproductive success of plants. Genotypes under present study showed varied flower size and were significantly different ($p \leq 0.05$) for flower length and width (Table 3). Maximum flower length was noted in REC-1 (12.23 mm), which was *at par* with REC-3 (11.46 mm); whereas, minimum flower length was recorded in REC-5 (7.88 mm). Similarly, maximum flower width was recorded in REC-1 (8.65 mm), which was *at par* with REC-3 (8.25 mm), while minimum width was recorded in REC-2 (6.95). These variation in flower size among the genotypes might be genotype dependent.

Sepals are important parts of the flower which protect the inner parts of the flower and prevent desiccation during its development. It was observed that sepals were small and greenish in colour irrespective of the genotypes. Sepals were four angled, fused together to form tubular and bell-shaped structure (Fig. 1b & c). It was observed that sepals and stamens are linked by a tissue of long tube extending below ovary. A hypanthium is a floral cup or calyx tube developing from common zonal growth at the base of perianth (congenitally fused and androecium) parts. Furthermore, any tubular structure bearing the calyx, corolla lobes and the stamens are considered to be a hypanthium.

The petals vary dramatically among species, which have been more commonly used to distinguish and classify the species. Petal characteristic also play a vital role in proper pollination by attracting pollinators. Irrespective of genotypes, *Sohshang* flower has four numbers of petals. Significant differences ($p \leq 0.05$) in petal size were observed within the five *Sohshang* genotypes (Table 3). REC-1 recorded maximum petal length (3.59 mm), which was *at par* with the REC-5 (3.42 mm) and REC-3 (3.36 mm). Shortest petal (2.81 mm) was found in REC-2. Similarly, maximum petal width was also noted in REC-1 (3.01 mm), which was *at par* with REC-5 (3.00 mm) and REC-3 (2.96 mm). These variations among genotypes for petal size could be due to genotypic effects.

The ovary position and number of ovule with respect to the other floral parts is often used as a trait to distinguish taxa, especially families. In all the genotypes, ovary appears to be inferior, although there was no connection observed between the ovary walls with the external wall. However, there was a constriction above the ovary (Fig. 1f). This inferior position of ovary was further stressed due to the presence of a nectary on the hypanthial slope, surrounding the style. Ovary characteristics also exhibited significant variation ($p \leq 0.05$) among genotypes (Table 3). At apical portion, REC-1 recorded highest ovary width (0.57 mm), while lowest (0.44 mm) was observed in REC-2 and REC-4. Ovary width at middle portion was recorded maximum in REC-3 (0.63 mm), while ovary width at the base was highest in REC-1 (0.52 mm). Maximum ovary length (1.25 mm) was recorded in REC-2, as compared to minimum in REC-5 (0.82 mm). These variations in ovary dimension might be a unique trait of each genotype. Irrespective of genotypes, one ovule was recorded, which is erect and inverted in position (Fig. 1d).

Style is the supportive stalk of stigma and the pathway for pollen tubes to grow from pollen grains adhering to the stigma. In all the genotypes, silvery and shining star shaped hairs on the style (Fig. 1g) and a disc located at the base of the style were observed (Fig. 1h). The base of the style was enclosed by the hypanthial slope. Significant variation was also observed in style dimension among different genotypes of *Sohshang* ($p \leq 0.05$; Table 3). Maximum style width at the base was recorded in REC-5 (0.58 mm), while minimum was noted in REC-4 (0.39 mm).

Table 3. Flower parts of different *Elaeagnus latifolia* genotypes.

Genotype	Flower		Petal			Ovary						Style			
	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Petal No.	Width (mm)			Length (mm)	Type	Ovule No.	Width (mm)			Length (mm)
						Apical	Middle	Base				Base	Apical	Middle	
REC-1	12.23	8.65	3.59	3.01	4.00	0.57	0.62	0.52	1.01	Inferior	1.00	0.47	0.52	2.05	6.83
REC-2	10.78	6.95	2.81	2.73	4.00	0.44	0.52	0.40	1.25	Inferior	1.00	0.46	0.51	0.59	7.57
REC-3	11.46	8.25	3.36	2.96	4.00	0.51	0.63	0.46	1.16	Inferior	1.00	0.50	0.49	0.64	7.51
REC-4	10.91	7.75	3.14	2.71	4.00	0.44	0.41	0.33	0.65	Inferior	1.00	0.39	0.40	0.59	6.45
REC-5	7.88	8.01	3.42	3.00	4.00	0.50	0.59	0.49	0.82	Inferior	1.00	0.58	0.74	1.19	8.05
$CD_{0.05}$	1.33	0.89	0.42	0.23	-	0.03	0.11	0.07	0.27	-	-	0.20	0.09	0.84	0.78

- = No analysis was done

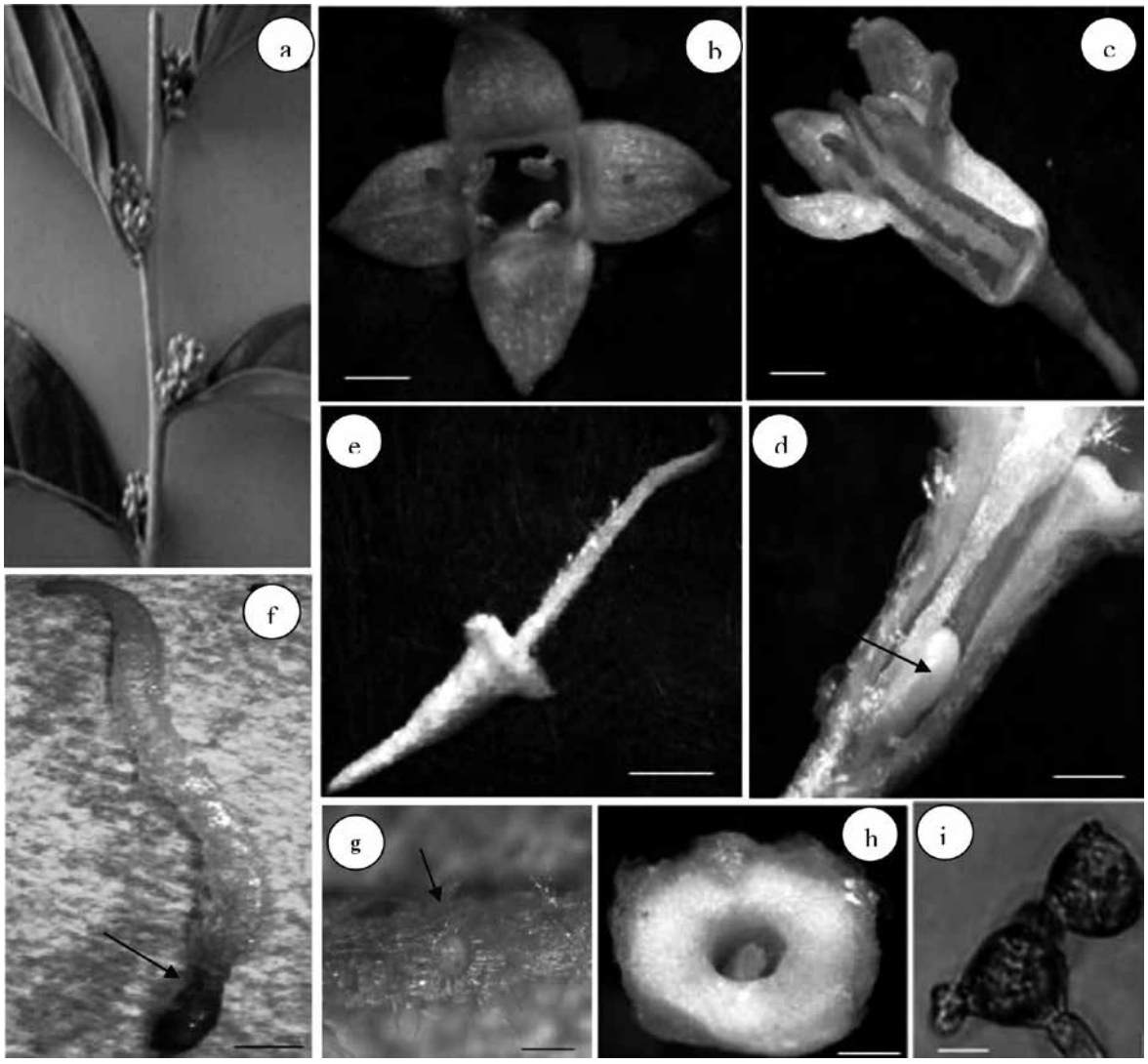

Fig. 1. (a) Cluster of flower buds in leaf axil, (b) Actninomorphic flower, (c) Location of style and stamens, (d) Erect and inverted ovule, (e) Flower stalk and style, (f) Constriction between the ovary and style, (g) Peltate hairs, (h) Disc at the base of the style, (i) Pollens.

REC-1 showed the highest style width in the middle (2.05 mm), while minimum (0.59 mm) was recorded in REC-2 and REC-4. Maximum style width at apical region was recorded in REC-5 (0.74 mm), and the minimum was recorded in REC-4 (0.40 mm). Style length ranged from 6.45 mm in REC-4 to 8.05 mm in REC-5. The present finding on style length range was similar to *Elaeagnus umbellate* (Murraya, 5).

It was noted that number of stamens in all the genotypes were four and no variations were observed (Table 4). Anthers and filaments dimension of different genotypes showed significant variations ($p \leq 0.05$). Anther length was maximum in REC-5 (1.35 mm), and least in REC-2 (1.09 mm). Similarly, maximum anther width was also recorded in REC-5 (0.58 mm), while minimum was recorded in REC-3 (0.30 mm). Similarly, variations were also observed within the filaments. REC-4 recorded the maximum filament width at the base (1.38 mm) and in the middle portion (0.90 mm) and least in REC-2 both in the base (0.37 mm) and in the middle portion (0.32 mm), respectively. Filament width at the apical portion showed non-significant variations. REC-5 recorded maximum filament length (1.84 mm), while minimum was observed in REC-2 (1.24 mm). These variations among genotypes could be attributed to genetic factors.

Stigma is the receptive part of the female reproductive system which binds pollen and mediates tube migration into the style. Irrespective of genotypes,

Table 4. Stigma, androecium and pollen characteristics of different *Elaeagnus latifolia* L. genotypes.

Genotype	Stigma		Androceium							Pollen size (µm)	
			Anther		Filament width (mm)			Filament length (mm)	No. of stamen		
	Width (mm)	Length (mm)	Length (mm)	Width (mm)	Base	Middle	Apical			Polar region	Equatorial region
REC-1	0.53	1.87	1.18	0.39	0.66	0.41	0.36	1.47	4.00	2.53	2.15
REC-2	0.29	1.23	1.09	0.41	0.37	0.32	0.22	1.24	4.00	2.05	1.72
REC-3	0.36	1.23	0.97	0.30	0.54	0.35	0.30	1.35	4.00	2.07	2.00
REC-4	0.54	1.50	1.15	0.36	1.38	0.90	0.39	1.43	4.00	2.03	1.95
REC-5	0.47	0.37	1.35	0.58	0.77	0.46	0.36	1.84	4.00	2.41	2.00
$CD_{0.05}$	0.14	0.39	0.19	0.03	0.27	0.08	NS	0.33	-	0.03	NS

NS = Non significant difference; - = No analysis was done

it was observed that the stigmas were elongated; curved and brown dotted (Fig. 1e & f). Stigma characteristics exhibited significant variation among different genotypes of *Sohshang* ($p \leq 0.05$; Table 4). Stigma width ranged from 0.29 (REC-2) to 0.54 mm (REC-4). Longest stigma was recorded in REC-1 (1.87 mm), while shortest was found in REC-5 (0.37 mm). Stigma dimension showed variation among genotypes might be due to genetic influences.

Pollen dimension may contribute to the systematic and evolutionary arrangement in the family. Pollen dimension in different genotypes of *Sohshang* was also significantly different ($p \leq 0.05$; Table 4; Fig. 1i). REC-1 recorded significantly highest pollen length in the polar region, which was *at par* with REC-5 (2.41 µm). However, minimum pollen length was observed in REC-2 (2.04 µm) in the polar region. The pollen size along equatorial region showed non-significant variations among the genotypes, although the size ranged between 1.72 (REC-2) to 2.15 µm (REC-1). The results confirmed the findings of Sarkissian and Harder (7) that pollen size normally varies little within a species. However, there are also reports of intervarietal variation in pollen grain size (Franchi *et al.*, 3).

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Influence of six dwarfing interstocks on the 'Fuji' apple under drought stress

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ABSTRACT

Investigation of growth and physiology of scion on various interstocks to drought stress is a valuable process for apple drought-tolerance stock selection. In this attempt, the 'Fuji' apple cultivar grafted on six dwarfing interstocks, including of M series (M26, M9-T337), CG series (CG24) and SH series (SH1, SH6 and SH40) on *Malus hupehensis* var. Pingyiensis Jiang rootstock were maintained as normal (-10 to -20 Kpa) (control) , moderate (-30 to -40 Kpa) and severe drought (-50 to -60 Kpa) stress levels. After 50 days treatment, comprehensive evaluation by subordinate function value (SF) was employed based on trunk growth, net photosynthetic rate, leaf water potential *etc*., and drought tolerance of 'Fuji' apple was ranked as following interstocks: SH6 > SH40 > SH1> M9-T337 > M26 > CG24. Moreover, the determination of 'Fuji' apple in responding to drought stress revealed that three antioxidant enzymes (SOD, APX and DHAR) and four genes (*DREB2A*, *ZAT10*, *SOD1*, *APX1*) constitute the putative key components for drought regulation.

Key words: Apple, drought stress, dwarfing interstock, antioxidant, drought-related gene.

INTRODUCTION

Apples are one of the most widely cultivated and economically important fruits worldwide. With the development of dwarfing, high-density cultivation method in modern production systems, the importance of dwarfing interstocks has been widely recognized (Pereira-Lorenzo *et al*., 5). Since the 1960s, an increasing number of dwarf apple rootstocks have been developed, examples of these include the M series (Malling, UK), MM series (Malling Merton, UK), O series (Ottawa, Canada), CG series (Cornell-Geneva, United States) and SH series (Shanxi, China) *etc*. However, the screening for high drought-tolerance interstocks remained unsatisfactory, especially in arid and semi-arid areas, where drought has been a major limiting factor in apple cultivation. For investigating the influence of different interstocks to the 'Fuji' apple under drought stress, and laying a foundation for tolerant interstock identification, the most widely used dwarfing interstocks in China from the M series (M26, M9-T337), CG series (CG24) and SH series (SH1, SH6, SH40) were used in this study. And the putative effectors for drought tolerance from physiology and molecular components were detected and analysed comprehensively.

MATERIALS AND METHODS

The investigation was carried out on 3-year-old 'Fuji' apple (*M. domestica* Borkh.) trees grafted on six types of dwarfing interstocks (M26, M9-T337, CG24, SH1, SH6 and SH40) on the base rootstock *Malus hupehensis* var. Pingyiensis Jiang separately, at the Beijing Academy of Forestry & Pomology Sciences (39°56´N, 116°56´E), Beijing, China. Soil water potential were designed for control (control, -10 to -20 Kpa), moderate drought stress (T1, -30 to -40 Kpa) and severe drought stress (T2, -50 to -60 Kpa), and monitored using data loggers (WatchDog-1000 series, Spectrum Tech. Inc., USA). During 50 days treatments, Trunk diameter and shoot length were measured and trunk growth rate (TGR) and shoot growth rate (SGR) were calculated according to the following formula: TGR = [(Final diameter) – (initial diameter)] / [initial diameter] × 100; SGR = [(Final shoot length) – (initial shoot length)] / [initial shoot length] × 100.

All leaf tissue evaluations were performed using uniform fully expanded mature leaves from the mid-stem area of each replicates per 'Fuji' apple. Leaf water potential (LWP) was measured with a Scholander-type pressure chamber (Soil-Moisture Equipment Corp, CA, USA) at 5:00 a.m. and leaf relative electrolytic leakage (REC) was assessment according to Bolat *et al*. (1). Net photosynthetic rate (*P*n) and relative chlorophyll content (RCC) was measured using a CI-340 handheld photosynthesis system (Camas, WA, USA) and SPAD-502 chlorophyll meters (Konica Minolta, Osaka, Japan) separately, at 10:00 a.m. under clear weather conditions. Meanwhile, leaves were cut off and ground to powder used for antioxidant enzymes activity detection, and total

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RNA extraction used for gene expression analysis by Real-time qPCR, performed on a Bio-Rad C1000™ thermal cycler with a CFX384™ Real Time system (Bio-Rad, USA). Statistical analyses were carried out using SPSS 11.0. Differences among treatments were separated by least significant difference (LSD) tests at a probability levels $p = 0.05$.

RESULTS AND DISCUSSION

Soil moisture had a direct effect on scion growth, whilst interstock had an inhibitory effect on water transport from root to scion; the water conductivity of different interstocks determined the trunk and shoot growth, leaf water potential, as well as *P*n, which in turn were reflections of scion drought tolerance ability during rootstock-scion interaction (Martínez-Ballesta *et al.*, 3). Our experiments showed that after 50 days of severe drought stress (T2), significant deference of TGR of 'Fuji' apple on all six interstocks was observed, from those on CG24 and SH1 interstocks showed the smallest TGR, implying they are highly sensitivity to drought stress, whereas those on SH6 performed the highest TGR in all three treatments (Table 1). Besides, drought stress led to significantly decreasing in SGR of all scions except those on M9-T337. The 'Fuji' apple on M26 and CG24 showed more than 50% lower SGR compared with their controls, and those on SH series showed higher SGR than others in treatment T2. Generally, drought stress restrained trunk growth and shoot elongation, whereas for those 'Fuji' apple on the interstocks M26 and SH6, SGR inhibited significantly under moderate drought stress, but TGR was affected slightly and even were promoted, suggesting drought stress not only influenced the production but also the partitioning of dry matter to various plant parts.

As a productivity indice, *P*n indicated the carbohydrate accumulation capacity and then are directly related to abiotic-stress tolerance of plant. Under drought stress, we found that *P*n was not significantly affected in treatment T1 with the exception of those on CG24 (Fig. 1A), whereas more than 60% decrease was observed in treatment T2 to all plants, especially the scions grafted on CG24 decreased to just 12.6% of the control, suggesting diverse stability in drought tolerance. On the contrary, the REC of 'Fuji' apple was about 50% higher in treatment T2 than those of the control except those on M26 and SH6 (Fig. 1B). The REC level of scion on CG24 interstock increased to about 3-fold, suggesting more serious leaf cell membrane damage existed than those on other interstocks.

LWP is usually considered to be a result of a balance between soil moisture and transpiration. Here, we addressed the relationship between LWP and dwarfing interstock in the pre-requisite of the same soil water content, base rootstock and scion cultivar. As shown in Fig. 1C, 'Fuji' apple exhibited divergence in LWP among six dwarfing interstocks in each treatment. Comparatively, the relative steadiness of LWP both in T1 and T2 was observed on the scions of SHs interstocks, whereas, those on other interstocks with 2.9- to 4.5-fold increment, suggesting a weaker ability in leaf water conservation. Meanwhile, drought stress led to double impact on the RCC of 'Fuji' apple as indicated in Fig. 1D. Moderate drought stress had a slight improvement on RCC to all 'Fuji' apple detected, whereas, severe drought stress caused significantly decrease, especially for those on M26.

Evaluation of drought tolerance is usually conducted based on comprehensive analysis of limited indices, and tolerance levels are ranked relatively, but not showed by soil water potential or drought duration (Liu *et al.*, 2). Based on above six indices from treatment T2, comprehensive evaluation by subordinate function value (SF) was employed to the tolerance analysis of 'Fuji' apple on different interstocks under drought stress. Further, the larger of SF in each indice or average for each interstock, the more stability of 'Fuji' apple to drought stress. Thus, we ranked the drought tolerance of 'Fuji' apple as

Table 1. Trunk growth rate (TGR) and shoot growth rate (SGR) of 'Fuji' apple under drought stress treatments.

Interstock	TGR (%)			SGR (%)		
	Control	T1	T2	Control	T1	T2
M26	5.25a	4.95a	2.03b	3.27a	2.55b	1.58c
M9-T337	5.03a	2.76b	2.61b	1.81a	1.66a	1.22a
CG24	8.81a	4.23b	3.60c	3.42a	2.24b	0.90c
SH1	8.78a	6.86b	4.84c	3.47a	2.32ab	1.90b
SH6	11.54a	12.04a	8.95b	2.39a	1.77b	1.61b
SH40	9.63a	7.66b	5.39b	3.09a	1.89b	1.66b

Control, plants grown at a normal soil water potential (-10 to -20 kPa); T1, moderate drought stress (-30 to -40 kPa); T2, severe drought stress (-50 to -60 kPa). The means followed by the different letters in a row indicate significant differences at 0.05 level (LSD test).

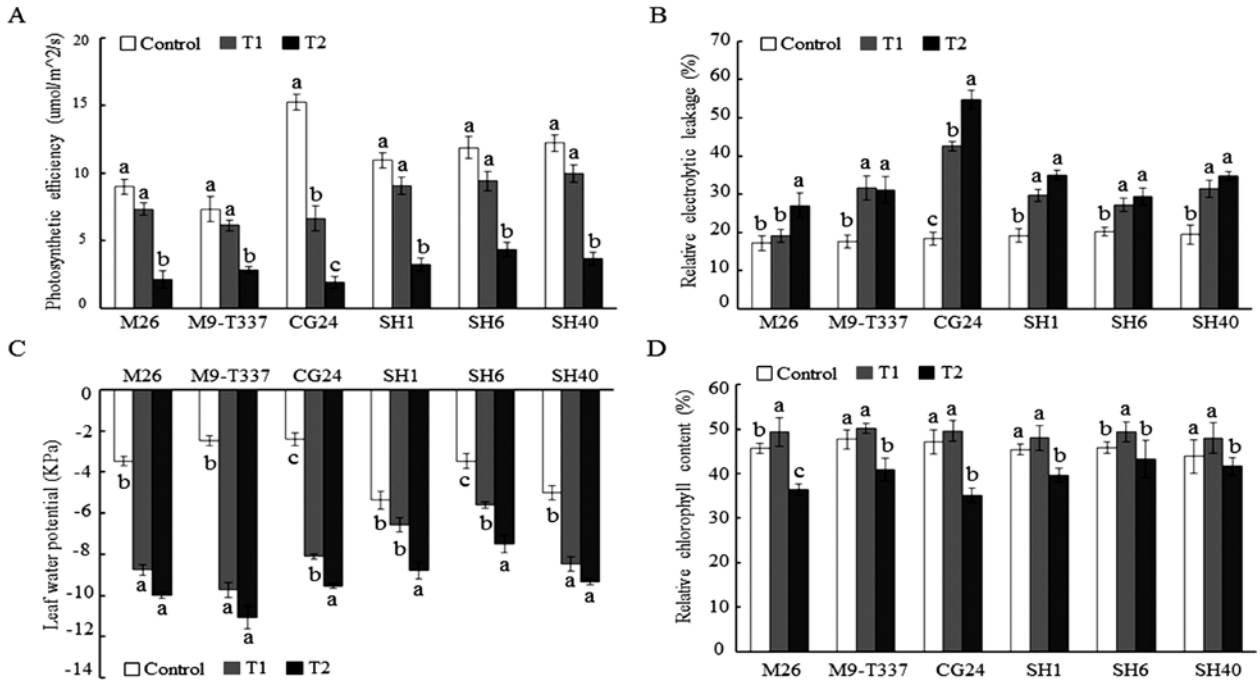

Fig. 1. Influence of six dwarfing interstocks on the leaf physiology parameters of 'Fuji' apple under drought stress. Control, plants grown at a normal soil water potential (-10 to -20 kPa); T1, moderate drought stress (-30 to -40 kPa); T2, severe drought stress (-50 to -60 kPa). Different letters in a group column indicate significant differences at 0.05 level (LSD test).

following interstocks: SH6 > SH40 > SH1> M9-T337 > M26 > CG24 (Table 2).

Antioxidant enzymatic systems play important roles in scavenging harmful oxygen species induced by multiple biotic and abiotic stresses. In this study, we assessed the activity changes of four types of antioxidant enzymes, including SOD, CAT, APX and DHAR in 'Fuji' apple grafted on SH6. With the process of drought stress treatment, the activities of antioxidant enzymes were usually induced and followed by decrease due to oxidation damage (Wang *et al*., 6), which was also well reflected in the changes of SOD, CAT and DHAR activities in the present study (Fig. 2). However, the levels of APX activities fail to be induced by drought stress and declined throughout the stress treatments, performing a higher-sensitivity response to drought stress.

A number of genes have been identified and demonstrated to be key regulars for drought tolerance (Xiao *et al.*, 7). In this study, we cloned 12 drought-related genes from 'Fuji' apple and determined by real-time qPCR. Results showed that the mRNA levels of four antioxidant enzyme genes were all induced by drought stress, and the changes of *DHAR2* gene

Table 2. Comprehensive evaluation of the adaptability of 'Fuji' apple on interstocks to drought stress by subordinate function value (SF).

Interstock	TGR	SGR	*P*n	REC	LWP	RCC	Av. of SF	Order
M26	0.00	0.68	0.09	1.00	0.30	0.16	0.37	5
M9-T337	0.08	0.32	0.37	0.85	0.00	0.71	0.39	4
CG24	0.23	0.00	0.00	0.00	0.43	0.00	0.11	6
SH1	0.41	1.00	0.53	0.71	0.64	0.56	0.64	3
SH6	1.00	0.71	1.00	0.91	1.00	1.00	0.94	1
SH40	0.49	0.76	0.72	0.72	0.49	0.80	0.66	2

TGR = trunk growth rate; SGR, shoot growth rate; *P*n = net photosynthetic rate; REC = relative electrolytic leakage; LWP = leaf water potential; RCC = relative chlorophyll content.

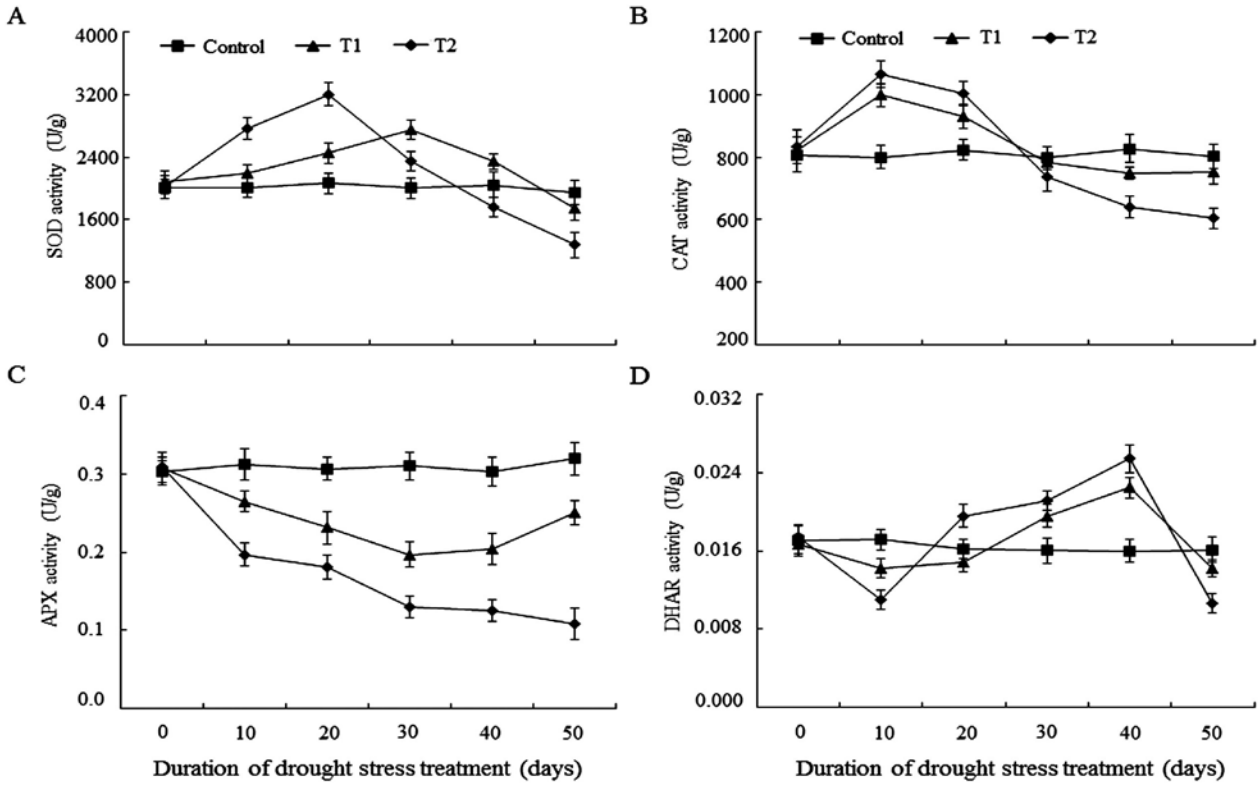

Fig. 2. Antioxidant enzyme activities of 'Fuji' apple under drought stress. (a) SOD, superoxide dismutase; (b) CAT, catalase; (c) APX, ascorbate peroxidase; and (d) DHAR, dehydroascorbate reductase.

expression showed a well conformity with enzyme activities (Fig. 3). Regarding DREB gene family, it was shown that *DREB1A*, *DREB2A* and *DREB2B* gene mRNA levels were upregulated significantly in treatment T2, whereas *DREB2C* were continuously down-regulated in both treatments. Besides, the expression of *NHX1*, *NPK1* and *LOS5* genes in treatment T2 peaked at 40 days and then decreased to initial levels, performing insensitive and none strong response to drought stress. Among the twelve genes, the expression of *SOD1*, *APX1*, *DREB2A* and *ZAT10* genes showed better positive reflection in response time and mRNA levels to drought stress than other genes detected. In treatment T2, the expression of *DREB2A* peaked at 20 days and then decreased sharply until the end of drought treatment. In contrast to the short-term action of *DREB2A*, *ZAT10* increased up to peak at 20 days and then decreased smoothly, which indicated a difference at functional stages during drought stress. The regulation pathway from *DREB2A* to *ZAT10* and then to antioxidant enzyme genes deserves further explorations (Mittler *et al.*, 4).

Overall, we investigated the influence of dwarfing interstocks on the 'Fuji' apple under drought stress, and ranked of six interstocks according to SF values calculated from six indices. Due to the restrictions of indice number, observation years and rootstocks *etc.*, additional experiments are needed to further determine the precise interaction between interstocks and 'Fuji' apple under drought stress. Further, the studies on mechanisms of drought response regulation and exploration of key drought-tolerance components involved in drought tolerance are still inadequate.

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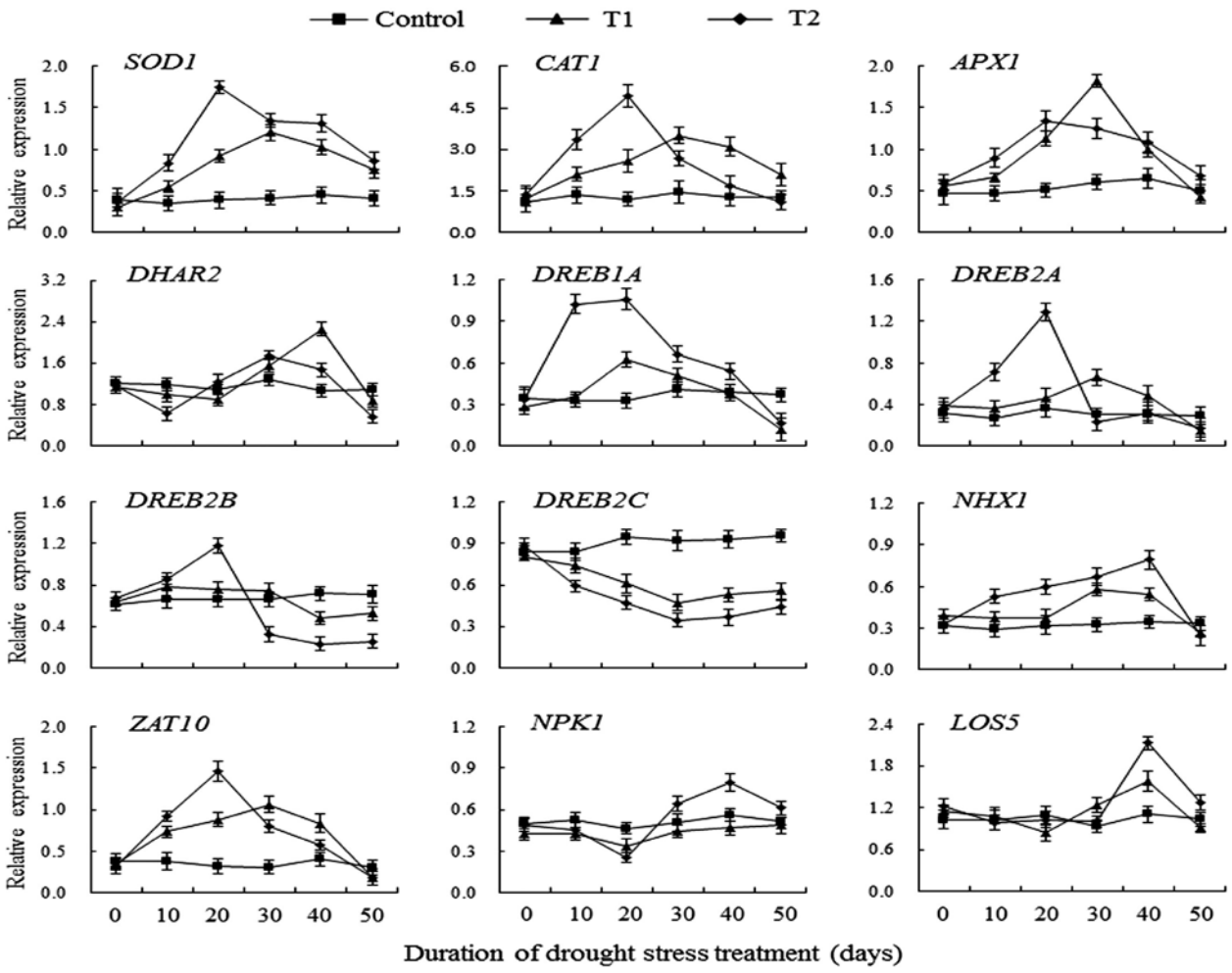

Fig. 3. Expression of 12 drought-related genes in 'Fuji' apple on SH6 interstock under drought stress.

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Effect of high density planting systems on physiological and biochemical status of rejuvenated mango plants of cv. Amrapali

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ABSTRACT

Mango trees grown under high density planting systems show a gradual decline in yield after 11-12 years due to overcrowding of canopies. To find out the effect of high density planting systems on physio-biochemical parameters of mango, an experiment was conducted on an old senile, rejuvenated high density cv. Amrapali orchard over two consecutive years. High density planting systems showed significant effect on physiological, biochemical and nutritional parameters of plants. Among the different planting systems, plants under cluster planting system recorded the highest leaf area (156.68 cm^2), transpiration rate (3.77 m mol^{-1} $m^{-2}s^{-1}$); and N (1.28%) P (0.20 %) and K (0.54%) contents. The maximum leaf relative water content (87.63%) was noted in plants under square planting system, while, the photosynthetic rate (8.36 µmol CO_2 m^{-2} s^{-1}) was highest in hedge-row planting system. The stomatal conductance (0.17 µmol CO_2 m^{-2} s^{-1}) was the highest in plants under double-hedge row planting system. The highest internal carbon concentration was recorded in paired planting system, while the maximum chlorophyll '*a*' (1.50 mg g^{-1}), chlorophyll '*b*' (0.65 mg mg g^{-1}) and total chlorophyll (2.02 mg g^{-1}) contents were found in plants under square planting system. The highest total phenolics (53.09 mg g^{-1}) content was recorded under paired planting system. The micronutrient contents in plants, *viz*. Cu, Zn, Fe and Mn also differed among different planting systems.

Key words: High density planting, mango tree, micronutrient, physiology.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most popular and choicest fruit crops of tropical origin. It belongs to the family Anacardiaceae and originated in Indo-Myanmar region. Owing to its delicious taste, appealing aroma, majestic appearance and rich nutritional value, this fruit occupies a superior position in the national and international markets. The fruit is an excellent overall nutritional source, rich in a variety of phytochemicals, vitamins and minerals. Mango is rich in antioxidant vitamins A, C and E. Besides that, vitamin B_6 (pyridoxine), vitamin K, other B vitamins (thiamine, riboflavin, niacin and pantothenic acid), essential nutrients (potassium, calcium, copper and iron) and 17 amino acids are also present in good levels. In mango, the concept of high density planting has gained momentum after the development of cultivar Amrapali (Dashehari × Neelum) a distinctly dwarf and regular-bearing hybrid. However, the mango plants grown under different high density planting systems show progressive decline in yield as well as fruit quality after 10-11 years of planting owing to overlapping/ intermingling of branches, poor light interception, low photosynthetic rate and high relative humidity within the tree canopy (Asrey *et al*., 2). Therefore, the time and severity of pruning, which is adopted for rejuvenation of mango orchards not only alter the physiological status of the plant but also modify its biochemical attributes, which is manifested by its flowering, bearing behavior and yield pattern. Previously several studies have been conducted on the effect of pruning in mango trees in relation to its micro-climate for better light penetrance, fruit set and yield performance. The physiological, biochemical and nutritional parameters in these earlier studies have received only little attention. However, beyond the routine information, there is an increasing interest among the researchers to know the effect of high density planting and pruning on plant physiological, biochemical and nutritional parameters of mango trees after rejuvenation. Hence, the present investigation was carried out.

MATERIALS AND METHODS

The field experiment was conducted in All India Coordinated Research Project (Fruit), Sabour, the permanent experimental site of Bihar Agricultural University, Sabour, Bhagalpur, Bihar, India (25°15'40" North, 87°2'42" East, elevation 46 m). The experiment was conducted on old senile orchard of cv. Amrapali mango planted in different planting systems. The

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experiment was carried out on rejuvenated plants that were planted in 1992 and become over-croweded and unproductive in 2008. Hence, rejuvenation pruning was done in July, 2009 with the help of mechanized pruner at a uniform height of 1.5 m. Thinning was done during 2010 and 2011 as per recommended practices. All the trees were maintained under uniform cultural practices. The experiment was laid out in randomized block design (RBD) with five treatments (*i.e.* planting systems) and five replications. The different plantings systems taken as treatments were (i) Square system (1,600 plants/ ha, and 9 plants/ plot), (ii) Hedge row system (2,670 plants/ ha and 15 plants/ plot), (iii) Double-hedge-row system (3,556 plants/ ha and 20 plants/ plot) 4. Paired planting (2,133 plants/ ha and 12 plants/ plot), and (v) Cluster planting (2,844 plants/ ha and 16 plants/ plot). Normal planting distance of 2.5 × 2.5 m and half-normal distance of 1.25 × 1.25 m were followed in this experiment. Normal plant spacing of 2.5 m × 2.5 m was maintained in square system of planting, while in hedge row system, spacing of 1.25 m × 2.5 m (P-P × R-R) was maintained. In double-hedge row system, 2 rows of hedge were planted at half-normal distance (1.25 m × 1.25 m). In case of paired planting, pairs of two plants at the distance of 1.25 m × 1.25 m were maintained. Cluster planting system accommodated cluster of 4 plants at 1.25 m × 1.25 m apart. Net area under each plot was 56.25 m^2 and total experimental area was 2,406 m^2.

Leaf relative water content (RWC) was determined following method of Weatherly (15). Leaf area was measured by laser leaf area meter (CI-203). Photosynthetic rate, stomatal conductance, transpiration rate and internal carbon ratio were recorded using photosynthesis system infra-red gas analyzer (LI-COR 6400 XT). Chlorophyll contents (chlorophyll *a*, chlorophyll *b* and total chlorophyll) of the leaves were analyzed at the time of fruiting (Barnes *et al*., 3), total phenols by Malik and Singh (5) using spectrophotometer (HALODB-20S UV-vis double beam spectrophotometer, Australia).

The macro- and micro-nutrients were estimated from shoot samples of mango at vegetative stage. For determination of total nitrogen content, 500 mg of oven dried sample was taken in a 250 ml Kelplus tubes. To this, 3.9 g digestion mixture containing 3.5 g K_2SO_4 and 0.4 g $CuSO_4$ was added and digested with 12 ml concentrated H_2SO_4 on the digestion unit till light green colour appeared. The digested sample was distilled and total nitrogen was determined by Kelplus KES 20LR. For estimation of phosphorous, potassium and micronutrients, tissue samples were wet digested with a diacid mixture of concentrated nitric acid and per chloric acid (9:4 v/v). Fifteen ml of diacid mixture was added to 500 mg of the prepared samples and pre-digested at a temperature of 100°C for an hour and at 250°C until the solution turned colourless and the volume reduced to 2-3 ml. The digested material was then diluted and filtered through Whatman No. 1 filter paper and volume was adjusted up to 100 ml. The filtrate obtained was used for the estimation of phosphorus, potassium, zinc, copper, manganese and iron.

Phosphorus was estimated by vando-molybdate colour reaction method. Per cent transmittance at 420 nm was measured with the help of spectrophotometer. Potassium (K) content was determined by a microprocessor based flame photometer using specific filter (K filter) and LPG flame. The different standard K solutions (5, 10, 20 to 100 ppm) were atomized to standardize the instrument. Suitably diluted di-acid digest was then fed to an atomizer capillary tube and concentration was directly read on the display monitor. Per cent K content was calculated on dry weight basis. Micronutrients (Cu, Fe, Mn and Zn) were determined by atomic absorption spectrophotometer (ECIL, Hyderabad) directly from the diacid digest using an air-acetylene flame. The concentrations of Cu, Fe, Mn and Zn were measured at 386.0 nm (lamp current 7 mA), 22.6 nm (lamp current 3 mA), 403.1 (lamp current 5 mA) and 213.9 nm (lamp current 5 mA) wavelengths, respectively. Final concentration was calculated in ppm by multiplying the concentration with suitable dilution factor. The data generated from the experiment were analyzed by following factorial RBD (Panse and Sukhatme, 8).

RESULTS AND DISCUSSION

After one year of rejuvenation, plants under high density planting system was recorded a profuse vegetative growth in all the directions. Therefore, in second year thinning was done as per recommended practices. Data was recorded in third year of rejuvenation, which showed quite interesting changes in plant physiology and nutrients.

High density planting systems after rejuvenation did not exert significant differences in leaf area and relative water content of leaf (Table 1). This might be due to single variety taken in all the planting systems. However, some differences were recorded among the treatments in respect to leaf area. Plants under cluster planting were recorded the highest leaf area (156.68 cm^2), which was at par with hedge row planting system (149.24 cm^2) and paired planting system (146.24 cm^2). The minimum leaf area (137.20 cm^2) was recorded in plants of square planting system. A possible reason for differences in the leaf area may be attributed to water stress as a result of competition of plants for air and water in closed

Table 1. Effect of high density planting systems on leaf area and leaf relative water content of Amrapali mango plants after rejuvenation.

Treatment	Leaf area (cm^2)	Relative water content (%)	Yield (t/ ha)
Square planting	137.32	87.63	11.05
Hedge-row planting	149.24	85.28	18.21
Double-hedge row planting	141.6	86.44	13.54
Paired planting	146.24	76.41	17.52
Cluster planting	156.68	75.5	20.88
CD at 5%	11.89	13.03	2.44

spacing. It is generally seen that leaf area of plant is increased in the plants which are under water limitation though this is not true with all the plants and may vary from species to species. However, in our investigation, similar results were found in cluster planting system, where higher leaf area was recorded because of water stress to plant, due to competition of plant for air and water because of narrow spacing.

The leaf relative water content was recorded highest (87.63%) in square planting system, which was closely followed by double-hedge row (86.44%) and hedge row planting (85.28%). The minimum leaf relative water content was found in cluster planting (75.50%), due to large leaf surface area and narrow spacing. The higher leaf relative water content in square planting system might be due to smaller leaf surface area, which lost minimum moisture, resulted into maximum water retention in the leaves. This result is in accordance with the findings of Singh *et al*. (13). In this study, planting systems having less number of plants/ plot (square planting system) showed lower leaf area and higher leaf water content, while, planting system having more number of plants/ plot exhibited the larger leaf area with minimum relative water content.

Rejuvenation of high density mago plants showed significant differences in rates of photosynthesis (Table 2). The maximum photosynthetic rate (8.36 µmol CO_2 $m^{-2}s^{-1}$) was recorded in plants of hedge-row planting system, which was at par with cluster planting (8.23 µmol $CO_2m^{-2}s^{-1}$), followed by square planting system (7.47 µmol $CO_2m^{-2}s^{-1}$) and double-hedge row planting system (7.26 µmol $CO_2m^{-2}s^{-1}$). In this study, high density planting systems showed significant effect on photosynthetic rate, stomatal conductance, transpiration rate and internal carbon concentration. A possible reason for high photosynthetic rate in hedge-row planting system might be due to the maximum non-flowering branches present in plants of hedge-row planting system. The non-flowering branches were reported to be high in internal carbon concentration, which are responsible for higher photosynthetic rate than flowering branches (Shivashankara *et al*., 11).

The significant variation in stomatal conductance was also found among plants under high density planting systems after rejuvenation. Among the treatments, maximum stomatal conductance (0.17 µmol m^{-2} s^{-1}) was recorded in double-hedge row planting system, which was at par with hedge-row planting system (0.14 µmol m^{-2} s^{-1}), whereas, the minimum stomatal conductance (0.10 µmol m^{-2} s^{-1}) was recorded in plants of square planting system. Stomatal conductance and net CO_2 assimilation rate in fruit species depend upon conditions such as solar irradiance, leaf temperature, soil and plant water status and mineral nutrition. Higher stomatal conductance in double-hedge row system plants and lower stomatal conductance in square planting system might be due to the fact that stomatal conductance always found higher in less vigorous plant and lower in the more vigorous plants (Murti and Upreti, 6). In double-hedge row planting system, the maximum number of plants was accommodated per unit area as a result; the height of plant increased more rather than plant vigour. Similarly, stomatal conductance was

Table 2. Effect of high density planting systems on photosynthetic rate, stomatal conductance, transpiration rate and internal carbon concentration of Amrapali mango after rejuvenation.

Treatment	Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$)	Stomatal conductance (µmol m^{-2} s^{-1})	Transpiration rate ($mmol^{-1}$ $m^{-2}s^{-1}$)	Internal CO_2 conc. (ppm)
Square planting	7.47	0.10	1.51	237.52
Hedge-row planting	8.36	0.14	2.25	243.52
Double-hedge row planting	7.26	0.17	1.81	224.55
Paired planting	6.05	0.12	1.89	245.38
Cluster planting	8.23	0.13	2.37	244.95
CD at 5%	1.22	0.017	0.33	16.50

lower in square planting system owing to vigorous growth of plant due to wider spacing than rest of the planting system.

The rate of transpiration also varied significantly among the planting systems. The higher transpiration rate was recorded in plants of cluster planting (2.37 mmol $m^{-2}s^{-1}$) and hedge row planting system (2.25 mmol $m^{-2}s^{-1}$). Plants under square planting showed the lowest transpiration rate (1.51 mmol $m^{-2}s^{-1}$) among the treatments. The higher transpiration rate in cluster planting might be attributed to maximum leaf area and lower leaf relative water content, which resulted into maximum transpiration of plant. Similarly, in square planting system, the minimum leaf area and higher leaf relative water content caused lowest transpiration from the leaf (Singh *et al.*, 13).

The internal carbon dioxide concentration was recorded maximum (245.38 ppm) in paired planting, while, it was minimum (224.55 ppm) in double-hedge row planting. The higher internal carbon dioxide concentration in paired planting system may be due to maximum light penetrance and low relative humidity within the tree, due to low canopy volume of the plant. This is an essential criterion for carbohydrate metabolism for higher fruit yield/ plant.

In the present study, the maximum chlorophyll '*a*' (1.50 mg g^{-1}) content was recorded in square planting, while, it was minimum (0.97 mg g^{-1}) in double-hedge row plants (Table 3). No significant difference in chlorophyll content was recorded between hedge-row, paired and cluster planting system plants. Among the treatments, highest chlorophyll '*b*' (0.65 mg g^{-1}) content was noted in plants of square planting system followed by hedge-row planting system (0.62 mg g^{-1}). The lowest chlorophyll '*b*' content was noticed in paired planting system (0.28 mg g^{-1}). The maximum total chlorophyll was recorded in square planting system (2.02 mg g^{-1}), which was at par with hedge row planting system (1.60 mg g^{-1}), while, it was minimum (1.21 mg g^{-1}) in double-hedge row planting system. The results depicted that plants under square planting system exhibited significantly higher content of chlorophyll '*a*', '*b*' and total chlorophyll than other treatments. Similar results were also reported by Abirami *et al.* (1). A positive correlation between vigour of mango plant and chlorophyll content was also reported by Pal *et al*. (7). Sharma and Singh (10) also reported that the value of total chlorophylls content was highest in leaves of vigorous un-pruned trees.

The total phenols content was estimated highest (53.09 mg g^{-1}) in plants of paired planting system, which did not differ significantly with square planting system (52.70 mg g^{-1}) (Table 3). The plants of double-hedgerow planting system showed lowest content of total phenols (41.08 mg g^{-1}) among the treatments.

Table 3. Effect of high density planting systems on chlorophyll content of Amrapali after rejuvenation.

Treatment	Chlorophyll (mg g^{-1})			Total phenols
	Chl '*a*'	Chl. '*b*'	Total	(mg g^{-1})
Square planting	1.50	0.65	2.02	52.70
Hedge-row planting	1.25	0.62	1.60	49.97
Double-hedge row planting	0.97	0.35	1.21	41.08
Paired planting	1.04	0.28	1.26	53.09
Cluster planting	1.03	0.44	1.40	41.68
CD at 5%	0.20	0.07	0.33	8.46

In present study, the highest total phenols content was found in paired planting system may be due to minimum canopy volume. Murti and Upreti (6) reported that phenols play a vital role in restricting vigour of plant and also confirmed that total phenols content in leaves showed significant inverse relationship with plant height and stem girth. In our study to similar results were also noticed. Higher total phenols content in the plants of paired planting was the possible cause of low vigorous growth, which resulted in minimum canopy volume and maximum canopy temperature within the plant, thus exhibiting maximum yield.

High density planting systems after rejuvenation had significant effect on tissue nutrient content of mango (Table 4). Pruning leads to high mobilization of N, P and K nutrients in plants (Singh *et al.*, 14). In this experiment, highest nitrogen content was recorded in cluster planting (1.28%), which was at par with double-hedge row planting system (1.22%). The minimum nitrogen (N) content was recorded in square planting (1.04%). Nitrogen is the main growth manipulating nutrient. It is found higher in reproductive shoots. The nitrogen content differed among the planting systems. This difference may be due to more number of flowered shoots in cluster planting than square planting. Devi and Tyagi (4) also found similar results that total nitrogen content was higher in flowered shoots than in non-flowered shoots. The phosphorus (P) content did not differ significantly among the treatment. However, Ram (9) also found higher P contents in the leaves during on year/ during flower bud differentiation than in the off year/ vegetative stage. The highest potassium (K) content was also found in cluster planting system (0.54%), which was at par with treatment of hedge row (0.53%), paired planting (0.52%) and double-hedge row (0.51%). The lowest K was found in square planting (0.42%). The potassium is not directly involved in the metabolism of the plant that might be one of the important reasons that high

Table 4. Effect of high density planting systems on tissue nutrient status in Amrapali after rejuvenation.

Treatment	Macro-nutrient (%)			Micro-nutrient (ppm)			
	N	P	K	Fe	Cu	Mn	Zn
Square planting	1.04	0.19	0.42	271.92	15.19	258.81	70.25
Hedge-row planting	1.14	0.19	0.53	236.00	23.37	326.97	58.81
Double-hedge row planting	1.22	0.18	0.51	173.95	19.41	306.16	53.67
Paired planting	1.14	0.20	0.52	213.22	24.73	200.43	73.49
Cluster planting	1.28	0.20	0.54	255.59	21.6	229.14	64.72
CD at 5%	0.08	NS	0.10	36.97	2.76	38.17	12.32

density planting system did not show significant differences in potassium content in tissues. Similar results were also reported by Singh and Rathore (12).

In case of micronutrients content, all the treatments differed significantly in zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) contents in leaf. Pruning showed marked influence on Zn, Cu, Fe and Mn content. Among the treatments, the highest Fe content was recorded in square planting system (271.92 ppm), which was at par with cluster planting system (255.59 ppm). The lowest Fe content was found in double-hedge row planting system (173.95 ppm).Iron is one of the micronutrients needed in small quantities by the plants to produce chlorophyll. In square planting system, higher Fe content resulted into higher chlorophyll content in the leaves and it also supported the contrary findings in double-hedge row planting system. Copper (Cu) is one of the micronutrients needed in very small quantities by plants. Copper activates enzymes in plants, which are involved in lignin synthesis and it is essential in several enzyme systems. It is also required in the process of photosynthesis, plant respiration carbohydrate and protein metabolism. In this study, copper content was recorded highest in paired planting system (24.73 ppm), which might be responsible for higher fruit yield/ plant while, it was recorded lowest in square planting system (15.19 ppm). Manganese (Mn) is an essential plant mineral nutrient, playing a key role in several physiological processes. It enhances the photosynthetic efficiency and dry matter production; provide resistance to biotic stresses. The highest manganese (Mn) content was recorded in hedge row planting system (326.97 ppm), which was at par with double-hedge row planting system (306.16 ppm). This higher Mn content in hedge-row planting resulted in higher respiration photosynthetic rate (Table 2). The lowest Mn content (200.43 ppm) was noticed in paired planting system. The highest zinc (Zn) content was found in paired planting system (73.49 ppm), which was statistically at par with the square planting system (70.25 ppm). The lowest Zn content was found in double-hedge row planting system (53.67 ppm). Zinc is one of the essential micronutrients required for optimum crop growth. Zinc transported in the xylem tissues from the roots to the shoots. Translocation of zinc takes place from older leaves to the younger leaves during fruit development phase. Zinc act as precursor for auxin, regulate starch formation and proper root development, and help in formation of chlorophyll and carbohydrates, helps in biosynthesis of cytochrome that's why it is the possible reason that the highest zinc content was found in paired planting system, which turned in to highest number of fruits per plant in comparison to other planting systems. While, it recorded lowest in double hedge row planting system resulted into low fruit yield. The severe pruning of Amrapali mango, planted under different high density planting systems, affected physiological and biochemical parameters significantly. The change in the physiological state of the plants after pruning not only the result of age but also the systems of planting.

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Rootstock induced changes in tree physiology and antioxidant enzymes activity in lemon cv. Kagzi Kalan

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ABSTRACT

Lemon cultivar Kagzi Kalan was evaluated on eight rootstocks under Delhi conditions during 2013-14. Rootstocks significantly affected all the physiological and biochemical parameters. Higher transpiration rate (*E*) and stomatal conductance (g_s) were observed in lemon trees budded on sour orange, *Jatti khatti (C jambhiri)*, Troyer citrange (*C. sinensis* x *Poncirus trifoliata*) and rough lemon (*C. jambhiri*) rootstocks. The higher values of photosynthetic rate (*A*) were observed in trees on sour orange and Troyer citrange rootstocks compared to other rootstocks. Lemon trees budded on *Billikhichlli* (*C. reshni*) and *Attani*-2 (*C. rugulosa*) had the highest intrinsic water use efficiency (WUEi), while it was lower in trees budded on *Jatti khatti* and rough lemon rootstocks. Higher relative water control (RWC) was recorded in trees on Troyer citrange and sour orange rootstocks, while highest excised leaf water loss (ELWL) was observed on sour orange followed by trees on rough lemon and Troyer citrange rootstocks. Trees on *Billikhichlli* rootstock had the highest superoxide dismutase (SOD) activity. However, the highest catalase (CAT) activity was found in the leaves of Kagzi Kalan on *Jatti khatti*. Leaves of Kagzi Kalan had the highest glutathione reductase (GR) activities on *Karna khatta* rootstock. The lowest values *E*, g_s, ELWL, and activities of SOD, CAT and GR were observed in the leaves of Kagzi Kalan lemon on *Attani*-2 rootstock.

Key words: Antioxidant enzymes, intrinsic water use efficiency, lemon, photosynthetic rate, relative water content, rootstocks.

INTRODUCTION

With the intensification of fruit production due to socio-economic considerations and in the perspective of climate change, the role of rootstocks in commercial fruit production has increased noticeably. Although rootstocks have several applications such as improving fruit quality, imparting adaptability to climatic and edaphic conditions, inducing dwarfing, environmental stress tolerance *etc*. The priorities of rootstock selection in the tropics and sub-tropics have been focussed mainly on vigour management and securing regular, high fruit yields. Poor soil health and / or toxic elements containing irrigation water may cause more ROS generation which are detrimental as excess ROS damage membranes, proteins, chlorophyll and nucleic acid (Apel and Hirt, 3). Rootstock scion combinations that have lower ROS production and greater activities of antioxidant enzymes are potentially better to sustain growth and productivity under changing scenario of climate and soil. Thus, in changing scenario of climate and soil health, it is imperative to investigate the role of rootstocks on physio-chemical alterations on scion cultivars.

Several reports have established the relationships between various physiological parameters of grafted trees and fruit quality (Naor *et al*., 10). These relationships are important as they provide a basis for selecting the best graft combination for particular environmental conditions with high quality fruit production. Furthermore, in citrus the effect of rootstocks on physiological and biochemical aspects influencing the plant development, productivity and environmental resistance are well documented in Valencia orange (Kaplankıran and Tuzcu, 9). Hence, selection of an appropriate graft combination is very crucial for the production of commercial citrus species. Lemon (*Citrus limon*) is one of the most important citrus fruits worldwide, mostly propagated through budding. *Jatti khatti* (*Citrus jambhiri*), *Karna khatta* (*C. karna*) and rough lemon (*C. jambhiri*) are frequently used rootstocks for lemon cultivars in India. Besides, several lesser known indigenous species, *viz*., *C. rugulosa* and citrus variants frequently found in mid hills of Himalayas are also used as rootstock. The objective of present study was to ascertain the role of rootstocks in alteration of physiological and biochemical parameters of lemon cv. Kagzi Kalan.

MATERIALS AND METHODS

Budded plants were transplanted in a commercial Kagzi Kalan lemon orchard on eight rootstocks (Table 1) at Experimental Orchard in the Division of

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Table 1. Rootstocks used in the experiment.

Common name	Botanical name
Rough lemon	*Citrus jambhiri* Lush
Attani-2	*Citrus rugulosa* (Accn. No. IC 285453)
Jatti khatti	*Citrus jambhiri* Lush
Billikhichlli	*Citrus reshini* Hort. ex Tan.
Sour orange	*Citrus aurantium* L.
RLC-4	*Citrus jambhiri* Lush (Accn. No. IC 274693)
Karna khatta	*Citrus karna* Raf.
Troyer citrange	*Citrus sinensis* x *Poncirus trifoliata*

Fruits and Horticultural Technology, ICAR-IARI, New Delhi in July 2010. The experimental site falls under trans-Gangetic plains of agro-climatic zones of India located at 77°12 'E longitude, 28°40 'N latitude and an altitude of 228.6 m above mean sea level. It has typical subtropical climate characterized by hot and dry summer followed by cold winter. May and June are the hottest months with the maximum temperature ranging between 41 to 44°C, and December and January are coolest months, with the minimum temperature ranging between 3 to 7°C. Trees on eight rootstocks were established at 4.5 m × 4.5 m distance. The experiment was externally guarded on all sides and located within a commercial planting. Soil type was a virgin inceptisol (alluvial soil) with a pH of 7.4 and $EC_{(1:2)}$ of 0.75 dS m^{-1}, a cation exchange capacity (CEC) 7.54 - 10.72 cmol kg^{-1}, organic carbon 0.48%, soil N, 240.23 kg/ha, P_2O_5, 58.65 kg/ha, K_2O 555.92 kg/ha.

Flood irrigation with water (E.C. 1.0-1.30 dS m^{-1}) was utilized during the first two years, and later was changed to a drip irrigation system at 6 l h^{-1} of water per tree, during 4-6 h, thrice a week. The experimental plants were applied 30 kg farmyard manure, 400 g N, 200 g P_2O_5 and 400 g K_2O per tree per year. An annual application of farmyard manure was done in January and urea, single superphosphate, and potassium sulphate were applied one month after flowering. Other cultural operations were carried out uniformly. Foliar micro-nutrients application and pest and disease management were performed in accordance with normal commercial practices.

Five fully developed leaves were collected from each of the selected trees at the middle of the tree four days after irrigation (drip), kept in properly sealed polythene bags. The bags then placed in ice box and brought into laboratory. These leaves were used to determine relative water content (RWC) and excised leaf water loss (ELWL). For ELWL, fresh weight was recorded using electronic balance. Leaf samples were left on working table for two hours at room temperature, thereafter the weight of wilted leaf samples was recorded. ELWL was calculated according to Barrs and Weatherley (4). Net CO_2 assimilation rate (*A*), stomatal conductance (g_s) and transpiration (*E*) were measured on five matured leaves from each treatment using an infrared gas analyzer (IRGA) (LI-6200, LI-COR Biosciences, Lincoln, NE, USA). Intrinsic water use efficiency (WUE_i) was calculated as the ratio of A to g_s (During, 5).

For antioxidant enzymes activities, 10 leaves from each treatment were collected freshly in ice box on 25th October and washed immediately with tap water followed by distilled water. One g of sample was weighed and homogenised in pre-chilled mortar and pestle by adding 5 ml chilled phosphate buffer (50 mM; pH 7.0). The homogenate was collected in oak-ridge tubes and centrifuged at 15,000 x g for 20 min at 4°C. The supernatant so obtained was sieved through two layers muslin cloth and stored in refrigerator which was used as extract for the estimation of following antioxidant enzymes. Soluble protein content of the leaf samples was determined according to Lowry *et al*. (8). The superoxide dismutase (SOD activity in leaf samples was measured according to Fridovich (6). The absorbance of each mixture was then read at 560 nm using a UV-VIS double-beam PC 8 scanning Auto-cell spectrophotometer (UVD-3200, Labomed Inc., Culver city, CA, USA). The complete reaction mixture without added enzyme extracts gave the maximum colour and served as a control. However, catalase (CAT) activity in each plant sample was measured according to Luck (9). Residual H_2O_2 was estimated by titrating the reaction mixture against 0.01M $KMnO_4$ until a faint pink colour persisted for at least 15 sec. Glutathion reductase (GR) was assayed according to Smith *et al*. (13). The reaction was initiated by adding 0.1 ml of 2.00 GSSG (oxidized glutathione) and the increase in absorbance at 412 nm was recorded at 25°C over a period of 10 min. on a on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, Inc, USA). Experiment was conducted in complete randomised block design (CRBD) with five replications. Data were analysed using analysis of variance OPSTAT, HAU, Hisar, Haryana (India). P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

There were significant difference ($P \leq 0.05$) in transpiration rate (*E*), photosynthetic rate (*A*), and stomatal conductance (g_s) of Kagzi Kalan lemon among the rootstocks tested (Table 2). Significantly higher *E* rate was measured on sour orange rootstock which was found non-significant with trees on *Jatti*

khatti and Troyer citrange rootstocks. Amongst all scion rootstock combinations, the highest values of '*A*' was observed in Kagzi Kalan trees on sour orange, which was non-significant with trees on Troyer citrange rootstock as compared trees on other rootstocks. Furthermore, values of 'g_s' were also observed higher on sour orange, Troyer citrange, and *Jatti khatti* rootstocks as compared to trees on other rootstocks. The lowest value of '*E*' and 'gs' were observed in trees on *Attani*-2 rootstock, while, '*A'* was found lowest on RLC-4 rootstock which was not differed significantly with trees on *Attani*-2 rootstock. Rootstock also significantly influenced intrinsic water use efficiency (WUEi) of lemon cultivar Kagzi Kalan (Table 2). Trees grafted on *Attani*-2 rootstock had the highest WUE_i of scion cultivar which was non-significant with trees on *Billikhichlli*, kanna khatta and Troyer citrange rootstocks. Significantly lower values of A/g_s were observed in trees grafted on *Jatti khatti* and rough lemon rootstocks. Rootstock also influenced relative water content (RWC) and excised leaf water loss (ELWL) of lemon trees significantly (Table 2). Higher values of RWC was observed in trees on Troyer citrange, which was statistically non-significant with trees on sour orange, *Billikhichlli* and *Attani*-2 rootstocks, while significantly lowest RWC was found on *Jatti khatti* rootstock. However, significantly highest ELWL was observed on sour orange rootstock followed by trees on rough lemon rootstock which was non-significant with trees on Troyer citrange rootstocks. Plants maintain a balance between carbon assimilation, storage and growth in response to development and environmental signals (Smith and Stitt, 14). Moreover, fruit set and further vegetative and fruit development in citrus are supported mainly by actual photosynthetic rates (Syvertsen and Lloyd, 15). In our study, leaves of lemon Kagzi Kalan budded on sour orange and Troyer citrange exhibited higher values of *A* and g_s than other rootstock-scion combinations at higher RWC. However, trees on *Attani*-2, *Billikhichlli* and Troyer citrange had the higher WUE_i. As the scion cultivar was same, differences in parameters might be due to a root-derived gradient, which were also reported in citrus (Rodriguez- Gamer *et al.*, 12) as well may be because of variations in cell size due to rootstock effect, which favours cell to cell water exchange. These findings are in agreement with those of grapevine (Naor *et al*., 10). Indeed it was observed that fresh detached leaves of lemon trees either on *Attani*-2 or RLC-4 had less ELWL than trees on other rootstocks might be due to role of these rootstocks in maintaining thicker leaf cuticle of lemon scion. Numerous studies have also shown that thicker cuticle is associated with more limited leaf water loss, and thus advantageous to plants. Furthermore, we found higher RWC and lower '*E*' and ELWL in Kagzi Kalan trees either on *Attani*-2 or *Billikhichlli* suggesting ability of these combinations to sustain better growth and yield under the conditions of limited water availability. It was also reported that lower ELWL, *E* and higher RWC in plant leaves could be considered as selection criteria to breed plant against drought conditions (Rahman *et al*., 11).

The superoxide dismutase (SOD) and catalase (CAT) activities also varied significantly ($P \leq 0.05$) on different rootstocks (Fig. 1A, B). Trees of Kagzi Kalan lemon on *Billikhichlli* rootstock had the highest SOD activity followed by trees on rough lemon and *Jatti khatti* rootstocks. The lowest SOD activity in leaves of Kagzi Kalan was observed on *Attani*-2 rootstock. The highest CAT activity was found in leaves of Kagzi Kalan on *Jatti khatti* followed by *Billikhichlli* which was non-significant with trees on sour orange

Table 2. Effect of rootstocks on transpiration rate (*E*), stomatal conductance (g_s), photosynthetic rate (*A*), intrinsic water use efficiency (WUE i), relative water conduct (RWC) and excised leaf water loss (ELWL) of lemon cv. Kagzi Kalan.

Rootstock	*E* (mol H_2O m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	*A* (μ mol m^{-2} s^{-1})	WUE_i (μ mol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	RWC (%)	ELWL (%)
Rough lemon	4.14	0.107	6.98	65.79	71.99	18.98
Attani-2	2.69	0.053	5.22	98.07	77.00	13.89
Jatti khatti	4.58	0.120	7.28	60.94	66.45	16.24
Billikhichlli	3.19	0.080	7.27	91.61	77.40	14.92
Sour orange	5.06	0.133	11.10	83.75	79.63	20.58
RLC-4	2.79	0.060	4.90	82.83	73.28	14.47
Karna khatta	3.67	0.083	7.40	89.01	70.29	17.18
Troyer citrange	4.55	0.113	10.30	90.80	82.17	18.54
LSD ($P \leq 0.05$)	0.69	0.02	1.02	14.49	1.56	1.43

rootstocks. Trees on *Attani*-2 rootstock had the lowest CAT activity. Notwithstanding, glutathione reductase (GR) activity was also affected significantly ($p \leq 0.05$) amongst rootstock-scion combinations (Fig. 1C). Activity of GR was found the highest when Kagzi Kalan lemon budded on *Karna khatta* followed by trees on *Jatti khatti* and RLC-4 rootstocks. The lowest GR activity was observed in leaves of Kagzi Kalan lemon on *Attani*-2 rootstock. There was also significant difference ($p \leq 0.05$) in total soluble protein content in leaves of Kagzi Kalan lemon on different rootstocks (Fig. 1D). Significantly maximum total soluble protein was recorded on rough lemon rootstock which was non-significant with sour orange, *Karna khatta* and Troyer citrange rootstocks. The lowest soluble protein content was observed on *Attani*-2 rootstock. The antioxidant enzymes could increase the ability of stress tolerance in scavenging ROS and, therefore, higher activity of these enzymes could increase the ability of stress tolerance and delay the senescence (Alscher *et al*., 2). The results of present study showed that Kagzi Kalan lemon trees had higher SOD on *Billikhichlli* suggesting trees on this rootstock could be more potential to remove O_2^- by catalysing its dismutation. However, lemon trees on *Jatti khatti* had higher CAT activity indicated higher H_2O_2 scavenging capacity by this combination. In our study, GR activity was higher on *Karna khatta* and *Jatti khatti* suggesting higher ability of these rootstock-scion combinations for ROS scavenging. Variation in antioxidant enzymes activities in different citrus rootstock species had also been reported by many workers (Almansa *et al*., 1).

In conclusion, our study indicate that rootstock genotypes influenced physiological and biochemical parameters of scion cultivars. *Attani*-2 and RLC-4 could be better rootstock under limited irrigation as lemon on these rootstocks had lower values of

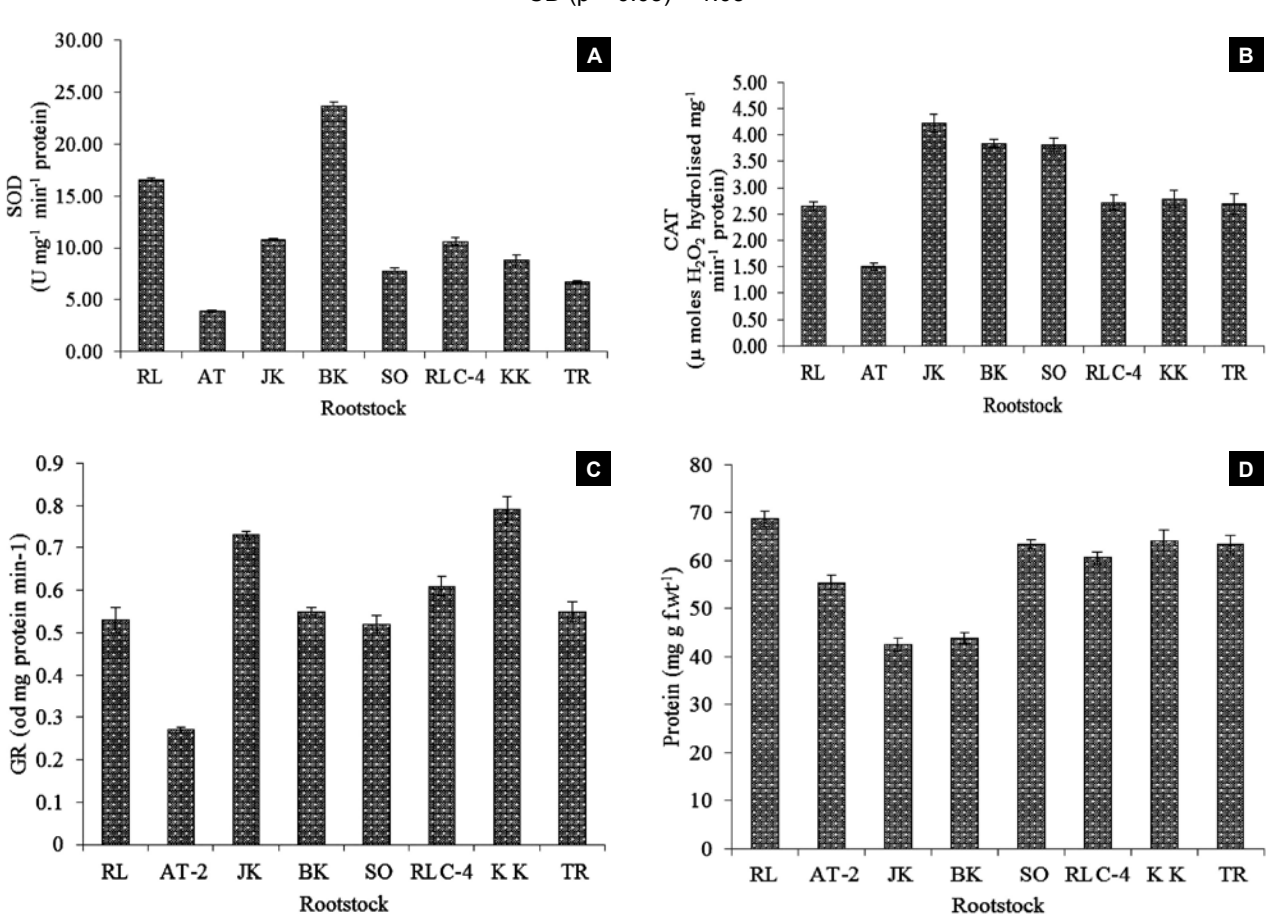

Fig. 1. Influence of rootstocks on superoxide dismutase (A), catalase (B), glutathion reductase (GR), (C) and protein content (D) in lemon cv. Kagzi Kalan trees. Vertical bar represent mean values of five replicates ± S.E. RL = rough lemon; AT = *Attani*-2; JK = *Jatti Khatti*; BK = *Billikhichlli*, SO = sour orange; RLC-4 = rough lemon collection 4; KK = *Karna khatta*; TR = Troyer citrange.

E, ELWL and higher RWC, while sour orange and Troyer citrange may be suitable rootstocks under normal soil moisture conditions because these rootstock had higher values of *E*, g_s, *A*, RWC and *ELWL*. Furthermore, trees on *Jatti khatti*, *Karna khatta* and *Attani*-2 may have higher capacity for ROS scavenging under irrigation with water having higher salt concentrations.

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Effect of irrigation and fertigation scheduling on growth, flowering, yield and economics of guava cv. Lalit under ultra high density planting system

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ABSTRACT

A study was conducted to find out the effect of irrigation and fertigation scheduling on growth, flowering, yield and cost economics of guava cv. Lalit elanted under ultra high density planting system. The experiment consisted of 15 treatments combination comprising three different levels of irrigation [50 % (I_1), 75% (I_2) and 100% (I_3) irrigation of pan evaporation] and five level of fertigation [20% (F_1), 40% (F_2), 60% (F_3), 80% (F_4) and 100% (F_5) or recommended of NPK. Among the different treatment combination, highest shoot gain after pruning (39.90 cm), plant spread N-S (1.72 m), increase in girth of primary branch (0.315 cm) and canopy volume (1.265 m^3) were recorded under I_3F_5 treatment combination. Whereas, highest flowers $shoot^{-1}$ (47.60), fruit weight (96.91 g), pulp weight (70.84 g), pulp: seed ratio (14.96), number of fruits $plant^{-1}$ (77.70) and yield (6.75 kg $plant^{-1}$ & 33.75 t ha^{-1}) were recorded in I_2F_4 treatment combination. However, treatment combinations I_2F_4 and I_2F_3 were found statistically at par with each other in respect of fruit weight, number of fruit $plant^{-1}$ and yield with each other in all the above parameters. Treatment combination I_2F_3 gave the maximum net return (Rs. 2,79,081) per ha under ultra high density planting.

Key words: Guava, drip irrigation, fertigation scheduling, evapotranspiration, ultra high density planting system.

INTRODUCTION

Guava (*Psidium guajava* L.) is one of the important fruit of the tropic and sub-tropic parts of the world due to its hardy nature and prolific bearing even in marginal land. Guava is considered as an apple of the tropics, because of its richness in vitamins especially vitamin C and minerals like Ca, P and Fe. It is mainly used as a table fruit but because of high pectin content, it has a very good potentiality of processing also and used for preparation of jam, jelly, nectar and other processed products. Due to high yield per unit area, ultra high density planting system of guava is gaining popularity among small as well as large farmers of southern Rajasthan. Among different cultivars of guava, farmers of the region prefer Lalit cultivar for ultra high density planting because it performs better though, fertigation is being followed in southern Rajasthan but the schedules are arbitrary due to lack of availability of scientifically worked out fertigation schedules.

Water is a scarce commodity, and it is important that 50 per cent of our arable land could be brought under irrigation. Thus, increasing demand for highly efficient irrigation system calls for the use of drip irrigation, which has also been found suitable under adverse climate, soil and irrigation water conditions. The drip-irrigation have ability to apply small but frequent irrigation, which has been found superior over flood method in terms of water saving, yield, quality and water use efficiency (Thakur *et al*., 14). Soil is considered as a reservoir for water under basin irrigation and the objective of irrigation is mainly to replenish the soil water, whereas under drip irrigation it is possible to apply small quantity of water based on evapotranspiration of the plant (Stegman, 13). Irrigation is often significantly exceeds the crop requirement, because the evapotranspiration accounts for nearly 30-50 per cent of applied water. Therefore, it is essential that plant should be irrigated on the basis of evapotranspiration.

Mineral nutrition is one of the most important inputs for increasing productivity and quality of fruits and accounts for nearly 30 per cent of the cost of cultivation. The limited root zone and the reduced amount of mineralization in the restricted wetted zone are the main reasons for the reduced nutrient availability to the plant with conventional method of fertilizer application under drip irrigation (Megen, 4). With drip irrigation both water and fertilizer can be applied more precisely in controlled quantity and at appropriate time directly to the root zone as per the crop need at different growth stage. Fertigation through drip can save fertilizers (25-40%), labour (50-60%) and water (50-60%), and increased yield (12-76%), water use efficiency (70-95%) and fertilizer use efficiency (Marwaha, 3; Thakur *et al*., 14). Therefore,

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the present study was undertaken to find out the efficient irrigation and fertigation schedule for guava under ultra high density planting system in southern Rajasthan.

MATERIALS AND METHODS

The field experiment was conducted during 2010-11 and 2011-12 on uniform four-year-old plants of guava cv. Lalit planted at the spacing of 2.0 x 1.0 m at Horticulture Farm of the Rajasthan College of Agriculture, MPUAT, Udaipur. There were three levels of irrigation, [50 % (I_1), 75% (I_2) and 100% (I_3) irrigation of pan evaporation] and five levels of fertigation [20% (F_1), 40% (F_2), 60% (F_3), 80% (F_4) and 100% (F_5) or recommended of NPK (225, 75, 150 g NPK plant^{-1} year^{-1} was applied alone and in combination (Shukla *et al*., 12). The experiment was laid out in factorial randomized block design with four replications. The treatment of different irrigation levels were given from June to March at one day interval. The daily USDA class A open pan evaporation readings were obtained from meteorological observatory, Agronomy Farm of RCA, Udaipur (Fig. 1 & 2). As per the treatments, water soluble fertilizer grade (NPK-19:19:19) was applied in 5 splits from fruit set to maturity stage and remaining nitrogen and potassium dose was supplemented through urea and MOP, respectively. The drip irrigation system was set up with main (75 mm) and sub-mains (50 mm) made up of high density polyethylene and laterals (12 mm) made up to low density polyethylene. The spacing between two adjacent laterals was 1.0 m. Two microtube type (1.2 mm) emitters were used on each plant for application. Water soluble fertilizers were injected in drip system through venturi. The data on gain of shoot after pruning (cm), tree height (m), plant spread from

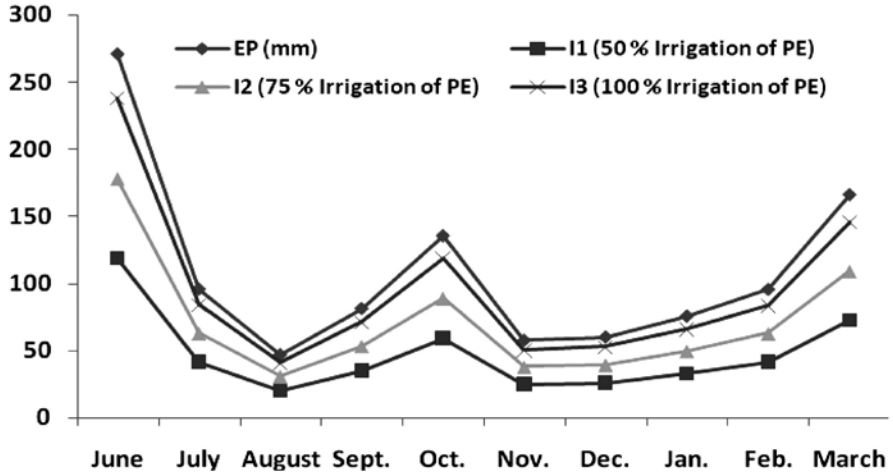

Fig. 1. Monthly crop water applied (L) and EP (mm) during 2010-11.

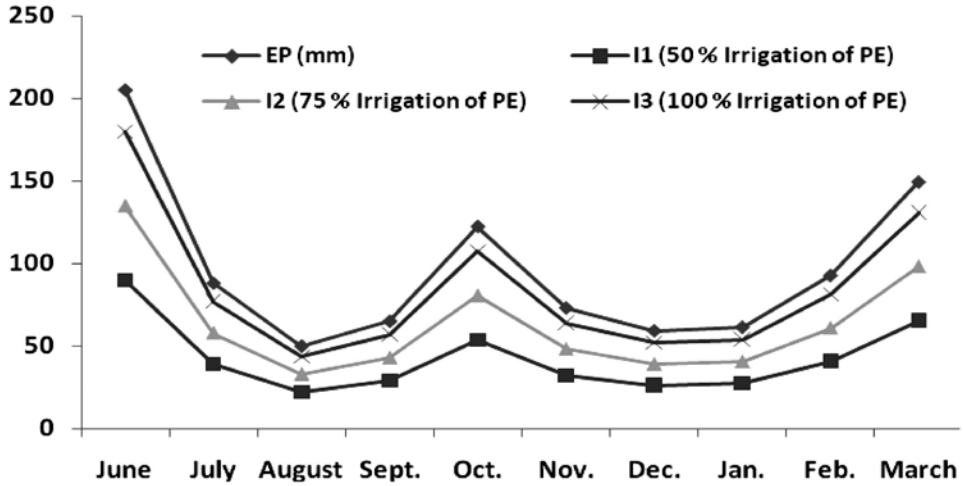

Fig. 2. Monthly crop water applied (L) and EP (mm) during 2011-12.

North-South and East-West (m) were recorded using metre scale, while girth of primary branches (cm) was recorded with Vernier calipers. Average increase in the girth of shoot was calculated by subtracting end value to initial value. Leaf area (cm^2) was measured with the help of leaf area meter (Systronics). Canopy volume was calculated as the method described by the Samaddar and Charkarbarti (8) and expressed in m^3. Fruit diameter, polar and equatorial was taken with the help of Vernier calipers. Mature fruits were harvested periodically in each treatment separately and the weight was recorded with the help of electronic balance and then the yield per plant was calculated. Estimated yield per hectare was calculated by multiplying the yield $plant^{-1}$ with number of plants per ha^{-1}. Total working cost (establishment cost/ annum, seasonal cost of drip system, cost of cultivation and cost of treatment along with interest and rental value) converted into per plant as well as per hectare. The net income was obtained by subtracting the total working cost from gross income.

RESULTS AND DISCUSSION

Varying quantities of water applied *via* drip irrigation 50, 75 and 100 per cent irrigation of PE level had a positive effect on different vegetative parameters. The stimulation on such growth characters were attributed to increasing quantities of water and maximum gain of shoot after pruning (33.13 cm), plant spread (1.81 m E-W & 1.55 m N-S), increase in girth of primary branches (0.233 cm), leaf area (67.60 cm^2) and canopy volume (0.93 m^3) were under I_3 (100% irrigation of PE level). However, I_3 and I_2 (75% irrigation of PE level) treatments did not differ significantly among themselves with respect of plant spread, increase in girth of primary branches and leaf area (Table 1). Judicious application of water directly to the root zone could improve plant growth and development. The favourable influence of I_3 on vegetative parameters may be due to excess moisture compared to I_2 and I_1 in the soil through drip irrigation treatment. This maintained the soil moisture at optimum level eliminating water stress to the plant resulting in greater vigour. The results are in accordance with the findings of Kachwaya *et al*. (2) on strawberry and Sarolia *et al*. (9) on guava.

Results in Table 1 indicated that growth parameters of guava trees were gradually stimulated with increasing levels of fertigation from F_1 to F_5. Fertigation level F_5 (100% RDF) showed higher shoot length gain (37.38 cm), plant spread (1.90 m E-W & 1.66 m N-S), increase in girth of primary branches (0.30 cm), leaf area (73.03 cm^2) and canopy volume (1.16 m^3). Increase in the vegetative growth with increasing fertigation levels might possibly be attributed to better supplementation and utilization of nutrients and moisture particularly in the plants with highest dose of NPK, when applied through fertigation. Which in turn enhanced cell division and formation of more tissues resulting in more vegetative growth leading to higher annual extension and plant spread. A direct relationship between nitrogen application and vegetative growth is a well established fact (Ramniwas *et al*., 7) in guava.

Among the different treatment combinations, I_3F_5 (100% irrigation of PE level + 100% RDF) resulted in maximum shoot gain (39.90 cm), plant spread N-S (1.72 m) and canopy volume (1.265 m^3) might be due to over all improvement in growth parameters with 100 per cent irrigation of PE level and application of 100 per cent RDF through fertigation (Table 1). The findings of present study are in accordance with Sharma *et al*. (11) and Varu *et al*. (15) on guava.

Among different levels of irrigation, maximum polar fruit diameter (5.34 cm), fruit weight (89.06 g), pulp weight (64.88 g), pulp: seed ratio (14.39), number of fruits $plant^{-1}$ (71.75) and yield (5.82 kg $plant^{-1}$ & 29.11 t ha^{-1}) were observed in treatment I_2 (75% irrigation of PE level) and minimum in I_1 level (50% irrigation of PE level) (Tables 2 & 3). The possible explanation for increase in fruit diameter and fruit weight by I_2 treatments might be due to increase in balanced vegetative growth with maximum harvest of solar light. The pulp weight is directly correlated to fruit size and weight therefore, the increase in size and weight of fruit due to this treatment is possible reason for increase in pulp weight. Further, per cent fruit set and retention were recorded maximum in I_2 level therefore number of fruits $plant^{-1}$ ultimately increased in this treatment. Bigger size fruits, higher fruit weight and maximum number of fruits $plant^{-1}$ were observed in I_2 level, which was, one of the reasons for achieving higher yield of guava under I_2 irrigation level. The outcomes of present study are also in line of Sarolia *et al*. (9) and Varu *et al*. (15) on guava.

Among different fertigation levels, F_4 (80% RDF) registered maximum fruit set (59.96%), fruit retention (62.33%), equatorial fruit diameter (5.65 cm), fruit weight (89.86 g), pulp weight (66.95 g), number of fruits $plant^{-1}$ (73.90) and yield (6.22 kg $plant^{-1}$ & 31.10 t ha^{-1}). However, treatment F_4 and F_3 (60% RDF) did not differ statistically concerning their influence on fruit set, fruit retention, equatorial diameter of fruit, fruit weight, pulp weight, seed weight and number of fruits $plant^{-1}$ (Tables 2 & 3). Prolonged availability of nutrients during the growth, flowering and fruiting period from fertigation might have improved the fruit set and retention. Nitrogen application might increase the supply of auxins to the fruits, which reduce abscission therefore increased fruit retention.

Table 1. Effect of irrigation and fertigation levels and their interaction on growth parameters in guava.

Treatment	Gain of shoot after pruning (cm)	Plant spread E-W (m)	Plant spread N-S (m)	Increase in girth of primary branches (cm)	Leaf area (cm^2)	Canopy volume (m^3)
I_1	28.20	1.70	1.45	0.186	61.98	0.72
I_2	31.98	1.76	1.53	0.225	66.89	0.86
I_3	33.13	1.81	1.55	0.233	67.60	0.93
CD at 5%	0.81	0.05	0.05	0.01	1.98	0.02
F_1	23.68	1.64	1.37	0.145	57.57	0.55
F_2	27.75	1.67	1.47	0.170	62.68	0.67
F_3	31.42	1.76	1.45	0.212	65.18	0.77
F_4	35.28	1.82	1.61	0.252	68.98	1.03
F_5	37.38	1.90	1.66	0.295	73.03	1.16
CD at 5%	1.04	0.06	0.060	0.007	2.56	0.03
I_1F_1	21.75	1.64	1.29	0.145	55.15	0.480
I_1F_2	26.10	1.63	1.44	0.160	59.70	0.580
I_1F_3	27.45	1.64	1.52	0.160	62.55	0.710
I_1F_4	31.65	1.73	1.45	0.215	65.00	0.805
I_1F_5	34.05	1.87	1.57	0.250	67.50	1.015
I_2F_1	23.90	1.60	1.43	0.140	57.95	0.555
I_2F_2	28.05	1.65	1.48	0.175	64.00	0.655
I_2F_3	33.20	1.79	1.36	0.225	66.75	0.730
I_2F_4	36.55	1.86	1.70	0.265	70.50	1.175
I_2F_5	38.20	1.91	1.71	0.320	75.25	1.190
I_3F_1	25.40	1.67	1.39	0.150	59.60	0.600
I_3F_2	29.10	1.75	1.49	0.175	64.35	0.780
I_3F_3	33.60	1.86	1.46	0.250	66.25	0.865
I_3F_4	37.65	1.88	1.69	0.275	71.45	1.115
I_3F_5	39.90	1.92	1.72	0.315	76.35	1.265
CD at 5%	1.80	NS	0.10	0.01	NS	0.05

I_1 (50%) I_2 (75%) I_3 (100%) irrigation of PE; F_1 (20%), F_2 (40%), F_3 (60%), F_4 (80%) and F_5 (100%) recommended dose of NPK.

The promotive effect of N and K in rapid production of leaves with better photosynthetic activity resulting in higher C: N ratio for flowering and better fruit set (Turner and Barkus, 20). Similar, results have also been reported by Shankar *et al*. (10) on guava.

Nitrogen is an essential constituent of chlorophyll the increase in chlorophyll would result in additional food manufacture, which would further result in to increased length, width and weight of fruits in treatments F_3 and F_4 as compared to F_1 and F_2. Furthermore, healthy and optimum vegetative growth with the application of treatment F_4 might have augmented photosynthesis, respiration and synthesis of more carbohydrate required for fruit growth, increase in vegetative growth resulted in production of more food material, which in turn may have been utilized for better development of fruits. Vegetative growth is directly correlated with physical attributes of fruit. However, maximum vegetative growth was attributed from F_5, but, under ultra high density planting there is no significance of more vegetative growth only optimum foliage is required with higher light interception for photosynthesis to produce maximum yield and good quality fruits. Per cent fruit set, per cent fruit retention, number of fruits $plant^{-1}$, fruit size and fruit weight were obtained highest from fertigation levels F_4 and F_3 during both the year of experiment which was responsible for higher yield. Results are in accordance with the findings of Patel *et al*. (5), Dantas *et al*. (1) and Pramanik and Patra (6) in guava.

Table 2. Effect of irrigation and fertigation levels and their interaction on flowers per shoot, fruit set, fruit retention, fruit diameter and weight of guava.

Treatment	Flowers shoot^{-1}	Fruit set (%)	Fruit retention (%)	Fruit dia. (polar) (cm)	Fruit dia. (equatorial) (cm)	Fruit wt. (g)
I_1	42.88	53.44	59.20	5.22	5.45	81.05
I_2	44.35	54.43	59.89	5.34	5.56	89.06
I_3	43.37	53.58	59.99	5.26	5.48	84.36
CD at 5%	NS	NS	NS	0.09	NS	0.94
F_1	41.53	49.41	56.08	5.17	5.25	77.23
F_2	43.15	52.21	57.21	5.25	5.50	82.48
F_3	45.13	56.53	62.08	5.34	5.64	89.84
F_4	44.50	56.96	62.33	5.33	5.65	89.86
F_5	43.37	53.98	60.76	5.27	5.46	84.68
CD at 5%	2.46	1.81	1.906	0.11	0.14	1.212
I_1F_1	43.43	49.26	57.84	5.05	5.10	73.80
I_1F_2	40.00	51.95	55.56	5.13	5.10	78.36
I_1F_3	44.98	55.80	60.92	5.31	5.33	86.11
I_1F_4	43.00	56.41	61.31	5.33	5.33	84.73
I_1F_5	43.00	53.79	60.35	5.28	5.26	82.22
I_2F_1	40.14	50.07	55.07	5.23	5.20	80.36
I_2F_2	45.91	52.10	58.08	5.31	5.30	85.88
I_2F_3	47.60	57.45	62.75	5.47	5.50	95.15
I_2F_4	45.00	58.29	62.64	5.44	5.21	96.91
I_2F_5	43.10	54.26	60.93	5.25	5.21	87.00
I_3F_1	41.00	48.92	55.33	5.24	5.23	77.54
I_3F_2	43.54	52.59	58.00	5.30	5.26	83.20
I_3F_3	42.81	56.35	62.58	5.24	5.20	88.27
I_3F_4	45.50	56.18	63.04	5.21	5.17	87.95
I_3F_5	44.00	53.90	60.99	5.29	5.33	84.82
CD at 5%	4.27	NS	NS	0.19	NS	2.09

I_1 (50%), I_2 (75%), I_3 (100%) irrigation of PE; F_1 (20%), F_2 (40%), F_3 (60%), F_4 (80%) and F_5 (100%) recommended dose of NPK.

Among different combination of irrigation and fertigation maximum average fruit weight (96.91 g), average pulp weight (70.84 g), pulp: seed ratio (14.96), fruits plant^{-1} (77.70) and yield (6.75 kg plant^{-1} and 33.75 t ha^{-1}) were obtained under I_2F_4 level, which remained at par with I_2F_3 (Tables 2 & 3). However, treatment combination I_2F_3 registered maximum number of flowers shoot^{-1} (47.60) might be due to ensure constant supply of water and balance nutrition to plant, favours better growth, development and dry matter accumulation. The reason of higher fruit diameter, fruit weight, pulp weight and seed weight under I_2F_4 and I_2F_3 treatment combinations may be due to availability of more constant soil moisture thereby their more translocation from root to leaves and other part of plant. The interaction level, I_2F_4 and I_2F_3 recorded significantly higher yield attributing characters might be due to their individual effect. The increase in number of flowers shoot^{-1}, fruit set, fruit retention, fruit size and fruit weight with the application of treatment combinations I_2F_4 and I_2F_3 is possible reason to increase in number of fruits plant^{-1} and yield. The results are in the confirmation with those of Varu *et al*. (21) in guava. The highest net return (Rs. 2,79,081.08) was obtained from I_2F_3 which was at par with I_2F_4. When the benefit: cost ratio was taken into consideration, it was highest (2.64) in I_1F_1 and I_2F_1. However, there is no significance differences were noted between treatment combinations I_1F_1, I_2F_1 and I_3F_1 in respect of B:C ratio. It is because of low

Table 3. Effect of irrigation and fertigation levels and their interaction on pulp weight, seed weight, pulp: seed ratio and yield of guava.

Treatment	Pulp weight (g)	Pulp: seed ratio	Fruits plant^{-1}	Yield tree^{-1} (kg)	Estimated yield ha^{-1} (tonnes)	Net return (Rs.)	B: C ratio
I_1	60.99	13.80	68.55	5.30	26.49	202679.25	1.87
I_2	64.88	14.39	71.75	5.82	29.11	233787.14	2.12
I_3	62.68	13.91	70.00	5.56	27.79	217558.63	1.98
CD at 5%	0.79	0.30	1.76	0.15	0.74	–	–
F_1	57.42	14.09	67.15	4.88	24.39	212176.02	2.63
F_2	61.13	13.78	67.82	5.24	26.19	216268.96	2.21
F_3	66.12	14.14	72.65	5.99	29.96	243967.61	2.11
F_4	66.95	14.25	73.90	6.22	31.10	240147.17	1.80
F_5	62.62	13.91	68.98	5.47	27.34	177481.94	1.18
CD at 5%	1.02	NS	2.28	0.19	0.95	–	–
I_1F_1	56.16	14.06	68.00	4.87	24.33	211711.92	2.64
I_1F_2	59.17	14.07	66.75	5.03	25.17	204351.05	2.09
I_1F_3	63.60	13.67	70.00	5.55	27.75	217811.37	1.89
I_1F_4	64.10	13.77	71.00	5.80	29.00	215311.09	1.62
I_1F_5	61.90	13.43	67.00	5.24	26.20	164210.82	1.09
I_2F_1	58.56	14.25	66.45	4.89	24.43	212547.42	2.64
I_2F_2	62.28	13.70	68.45	5.32	26.62	221440.58	2.26
I_2F_3	69.36	14.85	75.95	6.58	32.89	279081.08	2.42
I_2F_4	70.84	14.96	77.70	6.75	33.75	271913.82	2.04
I_2F_5	63.36	14.19	70.20	5.58	27.88	183952.80	1.22
I_3F_1	57.54	13.97	67.00	4.89	24.43	212268.72	2.62
I_3F_2	61.94	13.58	68.25	5.36	26.79	223015.25	2.27
I_3F_3	65.40	13.89	72.00	5.85	29.24	235010.37	2.03
I_3F_4	65.90	14.02	73.00	6.11	30.55	233216.59	1.75
I_3F_5	62.60	14.11	69.75	5.59	27.93	184282.20	1.22
CD at 5%	1.77	0.67	3.95	0.33	1.65	–	–

I_1 (50%), I_2 (75%), I_3 (100%) irrigation of PE; F_1 (20%), F_2 (40%), F_3 (60%), F_4 (80%) and F_5 (100%) recommended dose of NPK

treatment cost. However, on the basis of net return (Rs. 2,79,081.08) and B:C ratio (2.42) we recommend I_2F_3 treatment combination (Table 3).

Hence, 75 per cent irrigation of pan evaporation replenishment level along with supplementation of 60 per cent recommended dose of fertilizer, efficiently utilized water through liquid fertilizer (19:19:19) supplemented with urea and MOP in form of yield in guava cv. Lalit under ultra high density planting system.

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Response of different soil moisture regimes on sweet cherry under Karewa land of Kashmir valley

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ABSTRACT

The field experiment was conducted to ascertain the soil moisture regime under drip irrigation for Karewa land in sweet cherry cv. Bigarreau Noir Grossa with reference to qualitative and quantitative attributes. Results indicated that maximum yield of 73.26 kg / tree was recorded with 80% ET as compared to 100% ET which registered only 64.73 kg/ tree yield. The minimum yield of 50.50 kg/tree was noted when irrigation level was maintained at 40% ET. Further, the maximum fruit weight (4.51 g) was achieved with 100% ET followed by 80% ET and it decreased gradually to a minimum of 3.08 g with 40% ET. As far as qualitative characters are concerned, the maximum TSS of 23.02°Brix was observed when the irrigation level was maintained at 80% ET. This treatment also gave the highest TSS: acid ratio indicating an improvement in fruit quality. Fruit cracking was significantly reduced by maintaining the irrigation regime in between 80% (2.72%) to 100% (1.96%) ET as compared to irrigation level maintained at 60% (10.91%) and 40% ET (16.25%), indicating 80% reduction in fruit cracking. Sweet cherry plant with irrigation level maintained at 80% ET showed better nutrient uptake. The leaf Fe, Mn, Cu and Zn contents were maximum with 80% ET followed by 100% ET and minimum was recorded with 40% ET.

Key words: Drip irrigation, Karewa land, soil moisture regime, sweet cherry.

INTRODUCTION

Among the stone fruits, cherry characteristically needs more chilling period to break its dormancy and thereby its cultivation is restricted to the temperate climate only. By virtue of temperate climate, 90% of the total cherry production of India is confined to Kashmir valley of Jammu and Kashmir state. Srinagar district of Jammu & Kashmir contributes more than 60 per cent of cherry production. The landscape at higher elevation is locally known as *Karewas* offers a good drainage condition, which is prerequisite for cherry plantation. Orchards on these *Karewas* are totally dependent on rainfall and no water management is practiced for irrigation in sweet cherry resulting in low productivity with small fruits having inferior quality. In Kashmir valley, 80 per cent rainfall occurs from November to April which does not coincide with the fruiting season. This leads to nutrient deficiency in general and Zn and B deficiencies in particular at flowering stage and causes pollination problem, fruit drop and cracking of fruits. It has been observed that the soil water stress of 8 bar at flowering reduces the yield by 53% and after flowering by 35%. Therefore, it was envisaged to translate the full potentiality of natural resources into economic yield by way of adopting precise water management through drip irrigation practices. The uses of drip irrigation in cultivation of various fruit crops have lead to increase in yield and quality. The water requirement is relatively more in sweet cherry during spring and early summer, when fruits are under the process of development. However, regular irrigation is essential during the reproductive phase as irregular moisture condition causes dropping of flowers and reduction in fruit size. This problem is more intense in commercial varieties such as Double and Mishri, which require precise water and fertilizer management to maintain the desired pulp: stone ratio. The present experiment was undertaken to evaluate the different water regimes on vegetative growth, quantative and qualitative characteristics and fruit cracking in sweet cherry under drip irrigation.

MATERIALS AND METHODS

The experiment was conducted on 22-year-old sweet cherry (cv. Bigarreau Noir Grossa, locally know as Mishri) orchard at *Karewa* land of Gootlibagh of Srinagar district planted at a distance of 5 m x 5 m. The detailed initial nutrient status under different strata has been presented in Table 1. The drip irrigation system was installed with pre-calibrated four emitters/ plant with a flow rate of 4 l/ h. These emitters were placed equidistantly in east, west, north and south directions at a distance of 30 cm from

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Table 1. Depthwise initial soil nutrient status of experimental orchard.

Depth of soil profile (cm)	pH (1: 2.5)	Available macronutrients (kg/ha)			Available micronutrients (ppm)			
		N	P	K	Zn	Cu	Mn	Fe
0-20	6.7	230	11.9	410	1.22	2.00	24.21	45.89
20-40	6.8	220	9.2	379	0.93	1.99	18.22	41.56
40-60	6.7	194	8.9	343	0.72	1.98	15.23	40.11
60-80	7.9	170	8.2	290	0.56	1.52	11.78	36.32

the main trunk. Four irrigation treatments on a daily basis replenishing of ET based on USWB class 'A' pan evaporation losses were under taken comprising of T_1= 100% ET, T_2 = 80% ET, T_3 = 60% ET and T_4 = 40% ET in randomized block design replicated four times and two plants were taken in each replication. The data was subjected to statistical analysis as per the method suggested by Gomez and Gomez (8).

Water requirement of sweet cherry plants was estimated according to the formula given by Doorenbos and Purohit (7). The water received through rain was taken into account, while deciding the quantity to be applied for successive irrigation in all treatments. The irrigation system was operated daily and was stopped 12 days before expected harvesting date and during harvesting and later on was continued upto start of leaf falling (last week of September). No irrigation was applied from October to April in both years because of the fact that sufficient rain had taken place during that period. The other cultural practices were given uniformly.

Observation on major growth parameters were recorded once in a year during 2nd week of July and expressed in centimetres. Plant height was measured from ground level to the plant terminal. Trunk girth was measured by measuring tape at a height of 20 cm above ground level. The plant spread for N-S and E-W directions were measured through measuring tape. Number of fruits, fruit length, width, weight and yield per plant were recorded as per standard procedures. TSS was determined from freshly strained and thoroughly stirred juice using hand refrectometer and results were expressed in °Brix. Total sugars and total acidity were determined by using the standard titratable procedures (AOAC, 1). Leaf samples were collected for leaf analysis as per the procedure outlined by Chapman (5). For macro- and micro-nutrients except nitrogen estimation, well ground leaf tissue was digested in di-acid mixture containing HNO_3 and $HClO_4$ in 9:4 ratio for P, K and micronutrients. The phosphorous content was determined by using ammonium molybdate: ammonium metavan date (Chapman and Pratt, 6) using double beam UV-VIS spectrophotometer (ECIL, India) while potassium was determined by using flame photometer (Jackson, 9) and micronutrients were estimated using atomic absorption spectrophotometer (ECIL 4141, India). For leaf N estimation, known weight of samples were digested with H_2SO_4 using 10:1 K_2SO_4 and $CuSO_4$ as digestion mixture and digested at 390°C till clear digest is obtained. Digested samples were subjected to distillation with 40% NaOH and liberated ammonia was collected in H_3BO_3 using mixed indicator. Finally liberated ammonia was titrated against 0.1 N H_2SO_4 and N content in the leaves was expressed in percentage.

RESULTS AND DISCUSSION

Different water regimes had significant effect on vegetative growth of cherry plants (Table 2). There was a significant difference in shoot growth in both the years. The shoot growth rate was prominently faster in the trees receiving irrigation to replenish 100% ET during the 1st year. However the same treatment had deleterious effect on new shoot growth in the 2nd year of the experiment as compared to 80% ET. Trees replenished with 80 and 60% ET had positive response on shoot growth in both the years but the treatment 100% ET had negative response. This might be due to excess water available to plant which subsequently altered the root rhizosphere as cherry plant is sensitive to wet feet condition. Data revealed that there was no difference in shoot growth when plant was replenished the water with 80% ET than that of 100% ET. The shoot growth was suppressed when plants were replenished by 60 and 40% ET. The increase in vegetative growth might be due to the continuous supply of water, which maintains the soil moisture at optimum level eliminating water stress to the plant resulting in better availability of nutrient as well as their effective utilization by plant. Tree spread increased significantly in term of E-W and N-S direction with 100% ET and 80% ET. The least increase in both the direction was observed in 40% ET. The pooled data with regards to tree girth did not show significant increase with any of the treatment. This result is in conformity with the findings of Banyal and Rehalia (3) who also observed

Table 2. Effect of different water regimes on vegetative growth of sweet cherry.

Treatment	Shoot growth (cm)			Increased in tree height (%)			Increase in tree spread (%)						Increased tree girth (%)		
							East-West			North-South					
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
100% ET	26.00	25.33	25.66	7.80	8.90	8.35	6.30	6.73	6.51	6.33	7.23	6.78	4.23	4.36	4.30
80% ET	22.66	26.00	24.33	7.53	8.66	8.10	6.30	6.53	6.46	6.53	7.03	6.78	4.00	4.36	4.28
60% ET	20.66	23.00	21.83	6.96	7.63	7.30	5.96	6.23	6.13	6.23	6.60	6.41	4.20	4.13	4.06
40% ET	17.66	18.66	18.16	6.83	7.33	7.10	5.83	6.03	5.95	6.03	6.23	6.13	4.23	4.03	4.13
CD at 5%	1.52	1.96	1.79	0.52	0.42	0.67	0.19	0.31	0.28	0.23	1.66	0.44	NS	0.16	NS

A = 1st year; B = 2nd year; C = pooled

increased vegetative growth in different fruit crops by uniform distribution of soil water under drip irrigation.

Average fruit weight was maximum with 100% ET in both the years followed by 80% ET and least was in 40% ET (Table 3) indicating that plant of cherry does require precise water for better fruit size despite sensitive to water logged conditions. However, it was observed that the fruit of 100% ET showed shriveling after 3 days of harvesting and no such sign was noticed with other treatments. Hence, it is suggested to replace the evapo-transpiration of cherry plant with 80% ET for maintaining attractiveness of fruit and better shelf-life. The size of sweet cherry fruits is closely related to the content of water in the soil during last week before harvest (Blazkova *et al*., 4). Fruit width and length were also significantly higher with 100% ET followed by 80% ET. Increase in fruit weight under drip irrigation might be due to consistent moisture regimes in the root rhizosphere, which helps in optimum availability of different essential nutrients and its translocation to sink that accelerate the fruit growth.

Yield was significantly influenced by different water regimes (Fig. 1). The maximum yield of 73.26 kg/ ha was recorded with 80% ET as compared to 100% ET, which registered only 64.74 kg/ha and minimum was in 40% ET (50.50 kg/ha). Total yield in general decreased in 2nd year due to bad weather prevailing during the flowering, which restricted the bee activity for better pollination. It was further observed that an enhancement of yield by 44 and 28% was obtained under 80 and 100% ET, respectively as compared to 40% ET. An increase in yield under 80% ET might have resulted due to better water utilization (Manfrinato, 10), higher uptake of nutrient (Bafna *et al*., 2) and favourable effects on carbohydrate metabolism and better C: N ratio leading to more fruit production. Increase in apple yield under higher volume of water has also been reported by Rzekanowski and Rolbiecki (12).

It was found that the increase in TSS was maximum (23.04°Brix) when plant was replenished with 80% ET and was significantly superior over the other treatments. The results indicate that sweet cherry plants should be irrigated with 80% ET for better fruit quality. Cracking of fruits due to rain during harvesting is a serious problem and causes losses. The fruit cracking is believed to occur mainly because of sudden change in soil moisture content or due to rainfall after a long dry spell and this is common phenomenon in the Jammu and Kashmir resulting in huge losses. Most significant finding of the present experiment was reduction of fruit cracking from 16.25 to 1.96%. Replacing daily irrigation with 100 and 80% ET through drip reduced the fruit cracking to a great extent (Fig. 2). However the maximum cracking (16.25%) was observed with replenishing the irrigation at 40% ET followed by 60% ET (10.91%). Prasad *et al.* (11) also succeeded in reducing fruit cracking in

Table 3. Effect of different water regimes on physio-chemical composition of sweet cherry fruits.

Treatment	Fruit weight (g)			Fruit length (cm)			Fruit width (cm)			TSS (°Brix)			Acidity (%)			TSS:acid ratio		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
100% ET	4.53	4.50	4.51	1.80	1.80	1.80	1.90	1.91	1.90	22.00	21.50	21.75	0.64	0.66	0.65	34.31	32.61	31.79
80% ET	4.04	4.23	4.13	1.82	1.85	1.82	1.77	1.80	1.78	23.23	23.04	23.02	0.54	0.55	0.54	43.33	41.46	42.39
60% ET	3.50	3.69	3.59	1.72	1.74	1.72	1.81	1.81	1.80	20.81	22.46	21.40	0.60	0.64	0.61	34.89	35.14	35.01
40% ET	3.10	3.06	3.08	1.80	1.77	1.78	1.62	1.66	1.63	19.53	22.33	20.66	0.52	0.59	0.55	37.09	37.86	37.47
CD at 5%	0.25	0.14	0.14	0.15	0.02	0.07	0.23	0.05	0.09	1.70	0.68	1.55	0.05	0.04	0.04	3.59	3.73	3.27

A = 1st year; B = 2nd year; C = Pooled

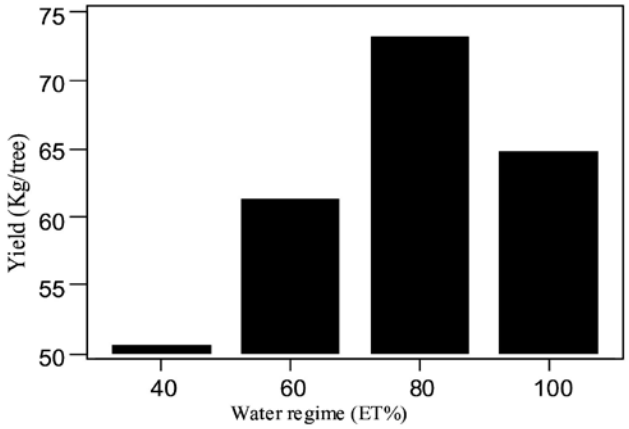

Fig. 1. Effect of different water regimes on fruit yield.

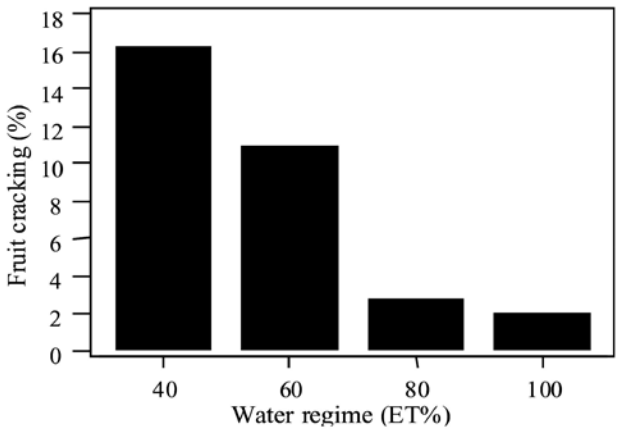

Fig. 2. Effect of different water regime on fruit cracking.

pomegranate under drip irrigation which supports the present finding.

The leaf N content was found to be maximum with 80% ET followed by 100% ET. Leaf N content was low during the 1st year of experiment compared to 2nd year. Results indicated that the continuous supply of nitrogenous fertilizer helps in nutrient build up in the cherry plants under optimum moisture regimes. It is interesting to note that the leaf nitrogen content was below the standard value in cherry leaf despite supply of full dose of N. This clearly suggests that the nitrogen dose recommended and practiced should be relooked for 25-year-old cherry plants. However, leaf P in all the treatments was found to be in sufficient range, while leaf K was sufficient in range upto 60% ET. The plants replenished water with 40% ET showed leaf K in deficient range. Hence, it is suggested that irrigation in sweet cherry must be replenished with 60% ET and above for better macro-nutrient uptake. As far as micronutrient content is concerned, leaf Fe, Mn, Cu and Zn was maximum with 80% ET followed by 100% ET and minimum was recorded with 40% ET. The leaf Fe, Cu and Mn were sufficient in range in all the treatments. However, the leaf Zn content was sufficient in 80 and 60% ET range and water replenished with 100 and 40% ET showed Zn deficiency (Tables 4 & 5).

The present finding indicate that sweet cherry plants cultivated on *Karewa* lands of Kashmir valley should be irrigated on a daily basis replenishing of

Table 4. Effect of different water regime on leaf macro-nutrient composition of sweet cherry.

Treatment	Nitrogen			Phosphorus			Potassium		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
ET (100%)	2.09	2.15	2.10	0.22	0.27	0.23	1.65	1.71	1.68
ET (80%)	2.35	2.39	2.35	0.26	0.28	0.26	1.72	1.83	1.77
ET (60%)	2.01	2.13	2.03	0.20	0.25	0.20	1.59	1.64	1.61
ET (40%)	1.93	2.17	1.98	0.18	0.23	0.18	1.33	1.46	1.39
CD at 5%	0.07	0.08	0.05	0.02	0.02	0.02	0.08	0.02	0.07

Table 5. Effect of different water regime on leaf micro-nutrient composition of sweet cherry.

Treatment	Fe			Cu			Zn			Mn		
	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
ET (100%)	195.67	203.33	199.50	15.66	15.66	15.66	20.00	17.00	18.50	68.33	68.33	68.33
ET (80%)	213.33	220.00	216.67	15.66	15.66	15.66	23.33	24.00	23.66	65.66	69.00	67.33
ET (60%)	193.33	198.33	195.83	15.66	15.66	15.66	19.66	21.00	20.33	57.66	60.33	59.00
ET (40%)	191.00	197.67	194.33	12.33	14.00	13.16	16.66	16.66	16.66	54.00	58.00	56.00
CD at 5%	9.07	15.97	8.24	1.52	NS	1.25	1.88	3.64	2.00	2.92	2.90	3.00

80% ET in order to achieve high yield, better quality, attractive fruit size and minimum fruit cracking.

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Heterosis and combining ability analysis in snowball cauliflower using indigenously developed CMS lines

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ABSTRACT

Five CMS lines, Ogu12A, Ogu13A, Ogu14A, Ogu15A and Ogu16A were selected among fifteen lines after BC_7 based on superior commercial, floral and seed setting traits. Line × Tester analysis was done by taking these five CMS lines as female parent with ten diverse lines of snowball cauliflower as testers. Data were recorded for 14 important vegetative and commercial traits. For days to 50% curd initiation and days to 50% curd maturity the CMS line Ogu15A exhibited significantly high GCA effect in negative direction (-14.52 and -14.38). For marketable curd weight and marketable curd yield significantly high GCA effects were recorded in the CMS line Ogu15A (0.11; 0.10) and Ogu13A (5.28; 4.75). Among the ten testers only two genotypes, Kt-2 and EC-162587 had significantly high GCA effect for marketable curd yield. Very high and significant SCA effect has been recorded in the combination Ogu16A × Kt-25 (20.25) and Ogu15A × Kt-2 (19.72) for marketable curd yield. The CMS line, Ogu15A was involved in the most of the heterotic combinations for earliness. Average heterosis was in desirable positive direction for 7 among 14 traits. The range of average heterosis for days to 50% curd maturity was -8.55 to 25.0% and it was -32.04 to 127.91% marketable curd yield. Ogu15A was involved in maximum number of combinations for earliness and higher productivity. Highest heterosis for marketable curd yield was recorded in the combination, Ogu15A × Kt-2 (127.91%), followed by Ogu14A × Sel-26 (90.90%) and Ogu15A × Sel-26 (86.18%). The CMS lines with better combiling ability are involved with most of the heterotic combinations for different traits thus can be used for development of F_1 hybrids.

Key words: Combining ability, heterosis, hybrid snowball cauliflower.

INTRODUCTION

Snowball or European summer cauliflower is the main vegetable crop in Indian sub-continent cultivated during winter season. In India, more than 90% of the cauliflower cultivated is F_1 hybrids. In cauliflower, F_1 hybrids are very popular mainly because of uniform maturity, high early and total yield, better curd quality with respect to compactness and colour, resistance to insect-pests, diseases and unfavorable weather conditions (Kucera *et al*., 7). Two pollination control mechanisms, *viz*. self-incompatibility (SI) and male sterility (particularly cytoplasmic male sterility; CMS) are widely used for production of F_1 hybrid seeds. So far, majority of cruciferous hybrid cultivars have been developed by using SI system (Watanabe and Hinata, 13). However, SI system has several disadvantages like, possibility of sibs in the hybrids and multiplication of SI parents through tedious bud pollination or treatment by enhanced concentration of CO_2 and NaCl spray (Jirik, 4; Kucera, 6; Sharma *et al*., 9). In case of snowball cauliflower, self-incompatibility system is very weak or not present at all (Watts, 14; Niewhoff, 8). In such situation, CMS system offers a good alternative (Kucera *et al*., 7; Sharma *et al*., 9) for production of F_1 hybrid seeds. All the hybrids cultivated in India are imported from different countries. Every year India loses a huge amount of revenue for import of hybrid seeds. Un-availability of suitable pollination control mechanism is the main constraint in developing indigenous F_1 hybrids. There is an urgent need to develop indigenous CMS/ SI lines and standardize technologies for F_1 hybrid seed production.

Good CMS system would be useful when they are transferred to the nuclear background of any cultivars/ lines with good general combining ability (GCA) and specific combining ability (SCA) besides possessing desirable agronomic characteristics. In this study, five superior Ogura based CMS lines were developed at our station and they had good floral and agronomic traits (data not presented). Their suitability in the heterosis breeding was tested through Line × Tester design.

MATERIALS AND METHODS

Five CMS lines, *viz.* Ogu12A, Ogu13A, Ogu14A, Ogu15A and Ogu16A were used as female parent (lines) and ten cauliflower genotypes, *viz*. Kt-22, Kt-25, Kt-2, HLSR-05, Sel-27, PSBK-1, EC-162587, Kt-15, Mukutmani, and Sel-26 were used as pollen parent (testers). Each of the lines was crossed with all the 10 testers individually in Line × Tester fashion

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(Kempthorne, 5) during 2012 to develop 50 hybrids. Sterile lines were raised under muslin cloth cage to avoid any natural pollination. Fully opened flowers of male sterile female lines were selected and pollination was performed by collecting fresh pollen from male parents covered with selfing bags of muslin cloth. Each male plant was covered individually before opening of flowers. Further, the 50 hybrids along with their parents were raised in randomized block design with three replications during 2013 at Baragraon Experimental Farm of IARI, Regional Station situated at an altitude of 1,560 m above mean sea level. All the standard agronomic practices for cauliflower as recommended by Singh *et al.* (11) in Kullu valley conditions were followed with a population density of 44,000 plants/ ha. Five randomly selected plants were labeled for recording the observations. Fourteen vegetative and commercial traits, *viz*., (i) days to 50% curd initiation, (ii) days to 50% curd maturity, (iii) plant height (cm), (iv) number of leaves, (v) leaf length (cm), (vi) leaf width (cm), (vii) gross plant weight (kg), (viii) marketable curd weight (kg), (ix) net curd weight (kg), (x) curd length (cm), (xi) curd width (cm), (xii) core length (cm), (xiii) marketable curd yield (t/ ha), and (xiv) harvest index (%) were recorded from the selected plants. These traits were recorded to estimate the suitability of the CMS lines in the production of high yielding, early maturity hybrids with other desirable traits. The data were subjected to combining ability analysis following the method suggested by Kempthorne (5). Mid-parental heterosis was calculated to work out the superiority of CMS based F_1 hybrids over their parents.

RESULTS AND DISCUSSION

Line × Tester analysis determined suitability of the CMS lines in heterosis breeding. The Line × Tester design is basically an extension of the top-cross analysis where instead of one tester (as used in top-crossing) more than one tester is employed. This analysis revealed GCA effects of the parental lines besides SCA effect of each cross. The estimate of GCA of a parent is an important indicator of its potential for generating superior breeding populations. A high GCA estimate indicates that the parental mean is superior or inferior to the general mean. This represents a strong evidence of favourable gene flow from parents to offspring at high frequency and gives information about the concentration of predominantly additive genes (Franco *et al.*, 3). The selected parental lines with better performance can be crossed in suitable combination to exploit heterosis. Such crosses with high SCA could be best utilized in heterosis breeding (Singh and Chaudhary, 12).

Mean squares of hybrids and Line × Tester were significant for all the fourteen traits (Table 1). Significantly high GCA in desirable direction for earliness related traits like, days to 50% curd initiation (-14.52) and days to 50% curd maturity (-14.38) was recorded in the CMS line, Ogu15A (Table 2). This indicated the genetic worthiness of the Ogu15A for the development of early maturity hybrids. For marketable curd weight and marketable curd yield, the lines Ogu15A (0.11) and Ogu13A (0.10) had significantly high GCA effect in positive direction. None of the lines had significantly high harvest index (Table 3). The

Table 1. Analysis of variance for combining ability for 14 horticultural traits based on Line × Tester design in snowball cauliflower.

Trait	Replication	Line	Testers	Line × tester	Error
DF	2	4	9	36	136
Days to 50% curd initiation	31.9	2290.7**	524.1*	193.6**	11.8
Days to 50% curd maturity	18.0	2131.6**	452.5*	168.9**	11.9
Plant height (cm)	0.6	32.1	117.6*	46.3**	8.9
Nos. of leaves	1.5	13.8	17.4	11.8**	1.7
Leaf length (cm)	4.5	62.1	115.3*	40.3**	6.2
Leaf width (cm)	4.7	41.3*	30.1*	12.1**	2.3
Gross plant wt. (kg)	0.1	1.94**	0.5	0.3**	0.1
Marketable curd wt. (kg)	0.1	0.40*	0.2	0.1**	0.1
Net curd wt. (kg)	0.01	0.29*	0.12	0.06**	0.01
Curd length (cm)	0.67	9.82*	1.64	2.59**	0.67
Curd width (cm)	0.45	2.85	5.03	3.31**	0.90
Curd depth (cm)	0.68	3.30	3.74**	1.26**	0.57
Marketable curd yield (t/ha)	104.43	961.61*	548.81	301.12**	37.97

*,** significant at 5 and 1% probability levels, respectively by F test

Table 2. Estimates of general combining ability effects of five lines based on Line × Tester design.

Trait	12A	13A	14A	15A	16A
Days to 50% curd initiation	8.51**	1.52	4.43**	-14.52**	0.05
Days to 50% curd maturity	7.43**	1.19	4.28**	-14.38**	1.47
Plant height (cm)	0.67	-0.74	1.31	-0.01	-1.24
No. of leaves	0.50	-0.02	-0.01	-1.09**	0.62
Leaf length (cm)	0.96	-0.28	1.40*	0.21	-2.29**
Leaf width (cm)	0.62	1.32**	0.36	-0.62	-1.68**
Gross plant wt. (kg)	-0.24**	0.23**	0.17**	0.13**	-0.30**
Marketable curd wt. (kg)	-0.12**	0.10**	0.04	0.11**	-0.14**
Net curd wt. (kg)	-0.10**	0.09**	0.01	0.09**	-0.10**
Curd length (cm)	0.02	0.96**	-0.38	-0.45*	-0.14
Curd width (cm)	0.28	0.27	0.01	-0.45*	-0.11
Curd depth (cm)	0.13	0.40*	0.14	-0.35	-0.32
Marketable curd yield (t/ha)	-5.78**	4.75**	2.07	5.28**	-6.32**
Harvest index (%)	0.52	-1.53	-3.13	2.17	1.97

*,** significant at 5 and 1% levels probability, respectively by F test

lines, Ogu13A and Ogu15A could be used in breeding programme for development of hybrids with higher yield. From the GCA analysis it was revealed the usefulness of the CMS line, Ogu15A in development of hybrids with earliness and higher yield. Among the testers significantly negative GCA effect was recorded in the genotype, HLSR-05 (-6.35), EC-162587 (-9.75) and Mukutmani (-5.78). Two tester genotypes, Kt-2 (8.76) and EC-162587 (8.59) had significantly high GCA effect for marketable curd yield. These tester lines could be used as pollen parent in development of cauliflower F_1 hybrid. Earlier, Dey *et al.* (2) also reported the CMS lines with better combining ability improve yield and earliness in cauliflower.

Among the 50 hybrids, 10 hybrids showed significantly negative SCA effect for days to 50% curd initiation, 10 hybrids had significantly negative SCA effect for days to 50% curd maturity (Table 4).

Table 3. Estimates of general combining ability effects of 10 testers based on Line × Tester design.

Trait	Kt-22	Kt-25	Kt-2	HLSR-05	Sel. 27	PSBK-1	EC-162587	Kt-15	Mukutmani	Sel. 26
Days to 50% curd initiation	5.53**	0.24	8.29**	-6.50**	-1.99	2.23	-9.75**	5.45**	-5.78**	2.28
Days to 50% curd maturity	5.02**	-0.52	7.68**	-6.35**	-1.12	2.63*	-9.06**	4.53**	-5.38**	2.55*
Plant height (cm)	0.95	1.49	3.28**	0.11	-4.52**	1.65	1.74	1.43	-5.23**	-0.92
Nos. of leaves	-0.93	0.68	-1.06*	1.35**	-0.79	-0.73	0.70	1.26**	-1.41**	0.94
Leaf length (cm)	-0.26	0.40	4.80**	1.25	-4.09**	-0.94	2.31	1.94	-3.89**	-1.51
Leaf width (cm)	-0.05	-0.03	1.25*	1.68**	-0.97	-0.50	2.30**	0.10	-1.83**	-1.93**
Gross plant wt. (kg)	-0.24**	0.11	0.27**	0.11	-0.12	-0.02	0.24**	-0.03	-0.22**	-0.08
Marketable curd wt. (kg)	-0.14**	0.07	0.19**	-0.09	-0.09	-0.01	0.19**	0.07	-0.21**	-0.01
Net curd wt. (kg)	-0.11**	0.05	0.11**	-0.06	-0.03	-0.04	0.14**	0.05	-0.12**	0.01
Curd length (cm)	-0.35	-0.17	0.11	-0.28	-0.53	0.38	0.02	0.20	0.14	0.47
Curd width (cm)	-0.54	-0.04	0.93**	-0.25	-0.38	0.69*	0.36	0.08	-0.99**	0.14
Curd depth (cm)	-0.31	-0.33	0.75**	-0.18	-0.80**	-0.01	-0.01	0.67*	-0.28	0.51
Marketable curd yield (t/ha)	-6.07**	3.53	8.76**	-4.03	-3.95	-0.89	8.59**	3.53	-9.16**	-0.29
Harvest index (%)	1.19	-0.33	2.50	-8.10**	-1.58	-0.71	3.47	5.30**	-4.36	2.61

*,** significant at 5 and 1% levels probability, respectively by F test

Table 4. Estimates of specific combining ability (SCA) effects of 50 hybrids based on Line × Tester design.

Hybrid	Days to 50% curd initiation	Days to 50% curd maturity	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Gross plant wt. (kg)	Marketable curd wt. (kg)	Net curd wt. (kg)	Curd length (cm)	Curd width (cm)	Curd depth (cm)	Marketable curd yield (t/ha)	Harvest index (%)
Ogu12A × Kt-22	-13.94**	-10.68**	0.88	3.06**	3.19	-0.99	0.06	0.05	0.01	-0.20	-0.17	-0.30	2.91	1.43
Ogu12A × Kt-25	2.95	4.03	-0.90	-1.31	-2.59	-1.56	-0.27	-0.18	-0.06	-0.68	-0.23	-0.52	-7.93	-0.32
Ogu12A × Kt-2	-3.26	-3.58	6.13*	0.50	4.89*	-1.25	0.35*	0.17	-0.04	-0.72	-0.75	-0.32	7.93	-2.69
Ogu12A × HLSR-05	4.67	3.89	1.58	0.16	1.28	-2.73*	-0.32	0.02	-0.02	-0.12	-0.51	-0.32	1.60	13.04**
Ogu12A × Sel-27	0.09	-2.34	-4.50	-0.81	-2.57	1.49	0.02	-0.05	0.08	0.38	-0.44	-1.56*	-2.12	-5.09
Ogu12A × PSBK-1	9.89**	8.83**	-0.66	-2.47*	-0.53	0.41	0.09	0.04	0.03	-0.05	1.33	0.12	-1.08	-4.99
Ogu12A × EC-162587	5.44	6.30*	1.64	1.14	-0.12	2.48*	0.19	0.08	0.10	2.10**	1.92*	1.34*	4.24	-1.62
Ogu12A × Kt-15	0.31	-0.13	4.95*	-0.57	1.86	1.70	0.14	-0.08	-0.08	0.05	-0.89	0.92	-3.30	-9.01
Ogu12A × Mukutmani	-0.99	-1.20	-8.23**	1.60	-5.54**	-0.51	-0.21	-0.08	-0.01	-1.42*	-0.40	-0.62	-3.51	3.68
Ogu12A × Sel-26	-5.16	-5.12	-0.89	-1.32	0.13	0.97	-0.06	0.02	0.01	0.67	0.16	1.28*	1.25	5.58
Ogu13A × Kt-22	7.24**	7.11*	-2.27	-3.44**	0.02	0.55	-0.06	-0.06	0.07	0.80	2.13**	0.65	-2.91	-1.74
Ogu13A × Kt-25	-4.50	-3.34	0.79	1.37	-1.84	1.24	0.08	0.08	0.09	-0.21	0.52	-0.26	3.60	1.92
Ogu13A × Kt-2	3.79	3.35	-4.50	-3.40**	-5.08*	-0.83	-0.58**	-0.34**	-0.07	0.48	-0.16	-0.40	-15.11**	0.62
Ogu13A × HLSR-05	0.99	-0.20	-0.98	-1.76	-1.43	-2.90*	-0.09	0.03	-0.09	-0.70	-0.48	-0.35	1.49	3.85
Ogu13A × Sel-27	13.24**	12.63**	7.10**	1.52	5.12*	0.27	0.38*	0.13	0.03	-0.04	-0.36	0.43	5.81	-3.73
Ogu13A × PSBK-1	-0.09	-1.20	1.01	1.19	1.89	1.09	-0.14	-0.10	-0.10	-0.72	-1.24	0.07	-3.69	0.18
Ogu13A × EC-162587	-7.76**	-9.06**	0.11	2.22*	-0.43	1.06	0.51**	0.17	0.09	-1.26	-0.98	-0.42	7.64	-6.36
Ogu13A × Kt-15	-0.78	-0.56	-2.68	5.42**	-4.77*	-1.95	-0.22	-0.04	-0.14	0.30	-0.01	0.08	-2.25	4.40
Ogu13A × Mukutmani	-14.44**	-12.30**	2.58	-0.95	7.63**	2.64*	0.20	0.38**	0.27**	1.81**	1.27	0.80	16.74**	13.12**
Ogu13A × Sel-26	2.33	3.58	-1.14	-2.15*	-1.10	-1.18	-0.08	-0.25*	-0.16	-0.44	-0.68	-0.59	-11.33**	-12.28*
Ogu14A × Kt-22	13.03**	11.93**	-3.13	0.07	-1.05	1.44	0.01	-0.14	-0.16	-0.25	-2.05**	-0.25	-6.24	-10.10*
Ogu14A × Kt-25	-1.34	-1.02	-0.14	-1.31	-0.85	-0.73	0.03	0.05	0.01	0.58	-0.07	0.07	2.25	2.25
Ogu14A × Kt-2	8.61**	9.20**	-2.25	1.20	0.21	-0.34	0.24	0.20	0.19*	0.94	0.65	0.42	9.09	3.71
Ogu14A × HLSR-05	-5.02	-4.02	1.63	2.04	0.10	3.14*	0.19	-0.12	-0.05	0.36	0.19	0.72	-5.50	-9.91
Ogu14A × Sel-27	-9.30**	-8.19**	-2.90	-0.82	-2.98	-0.84	-0.26	-0.12	-0.11	0.46	1.65*	0.50	-5.58	1.42
Ogu14A × PSBK-1	0.87	0.18	2.89	0.38	0.57	0.26	-0.10	0.01	0.01	-0.02	0.26	0.14	1.18	4.94
Ogu14A × EC-162587	-5.37	-5.75*	-1.74	0.17	-1.40	-2.66*	-0.55**	-0.16	-0.19*	-0.46	-0.38	0.17	-7.28	9.60

Contd...

Table 3 Contd...

Hybrid	Days to 50% curd initiation	Days to 50% curd maturity	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Gross plant wt. (kg)	Marketable curd wt. (kg)	Net curd wt. (kg)	Curd length (cm)	Curd width (cm)	Curd depth (cm)	Marketable curd yield (t/ha)	Harvest index (%)
Ogu14A × Kt-15	10.38**	10.61**	-2.09	1.96	0.60	-0.46	0.12	0.16	0.15	-0.73	0.23	-0.54	7.14	3.88
Ogu14A × Mukutmani	-7.98**	-8.36**	-0.16	0.02	0.02	-0.68	0.09	-0.12	-0.02	-0.56	-0.85	-0.70	-5.65	-10.65*
Ogu14A × Sel-26	-3.88	-4.57	7.89**	0.18	4.75*	0.89	0.23	0.24*	0.19*	-0.30	0.37	-0.55	10.60*	4.82
Ogu15A × Kt-22	-9.04**	-9.20**	1.53	0.96	-1.63	-0.76	-0.18	-0.02	-0.03	-0.02	0.38	0.01	-0.99	5.67
Ogu15A × Kt-25	-3.18	-3.08	-2.68	-0.59	-0.68	-0.76	-0.37*	-0.41**	-0.25**	-0.10	-0.73	0.05	-18.18**	-10.79*
Ogu15A × Kt-2	-6.63*	-6.26*	6.07*	2.02	6.96**	5.93**	0.79**	0.45**	0.16*	0.67	0.85	0.30	19.72**	-3.19
Ogu15A × HLSR-05	-0.16	-0.55	0.48	-0.99	-0.07	-0.88	0.51**	0.24*	0.31**	1.24	1.58*	0.36	10.50*	-2.01
Ogu15A × Sel-27	4.06	4.18	2.82	0.67	3.86	-0.60	-0.05	0.16	0.04	-0.10	0.38	0.28	7.28	11.42**
Ogu15A × PSBK-1	-2.00	-1.91	-3.17	1.05	-0.98	-0.88	-0.02	0.03	0.01	-0.49	-0.61	0.18	2.52	4.40
Ogu15A × EC-162587	3.65	2.75	-3.33	-3.24**	-1.57	-1.08	-0.47**	-0.22*	-0.14	-0.16	-0.48	-0.33	-9.98*	2.90
Ogu15A × Kt-15	0.77	1.39	-2.62	-1.54	-2.44	-0.22	-0.16	-0.07	0.04	0.43	0.22	-0.22	-3.36	1.68
Ogu15A × Mukutmani	7.88**	8.75**	2.22	-0.66	-0.59	0.31	-0.01	-0.24*	-0.21**	-1.58*	-1.73*	-0.20	-10.91*	-14.96**
Ogu15A × Sel-26	4.65	3.93	-1.32	2.33*	-2.84	-1.03	-0.02	0.07	0.06	0.12	0.12	-0.42	3.40	4.88
Ogu16A × Kt-22	2.71	0.84	2.99	-0.66	-0.53	-0.23	0.17	0.16	0.12	-0.31	-0.28	-0.10	7.23	4.73
Ogu16A × Kt-25	6.07*	3.42	2.92	1.84	5.98**	1.83	0.53**	0.46**	0.21**	0.41	0.51	0.66	20.25**	6.93
Ogu16A × Kt-2	-2.51	-2.70	-5.45*	-0.33	-7.01**	-3.50**	-0.80**	-0.48**	-0.24**	-1.38*	-0.58	0.01	-21.63**	1.54
Ogu16A × HLSR-05	-0.47	0.89	-2.71	0.54	0.10	3.37**	-0.29	-0.18	-0.14	-0.77	-0.77	-0.40	-8.10	-4.96
Ogu16A × Sel-27	-8.09**	-6.28*	-2.52	-0.55	-3.42	-0.32	-0.10	-0.12	-0.05	-0.69	-1.22	0.33	-5.39	-4.02
Ogu16A × PSBK-1	-8.68**	-5.91	-0.07	-0.16	-0.95	-0.88	0.17	0.01	0.04	1.30	0.25	-0.51	1.07	-4.53
Ogu16A × EC-162587	4.04	5.76	3.33	-0.29	3.53	0.20	0.32	0.12	0.14	-0.20	-0.07	-0.75	5.37	-4.52
Ogu16A × Kt-15	-10.68**	-11.31**	2.44	-1.33	4.74*	0.95	0.12	0.04	0.03	-0.06	0.43	-0.24	1.78	-0.95
Ogu16A × Mukutmani	15.53**	13.12**	3.60	-0.01	-1.52	-1.76	-0.06	0.07	-0.02	1.75**	1.71	0.73	3.33	8.80
Ogu16A × Sel-26	2.07	2.17	-4.53	0.95	-0.93	0.35	-0.05	-0.08	-0.09	-0.04	0.01	0.29	-3.92	-3.02

1. 12A, 2. 13 A, 3. 14A, 4. 15A, 5. 16A 6. Kt-22, 7. Kt-25, 8. Kt-2, 9. HLSR-05. 10. Sel-27, 11. PSBK-1, 12. EC-162587, 13. Kt-15, 14. Mukutmani, 15. Sel-26; *,** significant at 5 and 1% levels probability, respectively by F test

Table 5. Range and average heterosis among the 50 hybrids along with top 10 heterotic hybrids for 14 traits.

Heterosis and genetic combining ability	Days to 50% curd initiation	Days to 50% curd maturity	Plant height (cm)	No. of leaves	Leaf length (cm)	Leaf width (cm)	Gross plant weight (kg)	Marketable curd weight (kg)	Net curd weight (kg)	Curd length (cm)	Curd width (cm)	Curd depth (cm)	Marketable curd yield (t/ha)	Harvest index (%)
Range of Heterosis	-8.64-30.85	-8.55-25.00	-93.79-24.87	-16.62-44.76	-29.87-28.64	-25.13-40.03	-34.17-113.41	-32.04-127.91	-34.38-94.43	-11.97-31.66	-9.99-45.72	-34.85-303.05	-32.04-127.91	-35.73-38.40
Average Heterosis	7.24	6.49	-20.93	14.42	-0.57	-0.26	14.93	24.99	19.44	4.79	11.88	15.48	24.99	8.84
Top 10 heterotic F_1 hybrids	15A × HLSR05 118.8 (-8.64)	15A × Kt-22 116.8 (-8.55)	15A × Kt-2 51.10 (24.87)	14A × HLSR05 23.42 (44.76)	15A × Kt-2 52.17 (28.64)	14A × HLSR05 25.88 (40.03)	15A × Kt-2 2.94 (113.41)	15A × Kt-2 1.79 (127.91)	15A × HLSR05 1.04 (94.43)	13A × Mukut mani 14 (31.66)	12A × Ec162587 16.98 (45.72)	15A × HLSR05 26.83 (303.05)	15A × Kt-2 78.92 (127.91)	15A × Kt-22 68.41 (38.40)
	13A × Mukut Mani 107.2 (-8.23)	13A × Kt-15 140.53 (-7.52)	15A × Kt-22 44.24 (19.10)	13A × Kt-15 26.69 (42.38)	14A × Kt-15 44.14 (27.18)	15A × Kt-2 27.25 (32.43)	15A × HLSR05 2.51 (80.84)	14A × Sel-26 1.30 (90.90)	15A × Kt-2 1.08 (89.59)	12A × EC162587 12.89 (24.96)	12A × PSBK-1 16.72 (37.33)	12A × Kt-22 12.38 (84.60)	14A × Sel-26 57.53 (90.90)	15A × Sel-27 71.37 (37.35)
	15A × Kt-22 107.86 (-6.22)	15A × EC162587 114.66 (-7.04)	12A × Kt-15 48.82 (17.98)	15A × Sel-26 22.23 (33.92)	14A × Sel-26 44.83 (24.62)	12A × EC162587 26.09 (26.37)	14A × Kt-2 2.44 (74.38)	15A × Sel-26 1.217 (86.18)	15A × Sel-26 0.86 (86.81)	16A × Mukut mani 12.36 (19.32)	14A × PSBK-1 15.37 (23.54)	12A*Kt-15 8.41 (30.29)	15A × Sel-26 53.54 (86.18)	16A × Kt-22 67.28 (34.33)
	15A × Ec162587 105.26 (-5.60)	16A × Sel-27 131 (-6.19)	15A × Kt-15 40.56 (13)	14A × Sel-26 21.15 (30.17)	14A × HLSR05 42.95 (19.85)	13A × EC162587 25.38 (20.22)	14A × HLSR05 2.24 (58.68)	15A × PSBK-1 1.18 (82.90)	15A × Kt-15 0.89 (82.99)	13A × Kt-15 12.55 (19.03)	14A × Kt-22 11.81 (21.62)	14A × PSBK-1 6.95 (24.76)	15A × PSBK-1 52.06 (82.90)	16A × Kt-25 67.95 (34.25)
	15A × Sel-27 113.43 (-5.34)	13A × EC162587 118.43 (-5.86)	15A × Sel-26 39.51 (12.90)	16A × Kt-25 23.2 (29.85)	15A × HLSR05 41.59 (18.72)	14A × KT-15 20.68 (15.85)	14A × Sel-26 2.07 (56.87)	14A × Kt-2 1.47 (80.77)	15A × EC162587 0.79 (80)	13A × Kt-22 12.48 (16.81)	15A × EC162587 13.83 (21.45)	14A × Kt-2 7.99 (23.74)	14A × Kt-2 65.09 (80.77)	15A × EC162587 51.10,50.95 (33.11)
	-	15A × Sel-27 124.03 (-5.81)	12A × Kt-2 51.85 (11.72)	12A × Kt-22 22.67 (27.52)	12A × Kt-15 44.96 (18.03)	12A × Kt-15 23.11 (17.72)	13A × EC162587 2.73 (63.87)	16A × Kt-25 1.42 (65.50)	13A × EC162587 1.04 (70.11)	16A × PSBK-1 11.86 (14.94)	15A × Kt-15 14.25 (21.15)	13A × Kt-15 7.84 (22.79)	16A × Kt-25 62.62 (65.50)	15A × Sel-26 67.92 (32.58)
	-	15A × Kt-25 117.36 (24.03)	15A × HLSR05 42.34 (10.39)	12A × HLSR05 22.06 (27.43)	15A × Kt-15 39.90 (17.72)	15A × HLSR05 20.86 (12.57)	14A × Kt-15 2.01 (40.19)	15A × EC162587 1.11 (64.24)	14A × Sel-26 0.92 (68.90)	15A × Sel-26 11.23 (14.90)	15A × Kt-2 15.74 (20.75)	12A × Sel-26 8.60 (21.11)	15A × EC162587 49.04 (64.24)	15A × PSBK-1 65.22 (32.43)
	-	14A × Sel-27 130.33 (-4.12)	15A × Mukutmani 38.73 (8.66)	14A × Kt-2 20.16 (24.87)	12A × Kt-2 50.84 (13.62)	15A × EC162587 21.28 (12.48)	15A × PSBK-1 1.83 (38.11)	14A × Kt-15 1.316 (61.47)	14A × Kt-15 0.93 (62.86)	15A × HLSR05 11.58 (14.77)	14A × EC162587 14.39 (20.74)	14A × EC162587 6.98 (19.78)	14A × Kt-15 57.90 (61.47)	16A × Mukutmani 64.95 (31.48)
	-	14A × Mukutmani 125.9 (-3.40)	15A × PSBK-1 40.23 (5.24)	14A × Kt-25 19.4 (23.96)	14A × Kt-2 46.61 (12.68)	15A × Kt-15 19.93 (11.33)	15A × Sel-26 1.76 (35.79)	14A × PSBK-1 1.08 (59.21)	15A × PSBK-1 0.76 (60.28)	12A × Sel-26 12.25 (14.48)	16A × Kt-15 14.54 (19.98)	14A × Kt-15 6.94 (19.77)	14A × PSBK-1 47.52 (59.21)	14A × EC162587 69.31 (30.94)
	-	15A × Sel-26 127.46 (-2.62)	15A × Kt-25 40.57 (3.69)	15A × Kt-2 19.9 (20.61)	12A × HLSR05 43.69 (11.39)	14A × EC162587 20.68 (9.61)	14A × PSBK-1 1.78 (32.59)	15A × HLSR05 1.29 (58.95)	14A × Kt-2 1.02 (59.48)	13A × Sel-26 12.07 (12.76)	14A × Kt-15 14.73 (19.80)	14A × HLSR05 7.36 (18.25)	14A × EC162587 48.53 (55.35)	13A × Mukutmani 66.60 (24.31)

The number of hybrids with significant SCA effect in desirable direction was 5, 5, 7 and 5 for plant height, number of leaves, leaf length and leaf width, respectively. Highest SCA effect in negative direction for days to 50% curd maturity was observed in the hybrid, Ogu13A × Mukut mani (-12.30) followed by Ogu16A × KT-15 (-11.31) and Ogu12A × KT-22 (-10.68). For gross plant weight, marketable curd weight, net curd weight, curd length and curd width numbers of the hybrids with desirable SCA effect was recorded in 6, 4, 6, 2 and 4 hybrids, respectively. For marketable curd yield 5 hybrids had significantly high positive SCA effect. Highest positive SCA effect was recorded in the hybrid; Ogu16A × Kt-25 (20.25) followed by Ogu15A × Kt-2 (19.72) and Ogu13A × Mukutmani (16.74). For harvest index only three hybrids had significantly high positive SCA effect.

All the 14 traits under study showed varying degree of heterosis (Table 5). The range of heterosis for days to 50% curd initiation and days to 50% curd maturity was -8.34 to 30.85 and -8.55 to 25%, respectively. Highest negative heterosis for days to 50% curd maturity was recorded in the hybrid, Ogu15A × Kt-22 (-8.55%) followed by Ogu13A × Kt-15 (-7.52%) and Ogu15A × EC-162587 (-7.04%). Five among the top 10 heterotic hybrid for curd maturity had Ogu15A as female parent. The range of heterosis for plant height, number of leaves, leaf length, leaf width and gross plant weight was -93.79-24.87%, -16.62-44.76%, -29.87-28.64%, -25.13-40.03% and -34.17-127.91%, respectively. Whereas, the range of heterosis was -32.04-127.91%, -34.38-94.43%, -11.97-31.66%, -9.99-45.72%, -34.85-303.05%, -32.04-127.91% and -35.73-38.4% for marketable curd weight, net curd weight, curd length, curd width, core length, marketable curd yield and harvest index, respectively. Average heterosis was in desirable positive direction in 7 yield and yield related traits and harvest index. The average heterosis for marketable curd weight and net curd weight was 24.99 and 19.44%, respectively. Highest heterosis for marketable curd weight was recorded in the hybrid, Ogu15A × Kt-2 (127.91%) followed by Ogu14A × Sel-26 (90.90%), Ogu15A × Sel-26 (86.18%), Ogu15A × PSBK-1 (82.90%) and Ogu14A × Kt-2 (80.77%). Among the top 10 heterotic hybrids for curd yield, five had Ogu14A and 4 had Ogu15A as female parents. Moderate to low heterosis for various traits was mainly attributed to the narrow genetic base of Indian snowball cauliflower. Low genetic diversity is because of high degree of self-compatibility and consequent selfing to a considerable percentage (Watts, 14; Nieuwhof, 8). Moreover, most of the snowball cauliflower lines in India have derived from European materials. Genetic base of Indian snowball cauliflower is low as the base population had low genetic diversity. Astarini *et al.* (1) also reported narrow genetic base in cauliflower.

The lines with better estimates of GCA and SCA effects were involved in the hybrids with better performance for various traits. Therefore, it was concluded that careful selection of parental lines for good combining ability would help in developing more productive and early maturity F_1 hybrids. Singh *et al.* (10) also reported similar result in early Indian cauliflowers. Thus, the CMS lines, Ogu13A, Ogu14A and Ogu15A would be immensely useful in the development of heterotic hybrids for yield and early maturity after selection of suitable pollen parent line.

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Evaluation of hull-less seeded pumpkin lines for growth, yield and quality traits under subtropical conditions

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ABSTRACT

Hull-less seed is an important trait to use as snacks and oil of pumpkin seeds. The present investigation was undertaken with the objective to evaluate the advance breeding lines for growth, yield and quality traits of hull-less seeded pumpkin at PAU, Ludhiana. Experiment was conducted in the Department of Vegetable Science during 2014 and 2015 involving 25 genotypes. A wide range of genetic differences was observed for vine length (28.56 to 175.38 cm), days to 50% flowering (19.17 to 35.17) and days to harvest (58.00 to 77.00). Fruit yield ranged from 44.47 to 552.79 q/ha and seed yield from 1.84 to 8.35 q/ha. Quality traits like oil content (16.66 to 38.67%), dry matter (93.41 to 95.71%), crude fibre (2.33 to 8.00%), total ash (4.00 to 6.33%), protein (1.25 to 2.61%), total sugars (3.19 to 7.47%) and starch (1.66 to 10.90%) also showed considerable differences. Two years evaluation revealed that PWT-2, PWT-4, PWT-8, PWT-10, PWT-20, PWT-43 and PWT-44 were the promising. PWT-4 gave the highest seed yield (8.35 q/ha), oil content (38.67%), oil yield (3.44 q/ha), total sugars (5.67%) and fruit yield (291.9 q/ha). PWT-4 also gave good size of seed (9.27g/100 seeds), protein (2.27%) and starch (4.4%) contents. All these seven genotypes were significantly at par among themselves; however, PWT-4, PWT-20, PWT-10 and PWT-8 were better than check variety Lady Godiva for hull-less seed yield. Therefore, these genotypes can further be evaluated for yield, oil content and quality parameters for commercial release.

Key words: Fruit yield, hull-less seed, pumpkin, oil content, seed yield.

INTRODUCTION

Cucurbita genus (2n = 40) belongs to family Cucurbitaceae and comprised of five domesticated species (Hadia *et al*., 13). All these species are native of Americas (Whitaker, 26) and *Cucurbita pepo* found to be most variable among all (Paris and Nerson, 18). Pumpkins are mostly referred to cultivars having round fruits and used upon maturity for baking or feeding livestock, whereas, squashes to those having edible immature fruits. The seed of most cultigens of *Cucurbita pepo* possess a thick and leathery outer layer (Latifi *et al*., 16). However, a mutant called Styrian (hull-less) seed pumpkin (*Cucurbita pepo* subsp. *pepo* var. *styriaca*) lack complete lignifications of the testa, which makes it cost effective by evading expensive decorticating process. This mutant was emerged in 1880's in the South-East of the Astro-Hungarian Monarchy (Zraidi *et al*., 23). It is controlled by single recessive gene and led to a very thin outer hull (naked or hull-less seeds), as a result pumpkin turned into a snack and oilseed crop in Europe and USA (Idouraine *et al*., 14). Pumpkin seeds have a malleable, chewy texture and a subtly sweet, nutty flavour. Seed possess valuable dietary and medicinal qualities besides being a source of good-quality edible oils. Pumpkin seed extract has been reported to have anti-diabetic, anticancer, anti-mutagenic and antioxidant activities. Keeping in view the significance of oil and nut industry, this mutant was introduced in India and transferred into local genotypes. The advance breeding lines carrying hull-less seed trait were stabilized for growth, yield and quality. Therefore, present investigation was undertaken with the objective to evaluate the advance breeding lines for growth, yield and quality traits of hull-less seeded pumpkin.

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MATERIALS AND METHODS

The experiment was carried out for two consecutive years during summer season of 2014 and 2015 at PAU, Ludhiana. The experimental material comprised of 23 advance breeding lines (F_5 & F_6) of hull-less seeded pumpkin along with check varieties PCK-1 (hulled) and Lady Godiva (hull-less). These lines are derived from across of PCK-1 x Lady Godiva and an unknown snack seeded F_1 hybrid introduced from the USA. Seeds of 25 entries were sown on 5 February during 2014 and 12 February during 2015 in plug-trays. Upon attaining proper size seedlings were transplanted in the field by accommodating ten plants per replication of each genotype at 0.45 m distance between plants on both sides of 1.5 m wide beds. The experiment was laid out in randomized block design suggested by Snedecor and Cochran

(20) with three replications. Other cultural practices were followed as per PAU Package of Practices for the pumpkin (Anon, 3). The observations were recorded on different quantitative and quality traits, *viz.* vine length (cm), days to 50% flowering, node of first female flower, internodal length (cm), fruit shape index, flesh thickness (cm), days to harvest, number of fruits per plant, average fruit weight (kg), fruit yield per hectare (kg/ ha), No. of seeds per fruit, 100-seed weight (g), seed yield per hectare (g/ ha), oil content (%), dry matter (%), ash content (%), carbohydrates (%), protein (%) and fibre content (%). Oil content was estimated using method given by Folch *et al.* (11) and protein content, carbohydrates, total sugars and starch content following standard methods. Dry matter and ash content were determined by recommended methods (AOAC, 4). The level of significance was compared with the introduced variety Lady Godiva of hull-less seeded pumpkin.

RESULTS AND DISCUSSION

The results for growth and yield parameters given in Table 1 depicts that most of the genotypes were dwarf in nature having bush type growth habit. There were 19 genotypes significantly shorter in vine length than check variety Lady Godiva, which was trailing in nature. Bush types are preferred over vine type to accommodate more number of plants, early

Table 1. Performance of different hull-less seeded genotypes for growth and yield parameters.

Genotype	Vine length (cm)	Days to 50% flowering	Node of 1st female flower	Internodal length (cm)	Fruit shape index	Flesh thickness (cm)	Fruit cavity (cm)	Days to harvest	No. of fruits/ plant	Av. fruit wt. (kg)	Fruit yield (q/ha)	Seed yield (q/ha)
PWT-2	107.65*	25.00	7.67	5.10	0.96	1.45	8.92	64.50	2.16*	0.57	381.39*	6.52
PWT-3	61.82*	34.33	8.17	2.62*	0.72	1.55	12.37*	73.67	1.00	0.53	162.13	4.14
PWT-4	47.61*	32.33	5.33*	2.08*	0.63	1.78*	6.60	70.50	2.83*	0.42	294.95*	8.35*
PWT-8	69.02*	33.00	5.17*	3.07*	0.94	1.32	7.90	72.67	2.83*	0.32	267.12	7.71*
PWT-10	35.83*	28.50	5.83	2.27*	0.94	1.78*	9.02	67.50	1.83	0.55	384.81*	7.90*
PWT-11	57.33*	19.33*	7.17	3.26*	1.75*	1.45	6.72	58.00	1.83	0.58	346.72*	7.28
PWT-12	72.31*	35.17	7.33	4.52	1.44*	1.55	8.15	76.00	2.00	0.62	323.28*	6.43
PWT-15	175.38	24.17	7.17	6.87	1.00	1.20	8.65	63.33	1.83	0.21	147.97	5.17
PWT-16	110.25*	27.50	7.83	4.50	0.92	1.50	9.23	65.67	1.83	0.52	338.42*	6.32
PWT-17	108.02*	24.50	7.00	2.11*	1.26*	1.60	6.87	62.83	1.50	0.45	284.21*	4.77
PWT-18	41.11*	32.00	7.17	2.82*	1.31*	1.58	6.72	74.00	1.67	0.58	286.65*	7.27
PWT-19	46.18*	34.50	8.33	1.49*	1.02	1.63	8.64	77.00	2.17*	0.42	297.40*	7.66*
PWT-20	31.67*	22.33	6.83	5.22	1.02	1.25	8.13	63.33	2.50*	0.46	358.44*	8.16*
PWT-22	141.19	24.00	6.67	6.76	1.59*	1.42	8.09	64.17	1.83	0.54	249.05	3.39
PWT-23	131.77	21.67	5.83*	5.47	1.30*	1.33	9.38	61.67	1.83	0.55	190.94	7.15
PWT-25	149.04	23.67	8.00	6.34	0.78	1.40	9.13	62.83	1.17	0.53	177.27	3.18
PWT-26	61.89*	25.50	6.67	2.78*	0.94	1.85*	12.17*	65.83	1.00	1.15	391.30*	3.35
PWT-27	93.26*	26.17	6.83	4.29	1.18*	1.23	7.37	65.67	1.83	0.37	226.59	6.32
PWT-33	49.79*	29.00	8.00	2.71*	1.19*	1.70	9.25	66.00	1.83	0.66	317.91*	4.44
PWT-35	73.67*	21.83	7.50	3.15*	0.98	1.52	8.10	59.33*	1.17	0.50	183.13	4.06
PWT-39	59.97*	19.17*	7.50	2.61*	0.93	1.45	9.32	64.00	1.50	0.43	167.50	1.84
PWT-43	28.56*	31.83	8.17	1.57*	1.10*	1.43	8.00	70.67	2.50*	0.45	308.14*	7.52
PWT-44	119.07*	31.33	7.67	4.83	0.82	1.80*	9.67	70.83	2.00	0.94	418.51*	6.57
Lady Godiva	132.22	22.67	7.50	4.59	0.92	1.50	10.47	63.83	1.50	0.52	241.73	5.65
PCK-1	35.87*	23.33	5.17	1.93*	0.48	2.98*	8.12	60.17*	2.50*	0.38	225.61	6.65
$CD_{0.05}$	11.49	2.83	1.11	0.80	0.14	0.20	1.12	2.84	0.53	0.77	33.78	1.87

*Significantly better than check variety Lady Godiva

maturity, high yield and mechanical weed control. It was also observed that most of the genotypes start flowering within one month of transplanting in the field. The internodal length, another important indicator of dwarfness was also found short in majority of the lines compared with check Lady Godiva (4.59). The bush type pumpkins differ from the common trailing varieties by their much-shortened internodes. Node at which 1st female flower appears indicate the days to fruiting and PWT-8 borne it on 5.17th node of the plant. Among the evaluated hull-less lines, PWT-11 and PWT-35 were the earlier to harvest the mature fruits. There was 19 days difference in fruit maturity among the tested lines; therefore, genotypes of variable maturity can be identified from the available material.

Fruit shape index, *viz.* polar and equator diameter ratio determines shape of the fruit. The ratio of 1.00 shows complete roundness, more than 1.1 towards elliptical and less than 0.9 towards flat shape of the fruit. Generally, consumers prefer round fruits and 12 genotypes fall in this category, whereas, PWT-11, PWt-12 and PWT-22 were highly elliptical with 1.75, 1.44 and 1.59 fruit shape index, respectively. Flesh thickness is important for consumption as vegetable and was significantly more in PWT-4 (1.78 cm), PWT-10 (1.78 cm), PWT-26 (1.85 cm), PWT-44 (1.80 cm) and PCK-1 (2.98 cm) than Lady Godiva (1.50 cm). Therefore, such genotypes can be exploited as dual purpose, *viz.* vegetable as well as snack seeded pumpkin. The larger cavity of fruit can accommodates more number of seeds and was maximum in PWT-3 (12.37 cm) followed by PWT-26 (12.17 cm).

Number of fruits is an important trait that contributes toward yield. In present study, seven genotypes were significantly better in fruit number/ plant than the check variety. PWT-4 and PWT-8 borne maximum 2.83 fruits/ plant. Loy (20), Winkler (22) and Bavec *et al.* (7) recorded 1.2 to 3.5 fruits per plant. Fruit weight is another parameter revealing fruit yield and all the genotypes harvested at mature fruit stage were at par with maximum weight in PWT-26 (1.15 kg) and minimum in PWT-15 (0.210 kg). Bavec *et al.* (7) reported 5.1 kg and Cui and Loy (8) 1.0-1.5 kg fruit weight of hull-less seeded pumpkin in different studies. Fruit yield determines the total economic yield potential of a genotype. PWT-44 (418.50 q/ha) was highest in fruit yield followed by PWT-26 (391.30 q/ha) and PWT-2 (381.39 q/ha). Among the genotypes, 14 were significantly better than Lady Godiva for the fruit yield. Keeping in view the significance to use as snacks or oil, seed yield is most important parameter of hull-less pumpkin. In this study, PWT-4 (8.35 q/ha) was having highest seed yield followed by PWT-20 (8.16 q/ha), PWT-10 (7.90 q/ha), PWT-8 (7.70 q/ha) and PWT-19 (7.66 q/ha). All these five genotypes gave significantly better yield than check variety Lady Godiva. Bahlgerdi *et al.* (6) recorded 5.27, Winkler (22) 7.40 to 9.80 and Fruhwirth and Hermetter (12) 5.00-6.00 q/ha seed yield of hull-less seeded pumpkin.

Oil content of hull-less seeded genotypes is one of the most important traits due to its nutritional significance. The results on quality traits in Table 2 depicts that 17 genotypes were at par with Lady Godiva for oil content. Among them PWT-4 (38.67%) had the maximum oil content followed by PWT-15 (38.33%) and PWT-33 (38.33%). However, for oil yield PWT-4 was significantly better than Lady Godiva, with maximum oil yield, *i.e.* 3.23 q/ha followed by PWT-20 (2.58 q/ha), PWT-8 (2.18 q/ha) and PWT-44 (2.08 q/ha). These values depicted high potential for oil yield in hull-less seeded genotypes. Therefore, this stock can also be exploited as oil seed crop along with the snack seeds and as vegetable. The oil content values are in concordance with those of Stevenson *et al.* (21), wherein, it ranged from 9.8-52.1% in different species of *Cucurbita* and from 31.2-51.0% in different varieties of *C. pepo*. Ardabili *et al.* (5) reported 41.59% and Winkler (22) 45.8% average oil content in hull-less seeded pumpkin, whereas, Martinez *et al.* (17) reported oil contents of two naked-seeded *C. pepo* accessions as 35 and 37%. Pumpkin seeds are considered to be rich in protein. Among all the genotypes, 11 were significantly at par for protein content with check variety. In present study, PWT-11 (2.61%) was found to have maximum protein content followed by PWT-16 (2.58%), PCK-1 (2.50%) and PWT-15 (2.43%). The hullless seeds of pumpkin having high level of dry matter can give good storage for use by the snack and oil seed industry. Low moisture content reduces perishability, because higher moisture content may lead to susceptibility for microorganisms. Among all the genotypes, 22 were significantly at par with check variety, where, PWT-8 (95.71%) was found to have maximum dry matter followed by PWT-15 (95.58%) and PWT-2 (95.50%). The content of dry matter is the reciprocal of moisture and can be determined from the level of moisture in the seed. Adeel *et al.* (1) reported 5.9%, Eman and El-Kinawy (10) 6.8% and Jafari *et al.* (15) 4.7 to 5.4% moisture content in pumpkin seeds. Ash content is the index of total mineral in the seeds, which are expected to speed up metabolic processes, improve growth and development. Generally, ash content of seeds is higher than the fruits, which indicated seeds as good source of minerals. In present study, PWT-3, PWT-

Table 2. Performance of different hull-less seeded pumpkin genotypes for growth and yield parameters.

Genotype	Oil content (%)	Oil yield (q/ha)	Protein (%)	Dry matter (%)	Ash content (%)	Total sugars (%)	Starch (%)	Fibre (%)
PWT-2	31.67	2.06	2.39	95.50	5.00	6.10*	9.76	5.00
PWT-3	32.33	1.34	1.58	94.44	6.33	7.09*	6.62	5.33
PWT-4	38.67	3.23*	2.27	94.17	4.67	5.97*	8.80	4.5
PWT-8	28.33	2.18	1.88	95.71	4.33	5.54	2.08	5.33
PWT-10	23.33	1.84	1.91	95.28	5.33	7.28*	6.70	7.33
PWT-11	25.00	1.82	2.61	94.76	5.67	4.33	1.66	5.67
PWT-12	26.67	1.71	1.99	94.49	5.67	4.99	6.04	6.33
PWT-15	38.33	1.98	2.43	95.58	4.67	5.31	10.12	3.00
PWT-16	18.34	1.16	2.58	95.02	4.33	7.47*	8.54	2.33
PWT-17	18.34	0.87	1.67	95.07	5.00	6.27*	4.60	3.67
PWT-18	25.00	1.82	2.39	93.41	5.33	4.94	4.58	4.67
PWT-19	18.33	1.40	2.32	94.7	4.67	5.40	5.74	5.33
PWT-20	31.67	2.58	2.20	94.07	4.67	6.28*	5.90	6.33
PWT-22	18.33	0.62	2.29	93.77	4.67	6.23*	5.06	6.33
PWT-23	21.67	1.55	1.25	94.91	5.67	5.61	5.62	6.33
PWT-25	20.00	0.64	1.82	94.12	4.67	3.54	9.14	7.67
PWT-26	28.33	0.95	1.49	94.00	6.33	5.68	8.80	4.67
PWT-27	30.00	1.90	1.49	94.30	6.33	3.19	10.18	5.33
PWT-33	38.33	1.70	2.35	94.47	6.00	6.99*	10.12	7.00
PWT-35	30.00	1.22	1.60	95.43	4.67	6.27*	9.92	4.33
PWT-39	16.67	0.31	1.30	94.6	4.67	4.87	8.92	4.67
PWT-43	23.33	1.75	2.35	93.56	4.67	5.24	9.86	4.00
PWT-44	31.67	2.08	1.40	94.52	4.00	4.75	10.08	3.00
Lady Godiva	33.33	1.88	2.39	94.68	5.00	3.48	10.90	4.33
PCK-1	21.67	1.44	2.50	93.46	5.33	6.91*	5.64	8.00
$CD_{0.05}$	10.19	0.98	0.36	1.00	NS	2.38	2.04	2.16

*Significantly better than check variety Lady Godiva

26 and PWT-27 were found to have maximum ash content, *i.e.* 6.33%. The results are close enough to the total ash content (6.1%) of seed reported by Adeel *et al.* (1) and Ardabili *et al.* (5) in *C. Pepo* (5.34%), Alfawaz (2) in *C. maxima* (4.59%) and Shobha (19) in *C. mixta* (5.35%). Sugars are used as sweeteners to improve the palatability of foods and beverages and for food preservation. In this study, PWT-16 (7.47%) was found to have maximum sugar content followed by PWT-10 (7.28%) and PWT-3 (7.09%). Among all the genotypes, 11 were significantly better than Lady Godiva for sugar content. Starch is the storage form of carbohydrates in plants. PWT-24 (10.9%) was found to have maximum starch content followed by PWT-27 (10.18%) and PWT-15 (10.12%). However, 10 genotypes were significantly at par with Lady Godiva for starch content. Ardabili *et al.* (5) reported 25% carbohydrate contents in seeds of hull-less seeded pumpkin. Similarly, Adeel *et al.* (1) reported 25% and Elinge *et al.* (9) reported 28% carbohydrates content in seeds of *Cucurbita pepo*. Fibre containing food were known to expand the inside walls of the colon, easing the passage of waste, thus making it an effective anti-constipation, lowers cholesterol level in the blood and reduce the risk of various cancers. PCK-1 a hulled line has maximum (8.00%) fibre followed by PWT-25 (7.67%) and PWT-10 (7.33%). Among all genotypes, thirteen were significantly at par with Lady Godiva for fibre value. The results obtained for crude fibre contents in seeds of pumpkin were higher than 1.0% as reported by Elinge *et al* (9), which was less than the value (14.94%) reported

Table 3. Best performing seven hull-less seeded pumpkin genotypes for different traits.

Genotype	Seed yield (q/ha)	Oil content (%)	Oil yield (q/ha)	100-seed weight (g)	Fibre content (%)	Protein content (%)	Dry matter (%)	Ash content (%)	Total sugars (%)	Starch content (%)	Fruit yield (q/ha)
PWT-4	8.35*	38.67*	3.23*	9.27	4.50	2.27	94.17	4.67	5.97*	4.40	291.90*
PWT-20	8.16*	31.67	2.58	10.17*	6.33	2.20	94.07	4.67	6.28*	5.90	358.43*
PWT-10	7.90*	23.33	1.84	7.63	7.33	1.91	95.28	5.33	7.28*	3.35	381.35*
PWT-8	7.71*	28.33	2.18	6.65	5.33	1.88	95.71	4.33	5.54	2.08	267.12
PWT-43	7.52	23.33	1.75	7.05	4.00	2.35	93.56	4.67	5.24	4.93	304.90*
PWT-44	6.57	31.67	2.08	6.1	3.00	1.40	94.52	4.00	4.75	5.04	418.50*
PWT-2	6.52	31.67	2.06	7.99	5.00	2.39	95.50	5.00	6.10*	4.88	377.50*
Lady Godiva	5.65	33.33	1.88	9.08	7.00	2.39	94.68	5.00	3.48	10.89	241.73
$CD_{0.05}$	1.87	10.19	0.98	0.08	2.16	0.36	0.99	NS	2.38	2.04	45.42

*Significantly better than check variety Lady Godiva

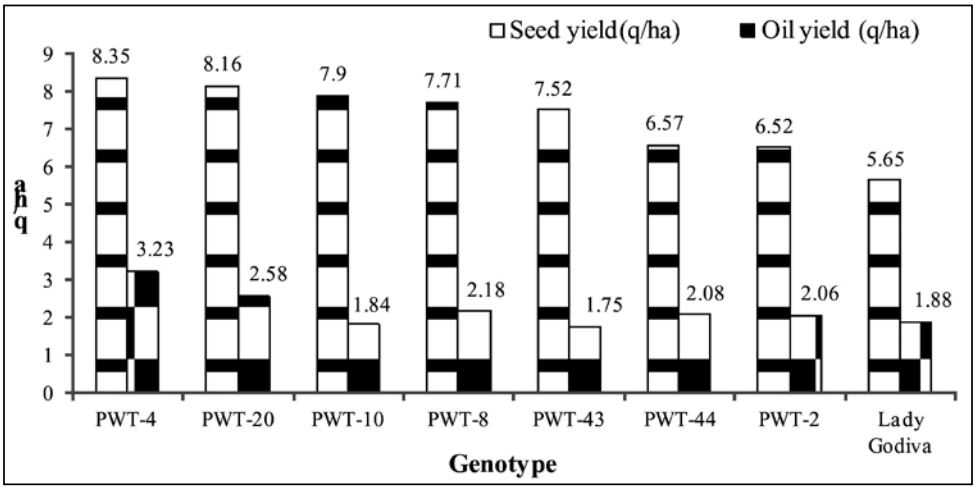

Fig. 1. Performance of best seven hull-less seeded pumpkin genotypes for seed and oil yield.

by Eman and El-Kinawy (10) and near to the value (2.49%) given by Ardabili *et al.* (5) in *Cucurbita pepo*. The fibre content of the pumpkin seeds was reported 2.5% by Adeel *et al.* (1) also.

Based upon seed yield, seven best hull-less seeded genotypes were short-listed and their performance for other quality parameters was compared (Table 3 and Fig. 1). PWT-4 gave highest seed yield (8.35 q/ha), oil content (38.67%), oil yield (3.23 q/ha), sugar content (5.67%) and fruit yield (291.9 q/ha). This genotype also has good size of seed (9.27 g/100 seed), protein content (2.27%) and starch content (4.4%). All these seven genotypes were significantly at par among themselves, but PWT-4, PWT-20, PWT-10 and PWT-8 were better than check variety Lady Godiva for hull-less seed yield. Therefore, these genotypes can further be evaluated for releasing them as varieties.

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Physiological and biochemical response of thermo-sensitive and tolerant tomato genotypes to high temperature stress

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ABSTRACT

High temperature stress induces considerable biochemical and physiological changes in the plants. The aim of the present investigation was to evaluate the physiological response of some selected tomato genotypes to high temperature stress. Twenty one diverse tomato genotypes collected from different sources were field evaluated at the Experimental Farm, Division of Vegetable Science, ICAR-IARI, New Delhi (2013 and 2014). Analysis of variance revealed substantial amount of genetic variability in the genotypes for all the traits. Relative water content (RWC) and membrane stability index (MSI) was recorded maximum in Pusa Sadabahar (83 and 86%, respectively) under heat stress condition. Highest proline content was recorded in wild genotypes, like SPM (*S. pimpinellifolium*) followed by SPR-1 (*S. peruvianum*). Tolerant genotypes like SPR-1 and SPM-2 showed the high value of chlorophyll *b* under heat stress condition as compared to sensitive genotypes. The highest phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were recorded for yield per plant followed by chlorophyll *b*, chlorophyll *a*, lycopene contents and total chlorophyll. High heritability coupled with high genetic advance as per cent over mean was recorded in yield per plant (98.84 and 79.30, respectively) followed by chlorophyll *a*, chlorophyll *b* and total chlorophyll content. This indicated the scope for improvement through simple selection for these traits.

Key words: Biochemical and physiological changes, heat stress, membrane stability index, *Solanum lycopersicum*.

INTRODUCTION

Tomato considered as a 'protective food' is being extensively grown as annual plant all over the world. It is mostly used for both fresh market and processing. It is an important source of vitamin A, vitamin C and lycopene. It is also an important source of ascorbic acid and β-carotene, which are potent antioxidants. In India, tomato is cultivated more extensively in comparison to other vegetables. The vegetative and reproductive processes in tomatoes are strongly modified by temperature alone or in combination with other environmental factors (Foolad, 8). When the ambient temperature exceeds 35°C, seed germination, seedling and vegetative growth, flowering, fruit set and fruit ripening are adversely affected (Wahid *et al*., 17). Heat stress also affects pollen grain viability, osmotic pressure, fruit set and yield (Saeed *et al.*, 15; Firon *et al*., 7). A critical analysis of the genetic variability is a prerequisite for initiating any crop improvement programme and for adopting of appropriate selection technique (Dhanwani *et al.*, 5). The genetic variability is determined with the help of certain genetic parameters, *viz.* genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and heritability estimates. For predicting the effect of selection, heritability estimates along with genetic advance are more useful than the heritability estimates alone (Johnson *et al*., 10). The present experiment was conducted with an objective to estimate the variability parameters on the basis of physiological and biochemical traits among the selected thermo-tolerant tomato genotypes.

MATERIALS AND METHODS

The field experiment was conducted for two consecutive years during summer (March-June) 2013 and 2014 at the Research Farm of Division of Vegetable Science, ICAR-IARI, New Delhi, which is located at 28°35 m N latitude and 77°12 m E longitude and at an altitude of 228.6 m above mean sea level. It has a semi-arid and sub-tropical climate characterized by extreme hot summer and cold winter. The experimental material consisted of 21 contrasting thermo-tolerant and diverse tomato genotypes (Table 1). These lines were selected based on past experiments and were grouped in heat tolerant or susceptible types. The experiment was laid out in randomized block design with three replications. All the recommended cultural practices were followed to raise a healthy crop. Five plants from each replicated plots were selected at random at the time of recording the data on various traits. Leaf samples were taken during the vegetative stage (when day temperature was more than 37°C and night temperature was more than 25°C) in the month of May-June (Table 4) for

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Table 1. Germplasm/ lines and standard released varieties used in the study.

Sl. No.	Genotype		Sl. No.	Genotype	
1.	Pusa Sadabahar	HT	12.	TH-348-4-R	HT
2.	Pusa Ruby	HS	13.	TH-348-4-2	HT
3.	Pusa 120	HS	14.	TH-348-4-5-1	HT
4.	Pusa Rohini	HS	15.	SPR-1*	HT
5.	Pusa Gaurav	HS	16.	SPR-2*	HT
6.	Pusa Sheetal	HT	17.	SPM**	HT
7.	Chico	HT	18.	SPM 1**	HT
8.	LP-2	HT	19.	SPM 2**	HT
9.	PSH-3	HT	20.	SPM 3**	HT
10.	TH-348-T2	HT	21.	SPM 4**	HT
11.	Balkan	HT			

HT = heat tolerant, HS = heat sensitive, * = *S. peruvianum*, ** = *S. pimpinellifolium*

various physiological studies like, membrane stability index (MSI) as described by Premachandra *et al*. (12). Relative Water Content (RWC) as suggested by Brass and Weatherley (3). Chlorophyll content of leaf (*a*, *b* and total) were estimated as suggested by Arnon (1), and total soluble solids content of fruit (TSS), ascorbic acid, acidity and lycopene content in the red ripe fruits were estimated according to the method described by Ranganna (13). Proline content was estimated as per the method described by Bates *et al*. (2).

The mean data were subjected to statistical analysis for estimation of variability, phenotypic and genotypic coefficients of variation (Burton and DeVane, 4), heritability (Falconer, 6) and genetic advance (Johnson *et al*., 10).

RESULTS AND DISCUSSION

Heat stress due to high ambient temperatures is a serious threat to crop production worldwide. Changes in seasonal temperature affect the crop yield, mainly through phenological development processes. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures. Selection of crops for tolerance to high temperature stress is proposed as the best and easiest strategy for breeding. The present study was carried out to evaluate the effect of high temperature on biochemical and physiological behaviour of tolerant and susceptible genotypes and to develop screening criteria for high temperature tolerance.

Analysis of variance showed highly significant difference between the genotypes for all the physiological and biochemical traits suggested thereby the substantial amount of genetic variability were existed in the materials under study (Table 2). The combined mean performance of 21 tomato genotypes for various biochemical traits are presented in Table 2. Relative water content (RWC) and membrane stability index (MSI) had high value under stress conditions in tolerant genotypes. All the genotypes recorded decreasing trend of RWC and MSI under heat stress conditions. However, the percent decrease in RWC and MSI was low in tolerant genotypes compared to heat sensitive genotypes. Heat tolerant genotypes, like Pusa Sadabahar recorded, the maximum values of RWC and MSI (83 and 86%, respectively) followed by SPR-1 and SPM (82 and 82.7%; 80 and 85% respectively) in both normal and heat stress conditions. In contrast to these, heat sensitive genotypes, like Pusa Ruby, Pusa 120, Pusa Rohini and Pusa Gaurav recorded low value of RWC and MSI (66 and 64%; 65 and 70.67%; 64.41 and 71%; 69 and 67%, respectively). Yadav *et al.* (18) found that at high temperature (27/37°C night/ day temperature) all the tomato genotypes (heat tolerant and susceptible) showed drastic and significant increase in MII, except Pusa Sadabahar. Pusa Ruby showed very high membrane injury index at high temperature. Low value of MII in tomato genotypes showed their tolerance to heat stress. Earlier Saeed *et al*. (15) recorded high thermostability (low membrane injury) in heat tolerant tomato genotypes.

Chlorophyll content of leaves, which is a vital component of photosynthetic activity in plant, was influenced significantly under heat stress conditions. Very high reduction in chlorophyll *a* and *b* was recorded in heat sensitive genotype, like Pusa Rohini (58 and 50%, respectively) as compared to heat tolerant genotype Pusa Sadabahar (11.5 and 28%, respectively). Tolerant genotypes showed high value of chlorophyll *b* under heat stress condition as compared to sensitive genotypes. It was emphasized from data that reduction in chlorophyll *b* is of prime importance, which give a better clue for its specific role in increasing tolerance to high temperature. Hence, relative reduction in chlorophyll *b* may be utilized as an indicator of down regulation of photosynthetic system in general. These results are in accordance with Hayat *et al*. (9) who reported that the tomato plants exposed to water stress exhibited a significant decline in photosynthetic parameters, MSI, leaf water potential, activity of nitrate reductase, carbonic anhydrase, chlorophyll and relative water content.

High chlorophyll *a*/*b* ratio was recorded in sensitive genotypes, like Pusa Ruby, Pusa 120, Pusa Gaurav (4.39, 4.17 and 4.46, respectively), while tolerant genotypes, namely, Pusa Sadabahar, LP2 and TH-348-T2 recorded low value of *a/b* ratio (3.87, 2.47 and 3.32, respectively). This showed that low value of *a*/*b*

Table 2. Mean performance of 21 thermo-tolerant tomato genotypes for physiological and biochemical traits under heat stress.

Genotype	RWC (%)	MSI (%)	Chl *a* (mg/g)	Chl *b* (mg/g)	Total chl (mg/g)	Chl *a/b* ratio	TSS (°Brix)	Lycopene (mg/ 100 g)	Ascorbic acid (mg/ 100 g)	Acidity (%)	Proline (µg/g)	Yield/ plant (g)
Pusa Sadabahar	83.36	86.00	1.50	0.40	1.90	3.87	5.33	2.35	21.40	0.42	347.33	685
Pusa Ruby	66.67	63.70	1.38	0.31	1.69	4.39	5.03	1.10	17.93	0.35	266.33	290
Pusa 120	65.06	70.67	1.04	0.22	1.22	4.17	5.20	1.24	17.33	0.33	280.42	280
Pusa Rohini	64.41	71.00	0.78	0.20	0.99	3.80	5.23	1.04	18.50	0.32	284.67	285
Pusa Gaurav	69.39	66.67	0.97	0.22	1.16	4.46	5.40	1.04	15.17	0.34	272.67	342
Pusa Sheetal	74.40	79.67	1.52	0.40	1.94	3.58	5.20	1.86	18.27	0.38	319.67	557
Chico	73.64	79.67	1.21	0.25	1.36	3.93	5.63	1.52	18.50	0.39	371.67	514
LP-2	75.07	82.33	0.94	0.38	1.32	2.47	6.37	2.09	15.73	0.36	356.53	610
PSH-3	78.17	76.00	0.69	0.19	0.96	3.84	5.77	1.88	15.20	0.44	357.01	548
TH-348-T2	75.82	80.67	1.43	0.43	1.81	3.32	5.90	1.59	13.90	0.34	395.33	542
Balkan	77.67	78.33	1.36	0.36	1.68	4.04	6.27	1.93	12.60	0.35	339.31	605
TH-348-4-R	76.42	81.67	1.36	0.34	1.72	3.84	5.37	1.71	18.33	0.37	354.01	594
TH-348-4-2	73.67	81.86	1.36	0.38	1.74	3.60	5.60	1.56	19.23	0.33	386.42	582
TH-348-4-5-1	79.14	83.33	1.09	0.40	1.51	2.58	6.30	1.83	23.13	0.35	394.17	568
SPR-1	82.67	82.67	1.54	0.44	1.98	3.51	7.00	1.85	21.27	0.30	398.32	152
SPR-2	77.33	85.00	1.90	0.36	2.27	5.25	7.67	2.08	22.17	0.40	385.67	160
SPM	80.87	85.67	1.36	0.39	1.72	3.79	8.07	2.13	24.37	0.45	417.33	170
SPM1	79.33	78.00	1.38	0.31	1.65	4.16	7.87	2.10	20.83	0.40	345.33	183
SPM2	76.03	79.67	1.31	0.44	1.66	2.94	7.70	1.97	20.47	0.34	352.67	180
SPM3	74.75	75.00	1.16	0.32	1.49	3.80	8.10	1.86	20.30	0.32	381.67	188
SPM4	72.66	76.67	1.59	0.41	2.00	3.90	8.27	1.88	21.07	0.37	381.39	190
Mean	75.07	78.30	1.28	0.34	1.61	3.77	6.35	1.74	18.46	0.36	351.81	391.60
CD at 5%	3.06	2.19	0.10	0.02	0.18	1.59	0.27	0.23	2.01	0.07	15.29	7.33
CV	2.44	1.67	6.81	9.90	6.52	9.32	2.53	7.99	6.53	11.40	2.60	0.70

ratio gives better tolerance under heat stress condition. Somkuwar *et al.* (16) under salt stress also reported that the low chlorophyll *a/b* ratio is an expression of large photosynthetic unit thereby increasing the light collecting capacity by a high content of light harvesting chlorophyll *a/b* protein complex.

There was no clear cut trend for TSS and acidity. However, slightly higher levels of TSS and acidity were recorded in tolerant genotypes under heat stress. TSS ranged from 5.03 (Pusa Ruby) to 8.27 (SPM-4). Acidity ranged from 0.3% (SPR-1) to 0.45% (SPM). Ascorbic acid content ranged from 12.60 mg/ 100 g in Balkan to 24.37 mg/100 g in genotype SPM under heat stress conditions. However, there was slight higher levels of ascorbic acid content in heat tolerant genotypes as compared to heat sensitive genotypes. Though high level of ascorbic acid and acidity is considered to give tolerance against heat stress. It was evident from the data that tolerant genotypes SPM showed higher value of acidity and ascorbic acid under heat stress. Lycopene content reduced significantly under heat stress conditions in all the genotypes under study. The tolerant and susceptible genotypes could not be distinguished on the basis of TSS and lycopene content as it could not mark significant differences in their values under normal and heat stress conditions in susceptible and tolerant genotypes.

Proline is the key osmolytes contributing towards osmotic adjustment. It can also improve stress tolerance by protecting and stabilizing membrane and enzymes during stress condition (Rudoplh *et al*., 14). Proline content was significantly influenced with

Table 3. Mean, range, PCV, GCV, heritability (h_2), genetic advance (GA) and genetic advance as per cent over mean of physiological and biochemical traits of 21 thermo-tolerant genotypes of tomato under heat stress.

Trait	Mean	Range		PCV	GCV	Heritability	GA	GA as % over mean
		Min.	Max.					
RWC (%)	75.07	64.41	83.36	7.24	6.82	88.63	9.93	13.23
MSI (%)	78.3	63.7	86.0	7.85	7.67	95.45	12.09	15.44
Chl *a* (mg/g)	1.28	0.69	1.90	22.71	22.26	96.15	0.58	44.97
Chl *b* (mg/g)	0.34	0.19	0.44	24.40	24.00	96.71	0.17	48.62
Total chl (mg/g)	1.61	0.96	2.27	21.94	20.95	91.16	0.66	41.20
Chl *a/b* ratio	3.77	2.47	5.25	18.06	15.47	73.36	1.03	27.29
TSS (°Brix)	6.35	5.03	8.27	18.13	17.95	98.05	2.32	36.61
Lycopene (mg/100 g)	1.74	1.04	2.35	22.46	20.99	87.35	0.70	40.41
Ascorbic acid (mg/100 g)	18.46	12.6	24.37	18.36	17.16	87.38	6.10	33.04
Acidity (%)	0.36	0.3	0.45	14.55	8.85	36.97	0.04	11.08
Proline (µg/g)	351.81	266.33	417.33	12.75	12.48	95.83	88.56	25.17
Yield/ plant (g)	391.67	152.00	685.00	39.15	38.79	98.84	308.01	79.30

Table 4. Standard meteorological months average weather data during March-June.

Weather parameter month	March		April		May		June	
	2013	2014	2013	2014	2013	2014	2013	2014
Max. temp. (°C)	26.9	29.8	34.8	36	38.7	41.9	41.7	37.0
Min. temp. (°C)	12.7	13.6	18.0	19.3	22.6	24.9	26.6	26.6
Average temp. (°C)	19.8	21.7	26.3	27.7	30.6	33.4	34.1	31.8
Relative humidity max (%)	69.1	87	56.8	67.2	58.4	52.3	53.1	80.1
Relative humidity min (%)	38.5	35.3	29.1	27.5	28.5	25.7	27.8	56.6
Rainfall (mm)	63.5	0	16.4	0.07	79.6	0	59.6	5

increase in temperature. The highest proline content was recorded in wild genotypes SPM (417 µg/g) followed by SPR-1 (398 µg/g) and TH-348-4-5-1 (394 µg/g). Hence, level of proline could be used as index for determining heat tolerance in tomato.

Phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) in all traits (Table 3). High value of PCV and GCV were recorded for yield per plant (39.15 and 38.79), chlorophyll *a* (22.71 and 22.30), chlorophyll *b* (24.40 and 24.00), total chlorophyll (22 and 21) and lycopene content (22 and 21). In accordance to our finding, Joshi *et al*. (11) also observed low value of PCV and GCV for TSS.

A critical perusal of data showed that proline content and MSI had very less difference in PCV and GCV, showing that variation in these traits were mainly due to genotypes and these traits were less affected by environment. However, very wide differences in PCV and GCV were recorded in acidity showed that environment played a major role in total variation rather than genotypes itself. Heritability (h^2) in broad sense was found high in most of the traits. Yield per plant recorded maximum heritability (98.84%) followed by TSS (98%), proline (96%) and MSI (95%).

Heritability alone gives information regarding magnitude of inheritance of the traits, but not the amount of genetic progress that would result from selecting the best individual. Therefore, a suitable selection procedure can be followed only when the broad sense heritability is coupled with high genetic advance. The genetic advance measures the genetic gain after selection. High heritability coupled with high genetic advance as per cent over mean was recorded in yield per plant, chlorophyll *b*, chlorophyll *a* and total chlorophyll contents. This indicated the scope for improvement through simple selection for these traits and it may be highly effective as these traits are less influenced by environment. Similarly a joint consideration of heritability, GCV and genetic advance

revealed high value for yield per plant, chlorophyll *b*, chlorophyll *a* and total chlorophyll content.

Hence, based on findings it could be concluded that genotypes Pusa Sadabahar, TH-348-T2 and LP-2 recorded low value of chlorophyll *a/b* ratio indicating better tolerance under heat stress condition. Whereas, RWC and MSI under heat stress conditions were recorded the maximum in Pusa Sadabahar, SPR-1 and SPM. Pusa Sadabahar and SPM were found most tolerant genotypes from cultivated and wild accessions, respectively. Therefore, they could produce significantly higher yield as compared to heat sensitive genotypes under stress, which can be utilized for further crop improvement programme.

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Evaluation of physiological and yield traits in cowpea for screening of drought tolerance lines

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ABSTRACT

Drought (soil and/ atmospheric water deficit) is the most environmental constraints among abiotic stresses. Cowpea is inherently more drought tolerant than other vegetables, but it also suffers to a considerable yield loss when the moisture deficit is imposed during flowering and pod setting. The experiment was carried out at IIVR, Varanasi during spring-summer of 2012 and 2013. A total of 29 diverse cowpea genotypes, comprising of vegetable and grain types were selected for study. Drought stress was imposed 35 days after sowing by withholding the irrigation for 25 days. All genotypes were also kept under well watered control. Experimental findings revealed that under drought stress, some genotypes, *viz.*, EC-30590, EC-37988, EC-390241, EC-15296, EC-472283 and Gomti expressed significantly higher relative leaf water content (>80%), photosynthesis (14.7 to 18.2 µmol m^{-2} s^{-1}), stomatal conductance (0.443 to 0.818 µmol m^{-2} s^{-1}), quantum yield of PSII photochemistry, *i.e. Fv*/ *Fm* (0.467 to 0.727) and transpiration rate (0.0321 to 0.0467). These genotypes also showed less yield and dry matter reduction under drought stress as compared to susceptible cultivars/genotypes. The commercial cultivars such as, Akra Garima, Kashi Nidhi, Kashi Shyamal and Kashi Kanchan were found more susceptible to drought. It may concluded that genotypes EC-30590, EC-37988, EC-390241, EC-15296, EC-472283 and Gomti were fairly drought tolerant, and may be utilized for cultivation under water limited condition or for breeding of drought tolerant cultivars.

Key words: *Cowpea*, *Vigna unguiculata*, gas exchange, physiological traits, drought tolerance.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is an important legume vegetable and pulse crop mostly grown in the arid and sub-arid zones of the tropical world where the production mostly depends upon rain or water supply. Its green pod is a good source of protein, mineral and dietary fibers in many developing countries. Abiotic stresses are the primary cause of crop loss worldwide, and are responsible for over 50% reduction in agricultural production (Wang *et al*., 15). Among the abiotic stresses, drought causes around 17% of total losses. Cowpea is inherently more tolerant to drought than other vegetables (Singh *et al*., 14); however, it is sensitive to drought, particularly during pod set and pod filling (Garg *et al*., 8; Abayomi and Abidoye, 1). During the vegetative phase, cowpea react to drought by limiting growth and reducing leaf area, changing leaf orientation and closing the stomata, whereas during flowering and podding, drought causes flower and pod abscission. During drought stress, plant experience a number of physiological and metabolic changes such as, reduction of photosynthetic activity, accumulation of organic acids and osmolytes, and changes in carbohydrate metabolism. Cowpea exhibits broad adaptation mechanism to drought such as drought escape, drought avoidance by decreasing leaf area, dehydration avoidance and vegetative stage drought tolerance by delaying leaf senescence (Hall, 10). Significant genotypic variations in cowpea have been observed on leaf gas exchange and yield parameters, which can give some indications of superiority among cowpea genotypes for agronomic fitness under drought (Anyia and Herzog, 2; Abayomi and Abidoye, 1). The objective of this study was to evaluate the dynamics of photosynthetic and yield parameters in 29 diverse cowpea genotypes (vegetable or pulse type) under well watered and drought stress condition, and to identify genotype(s) suitable for growing under limited water condition or utilization of such genotypes for evolving drought tolerant cultivars in vegetable type cowpea.

MATERIALS AND METHODS

A field experiment was carried out at Indian Institute of Vegetable Research, Varanasi during spring-summer of 2012 and 2013. A total of 29 diverse cowpea genotypes, mostly erect bushy types comprising both vegetable and pulse types were taken for study. Seeds of cowpea were sown on 5th March each year in flat beds at row-to-row spacing of 30 cm and plant-to-plant 20 cm. Drought stress (DS) was induced before flower initiation (35 days

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after sowing, DAS) by maintaining constant low soil water potential for 15 days (withholding irrigation for 20 days). All genotypes were also kept under well watered (WW) control, wherein irrigation was applied at 5-6 day intervals. The average values of weather parameters during the experiment period (5th March to 5th June) in 2012 and 2013 were- maximum temperature 38.7°C, minimum temperature 21.9°C; maximum RH 71%, minimum RH 26%; sunshine hour 7.8, open pan evaporation 10.1 mm and no rainfall. The average soil moisture content before release of the stress was 3.8, 7.4 and 8.8%, respectively in 0-15, 15-30 and 30-45 cm depth. Moisture content (25 cm depth) at field capacity (-0.33 bars) and permanent wilting point (-15 bars) was 22.3 and 6.8%, respectively. Soil moisture and temperature was recorded at 25 cm depth with soil moisture and temperature sensor (Decagon Devices, Pullman, WA, USA).

Gas exchange parameters (photosynthesis, stomatal conductance and transpiration) were measured with portable photosynthesis system (LICOR 6200, Lincoln, Nebraska, USA), and chlorophyll fluorescence (F_v/F_m) was measured in 20 min. dark adapted leaves by Plant Efficiency Analyzer (Hansatech Instrument Co. Norfolk, UK). These measurements were made on the 3rd or 4th fully expanded leaves from the apex between 10:00 and 12:00 h, just before release of drought stress (on the 15th day of stress). Gas exchange parameters, chlorophyll fluorescence, relative water content in leaf (RWC) and plant canopy temperatures were recorded at 55 DAS. The plant canopy temperature was recorded by Infra-red gun between 14:00 to 15:00 h at 3-4 days intervals started 5 days after irrigation. Total dry matter (TDM) production and yield were worked out between 55-75 DAS from three plants in each genotype.

RESULTS AND DISCUSSION

A total of 29 cowpea genotypes were taken for study; however, in this paper the results have been presented for six most tolerant and four most susceptible lines/ genotypes to drought stress. The rest of the genotypes showed intermediately or mixed response towards drought. Gas exchange or photosynthetic parameters such as photosynthesis rate, stomatal conductance, transpiration, and fluorescence in term of quantum yield of PS-II photochemistry (*Fv*/*Fm*) were significantly varied both under stressed and non-stressed conditions (Table 1). All photosynthetic parameters declines in all genotypes of cowpea as drought stress induced, but the reduction in EC-30590, EC-37988, EC-390241, EC-15296, EC-472283 and Gomti genotypes were less as compared to other cultivars. Under drought stress, these genotypes expressed significantly higher photosynthesis (14.7 to 18.2 µmol m^{-2} s^{-1}), stomatal conductance (0.654 to 0.761 mol m^{-2} s^{-1}), transpiration rate (0.0385 to 0.0467 mol m^{-2} s^{-1}) and F*v*/F*m* (0.625 to 0.727); whereas commercial cultivars such as Arka Garima, Kashi Nidhi, Kashi Shyamal and Kashi Kanchan exhibited sharp reduction in these photosynthetic traits. Under drought stress condition, the least reduction in photosynthetic rate (10%), stomatal conductance (31%), transpiration (10%) and F*v*/F*m* (8%) was observed in EC-15296 followed

Table 1. Effect of drought stress on gas exchange and fluorescence parameters in cowpea.

Line/ genotype	Photosynthesis (µmol m^{-2} s^{-1})		Stomatal conductance (mol m^{-2} s^{-1})		Transpiration (mol m^{-2} s^{-1})		F*v*/F*m*	
	WW	DS	WW	DS	WW	DS	WW	DS
EC 30590	24.0	17.3	1.404	0.818	0.0551	0.0467	0.788	0.625
EC 37988	17.6	10.3	0.968	0.443	0.0430	0.0321	0.707	0.467
EC 390241	19.6	16.5	1.079	0.754	0.0440	0.0395	0.825	0.705
EC 15296	20.3	18.2	1.205	0.827	0.0515	0.0415	0.791	0.727
EC 472283	21.4	14.7	1.019	0.713	0.0507	0.0385	0.812	0.725
Gomti	19.2	14.8	0.994	0.761	0.0414	0.0392	0.771	0.544
Arka Garima	14.5	9.3	0.855	0.297	0.0471	0.0291	0.581	0.389
Kashi Nidhi	19.3	4.6	1.303	0.220	0.0410	0.0266	0.450	0.418
Kashi Shyamal	18.6	6.3	1.212	0.196	0.0415	0.0185	0.712	0.377
Kashi Kanchan	21.7	4.4	1.250	0.177	0.0421	0.0221	0.728	0.391
$CD_{0.05}$	2.13	1.27	0.240	0.108	NS	0.0132	0.068	0.056

by EC-390241; whereas the maximum reduction in photosynthesis (80%), stomatal conductance (86%), transpiration (47%) and F*v*/F*m* (46%) as compared to WW was observed in cultivar Kashi Kanchan followed by Kashi Nidhi.

In corroborate to our findings, earlier Anyia and Herzog (3) also reported that drought stress caused a reduction in the leaf assimilation rate, transpiration rate and stomatal conductance in cowpea with genotypic variances of 75.4, 57.9 and 83.3%, respectively. According to them, drought tolerant genotypes maintained higher RWC or leaf water potentials by stomata closure and reduction in leaf area. In our study also the tolerant genotypes showed only 6.0 to 9.2% decline in RWC under DS conditions, while susceptible cultivars exhibited a sharp decline (18.4 to 23.3%) in RWC as compared to WW (Fig. 1). Findings of Hamidou *et al.* (11) revealed that the cowpea genotypes showing drought avoidance mechanism by decreasing the stomatal conductance and transpiration. They also reported that accumulation of solutes mostly proline and maintenance of total protein may contribute for turgor maintenance and protection of photosynthetic apparatus (PS II) against denaturation during water deficit. Reductions in leaf water potential as a consequence of drought positively correlated with a decline in assimilation rate, which is associated with stomatal closure. In our study, tolerant genotypes maintained relatively higher leaf water content (>75%) before release of stress than the susceptible genotypes (68-71%). Drought induces an array of morphological, physiological, biochemical and molecular responses, in which photosynthesis is one of the primary physiological target (Chaves, 6). Furthermore, relatively higher values of F*v*/F*m* in tolerant cowpea genotypes may be due to the increased activity and concentration of superoxide dismutase isoforms (Mn-SOD and Fe-SOD) induced by water deficit, which is associated with protection of photosystem II photochemistry and whole plant growth against oxidative stress (Brou *et al*., 5).

Canopy temperature is an important trait to work out the crop water stress index (CWSI) as it is the relationship between canopy-air temperature difference and the air vapour pressure deficit. Gonzalez-Dugo *et al.* (9) demonstrated that canopy temperature variability may be used an indicator for drought stress severity, particularly for low and moderately stressed crops. In our study, canopy temperatures of stressed and non-stressed plant varied significantly among the cowpea genotypes (Fig. 2). Genotypes such as EC-30590, EC-37988, EC-390241, EC-15296, EC-472283 and Gomti (tolerant to drought stress) registered an average increase in canopy temperature by 1.5°C, while in susceptible cultivars an increase by 2.5°C was recorded over respective WW plants. Soil temperatures recorded at 20 cm depth revealed that it varied significantly in

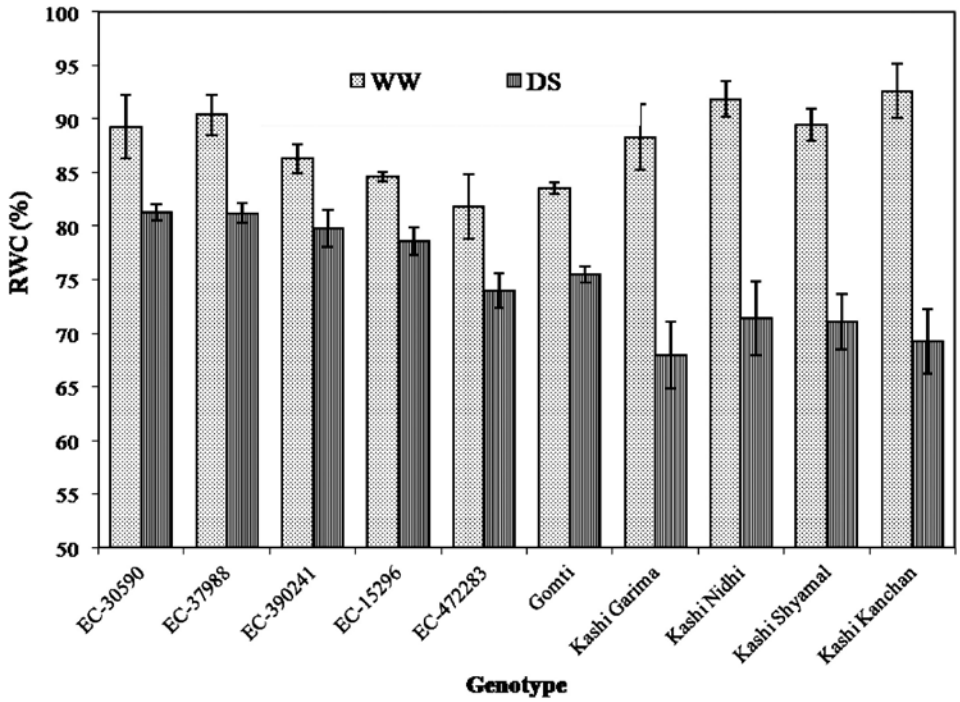

Fig. 1. Effect of drought stress on relative leaf water content in cowpea genotypes.

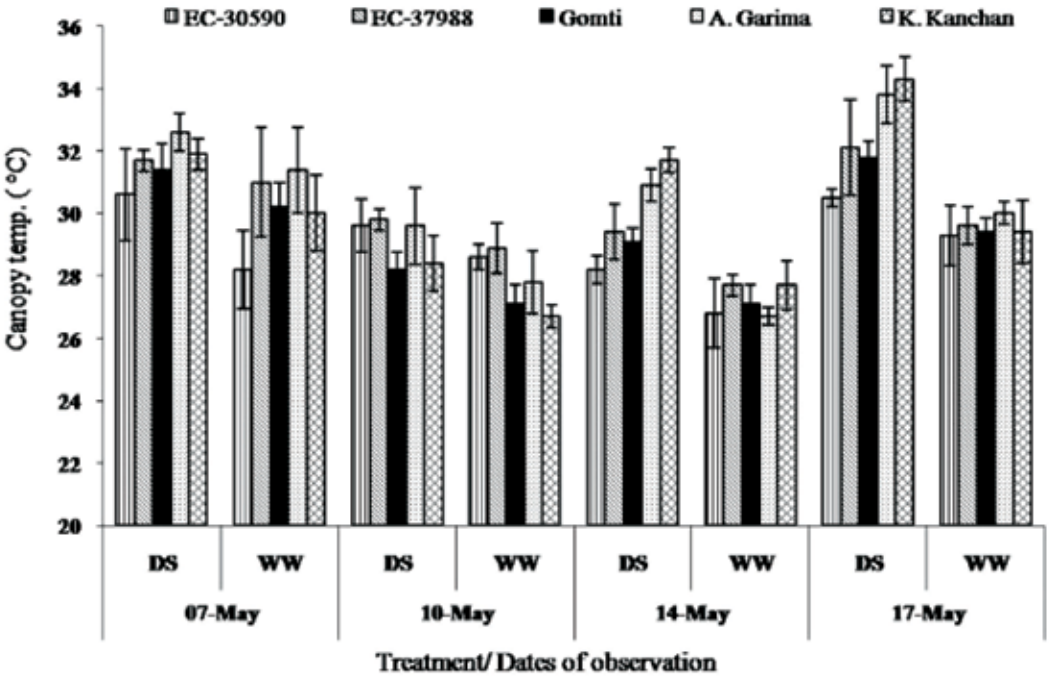

Fig. 2. Effect of drought stress on plant canopy temperature in cowpea genotypes.

stressed *vis-a-vis* non-stressed plots, but did not vary among the cowpea genotypes. The average mid-day soil temperatures in WW plot was 32.4°C (n = 9), while in DS plots it was 37.7°C.

Leaf /canopy temperature has been related to crop and soil water stress based on the fact that under stress-free conditions the water transpired by the plants evaporates and cools the leaves, while in water-deficit situation little water is transpired thus, leaf temperature increases. When the plant evapotranspiration (ET) rate is reduced, such as by soil water depletion, the rate of heat removal is reduced and the canopy temperature increases. This process links canopy temperature with crop water stress and ET. Colaizzi *et al.* (7) also showed that canopy temperature is strongly correlated to important quantifiable crop outputs such as yield, water use efficiency, seasonal ET, midday leaf water potential and irrigation rates.

Yield attributes such as TDM and pod yield were also varied significantly both under WW and DS conditions. It is obvious from Table 2 that TDM production under WW ranged between 87.54 (Arka Garima) to 188.68 g (Gomti), while in DS condition it ranged between 68.2 (Kashi Kanchan) to 168.60 g (Gomti). A significant reduction in TDM (28-43%) was observed in susceptible genotypes, whereas only 7-19% reduction in biomass under drought stress was observed in tolerant genotypes. Similar trends were also noticed in pod production. Both under WW and DS conditions, the susceptible or otherwise commercial cultivars have produced higher yields than the tolerant genotypes which were obliviously due to their higher genetic yield potentials. The cowpea genotypes that showed tolerance to drought in this study were either pulse grain type or shy bearing. Decline in yield due to drought stress was less (about 11%) in tolerant genotypes than in susceptible genotypes (53% reduction). Anyia and Herzog (2) reported that across the cowpea genotypes water deficit condition caused reduction in biomass between 11 to 40%. Similar to our findings, Bastos *et al.* (4) also reported that a water deficit reduced the yield of cowpea genotypes to the tune of 60% as compared to well irrigated plants.

Maintenance of high leaf turgidity, net photosynthetic rate and stomatal conductance during stress period along with less alteration in leaf metabolites in the tolerant genotypes were reflected in its yield compared to other lines/ genotypes. Mendes *et al.* (13) revealed that the water deficit condition did not influence the source capacity (leaf number, leaf area and specific leaf area) and reproductive efficiency, but reduced the sink size (number of pods, number and weight of seeds per plant) in cowpea. According to Likoswe and Lawn (12) cowpea maintains leaf water status above lethal levels for longer periods through different means, and cowpea

Table 2. Effect of drought stress on biomass production and yield in cowpea genotypes.

Variety/ Genotype	Total dry matter (g/ plant)		Single pod weight (g)		Pod yield/ plant (g)	
	WW	DS	WW	DS	WW	DS
EC 30590	88.12	71.30	6.41	6.15	70.6	65.4
EC 37988	115.23	114.13	3.90	3.31	80.0	60.5
EC 390241	180.50	158.44	4.47	4.67	47.6	42.0
EC 15296	156.80	132.42	3.20	2.76	73.3	64.3
EC 472283	134.40	124.54	3.87	3.67	67.3	65.6
Gomti	188.68	168.60	3.91	3.57	125.5	115.0
Arka Garima	87.54	49.41	5.67	4.47	190.3	90.6
Kashi Nidhi	118.20	84.60	7.97	7.35	220.3	135.5
Kashi Shyamal	134.40	85.60	7.55	6.80	225.7	105.0
Kashi Kanchan	96.22	68.20	9.80	8.67	283.5	88.6
SEm ±	3.25	2.44	0.31	0.27	2.11	1.85
$CD_{0.05}$	8.91	7.06	0.95	0.82	5.60	4.87

produced higher TDM under water deficit as compared to soybean and pigeon pea.

In conclusion, the genotypic variations in the gas exchange measurements, relative leaf water content RWC), canopy temperatures and yield traits were observed in this study indicates that cowpea genotypes EC-30590, EC-37988, EC-390241, EC-15296, EC-472283 and Gomti have shown tolerance against drought, and may be utilized for breeding drought tolerant cultivars in bush type vegetable cowpea. These genotypes showed significantly higher photosynthetic traits, RWC, biomass and yield, and relatively lower canopy temperatures than the susceptible or otherwise commercial cultivars.

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Growth and yield performance of cauliflower as influenced by NPK fertilization combinations under Western plain zones of Uttar Pradesh

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ABSTRACT

The present investigation was carried out during 2016-2017 to delineate the best performing combinational doses of NPK on two cauliflower varieties (Pusa Snowball K-1 and Pusa Himjyoti). Four different combinations of NPK kg ha^{-1}, *viz.* T0 = 0:0:0, T1 = 80:30:60, T2 = 120:60:80 and T3 = 150:70:100 NPK with three replications using two-way factorial analysis. Results revealed that NPK application of 120:60:80 kg ha^{-1} was found to be performing better for yield contributing characters of cauliflower var. Pusa Snowball K-1 with maximum plant weight (1.50 kg/ plant), net curd weight (659 g/ plant), curd diameter (13.33 cm), curd height (10.12 cm), stalk length (3.25 cm) and curd yield per plot (14.29 kg/plot), whereas, treatment T3, when applied on Pusa Snowball K-1 resulted in better growth in terms of plant height (37.16 cm), plant spread (47.23 cm) and number of leaves (14.93). The study also revealed that ascorbic acid as well as β-carotene contents in curds showed a decreasing trend with increasing level of NPK inputs irrespective of variety. Under Western plain zones of Uttar Pradesh better performance of Pusa Snowball K-1 with NPK application of 120:60:80 kg ha^{-1} could be suitable combination for cauliflower cultivation.

Key Words: Cauliflower, curd quality, growth, Pusa Himjyoti, Pusa Snowball K-1, yield.

INTRODUCTION

Cauliflower being a heavy feeder of nutrients requires balanced and sufficient supply of nutrients for better growth and higher yield. Hence, cauliflower production needs efficient nutrient management for achieving maximum yield. Among various factors responsible for low production of cauliflower, nutrition is of prime importance. Among the major nutrients, nitrogen is the main limiting nutrient in cauliflower along with other micro-nutrients (Ali *et al*., 1). An adequate supply of nitrogen is associated with vigorous vegetative growth. Phosphorus (P) is important not only for floral initiation but also influence the plant growth. It is an essential constituent of many vital compounds. An adequate supply of phosphorus tends to counter the deleterious effects of an excess of N. It hastens maturity, improves fruit quality, favours root growth and may increase disease resistance in plants. Potassium plays an important role in the water economy of plants and reduces the tendency to wilt. It also hardens supporting tissues and thereby reduces lodging. It may reduce susceptibility to disease and it improves the quality of fruits and other storage organs like swollen roots and tubers. A balanced N to K ratio is particularly important in plant nutrition, as K tends to reduce the adverse effects of excessive N. Balanced dose of nitrogenous, phosphatic and potassic fertilizers is required to increase crop productivity without any adverse effect on environment. The present investigation was conducted at ICAR-IIFSR during 2016-17 to find out the ideal level of nutrients on growth, yield and quality of cauliflower var. Pusa Himjyoti and Pusa Snowball K-1.

MATERIALS AND METHODS

The present investigation was carried out on cauliflower (*Brassica oleracea* L. var. *botrytis*) following two-way factorial analysis with four different combinations of NPK, two cultivars Pusa Snowball K-1 and Pusa Himjyoti with three replications for each treatment at Siwaya Farm of ICAR-IIFSR, Modipuram, Meerut during 2016-17. The experiment comprised of four combinations of NPK, *viz.*, control T0 (0:0:0), T1 (80:30:60 kg ha^{-1}), T2 (120:60:80 kg ha^{-1}) and T3 (150:70:100 kg ha^{-1}). The soil of the experimental plot was sandy loam with pH 7.57, organic carbon 0.55%, available N content 118.54 kg ha^{-1}, available P_2O_5 content 11.83 kg ha^{-1} and available K_2O 154.61 kg ha^{-1}. Five-week-old seedlings of cauliflower var. Pusa Snowball K-1 and Pusa Himjyoti were transplanted at a spacing of 60 cm x 60 cm there by accommodating 24 plants in a plot size of 10 m^2 (5 m × 2 m). Half dose of nitrogen, full dose of phosphorus and potash were applied in experimental plots and thoroughly mixed in soil before transplanting. Remaining half dose of nitrogen was applied one month after transplanting. All the crop

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management practices were adopted during cropping season. Observations on growth, yield and quality of cauliflower were recorded as per the standard procedures. Before and after harvest of the crop, soil samples from the depth of 0-15 cm were collected from each plot. Samples were dried, grinded, mixed thoroughly and used for determination of available NPK nutrient contents using standard methods, *viz*. nitrogen by alkaline potassium permanganate method (Subbiah and Asija, 13), phosphorus by Olsen's method (Olsen *et al*., 8) and potassium by ammonium acetate method using flame photometer (Hanway and Haidal, 4). The results obtained were subjected to two-way factorial (Factor 1: 4 levels of NPK: Factor 2: 2 varieties) analysis using SPSS software (ver. 16.0). Means of different treatments and their interactions were compared using Duncan multiple range test (DMRT) at 5% level of significance (Steel and Torrie, 12).

RESULTS AND DISCUSSION

The results presented in Table 1 depicts the effect of different treatments on growth characteristics and yield attributes of two cauliflower varieties. Plant height of at 30 DAT (days after transplanting) and plant spread at 30 and 45 DAT were significantly influenced by NPK fertilization. The maximum plant height of 23.43 cm at 30 DAT and 33.81 cm at 45 DAT has been recorded for treatment T3, whereas the minimum plant height of 18.40 and 29.33 cm were recorded for control (T0), respectively at 30 and 45 DAT. Among the two varieties, Pusa Snowball recorded better growth attributes than Pusa Himjyoti. Significantly higher values of plant height and plant spread at 30 and 45 DAT were recorded in Pusa Snowball K-1 as compared to Pusa Himjyoti. Pusa Pusa Snowball K-1 registered plant height of 24.67 cm at 30 DAT and 34.71 cm at 45 DAT. Combined effect of different NPK levels and variety on plant height at 30 and 45 DAT revealed significant interaction effect. Pusa Snowball K-1 with the application of T2 @120:60:80 recorded the maximum plant height (25.71 cm) at 30 DAT and T3 @150:70:100 recorded the maximum plant height of 37.16 cm at 45 DAT (Table 1). This might be due to the higher availability of nutrients, which promoted more vegetative growth in the plants receiving higher doses of NPK. Similar findings have been recorded by Verma and Yadav (15) in cauliflower and Choudhary and Choudhary (2) on cabbage; and Mahmud *et al*. (7) on broccoli.

The results also revealed the significant effect of NPK level on plant spread. The maximum plant spread of 27.79 cm at 30 DAT and 42.22 cm at 45 DAT has been recorded for treatment T3. Comparison of both the varieties in terms of plant spread at 30 and 45 DAT suggested significant higher values for Pusa Snowball K-1 over Pusa Himjyoti had significant difference for plant spread at 30 and 45 DAT (31.24 cm at 30 DAT and 45.06 cm at 45 DAT). Significant interaction effect of variety and NPK level was observed for plant spread. The results revealed the significant effect of NPK level on number of leaves at 30 and 45 DAT and plant stand (%) at harvest (Table 1). The highest number of leaves (11.57) has been recorded for treatment T3 at 30 DAT and 14.13 at 45 DAT. Pusa Snowball K-1 recorded higher values for number of leaves (12.65 at 30 and 13.89 at 45 DAT) as compared to Pusa Himjyoti (8.63 at 30 and 12.27 at 45 DAT). Significant interaction effect of variety and NPK level was observed in number of leaves, where higher number of leaves (13.96 at 30 DAT and 14.93 at 45 DAT were recorded for treatment T3, which were at par with T2. Similar results were also recorded for plant stand with highest value (92.13%) for treatment T2 and lowest (86.34%) for control (Table 1). This could be attributed to enhanced vegetative growth and subsequent higher yield. The results are in line with the findings of Kodithuwakku and Kirthisinghe (5) and Prasad *et al*. (9).

Main and interaction effect of variety as well as different levels of NPK on yield performance of cauliflower was studied. There was significant main and interaction effect of NPK level as well as variety on gross plant weight, net curd weight, curd diameter, curd height, stalk length and curd yield in the present study (Table 2). Treatment T2 recorded maximum gross plant weight (1.41 kg) and net curd weight (497.45 g). Among the two varieties, Pusa Snowball K-1 was better than Pusa Himjyoti and recorded higher gross plant weight (1.37 kg) and net curd weight (553.45 g). Maximum gross plant weight (1.50 kg) and net curd weight (659.00 g) was recorded for Pusa Snowball K-1 with treatment T2. The results revealed that yield attributes of both cauliflower varieties up to NPK supplementation at 120:60:80. However, further increase in NPK levels had negative impact. These results are also in close conformity with the findings of Mahmud *et al*. (6), El-All and Shabrawy (3) and Singh *et al*. (11) on brocooli. The result indicated that the treatment receiving high dose of nitrogen fertilizer resulted in higher vegetative growth, while at optimum doses of nitrogen, phosphorous and potash resulted in reproductive growth in terms of higher curd yield, curd weight, curd diameter, curd height and stalk length.

Table 1. Effect of different NPK combinations on growth attributes of cauliflower.

Treatment#	Plant height		Plant spread		No. of leaves		Plant stand (%)
	30 DAT	45 DAT	30 DAT	45 DAT	30 DAT	45 DAT	
NPK level							
T0 (control)	18.40^{a}	29.33^{a}	23.93^{a}	38.23^{a}	9.95^{a}	12.52^{a}	86.34^{a}
T1	21.06^{b}	30.76^{b}	25.95^{b}	39.21^{a}	10.48^{b}	12.65^{a}	88.89^{b}
T2	23.16^{c}	32.90^{c}	27.44^{c}	41.27^{c}	10.55^{b}	13.03^{b}	92.13^{c}
T3	23.43^{c}	33.81^{c}	27.79^{c}	42.22^{c}	11.57^{c}	14.13^{c}	87.35^{a}
SEM±	0.17	0.32	0.26	0.36	0.11	0.11	0.35
Variety							
V1 (Pusa Snowball) K-1	24.67^{b}	34.71^{b}	31.24^{b}	45.06^{b}	12.65^{b}	13.89^{b}	89.39^{b}
V2 (Pusa Himjyoti)	18.35^{a}	28.69^{a}	21.32^{a}	35.40^{a}	8.63^{a}	12.27^{a}	87.96^{a}
SEM±	0.12	0.23	0.18	0.25	0.04	0.08	0.25
NPK level × variety							
V1T0	23.33^{e}	32.53^{d}	29.06	42.23	11.77^{d}	13.52	87.04
V1T1	24.26^{f}	32.71^{d}	30.70	43.94	12.42^{e}	13.35	89.82
V1T2	25.71^{g}	36.43^{e}	32.49	46.82	12.44^{e}	13.74	93.06
V1T3	25.39^{g}	37.16^{e}	32.71	47.23	13.96^{f}	14.93	87.67
V2T0	13.46^{a}	26.12^{a}	18.81	34.22	8.13^{a}	11.52	85.65
V2T1	17.86^{b}	28.80^{b}	21.20	35.48	8.55^{ab}	11.94	87.96
V2T2	20.61^{c}	29.37^{bc}	22.38	35.71	8.66^{b}	12.31	91.21
V2T3	21.46^{d}	30.46^{c}	22.87	37.21	9.19^{c}	13.32	87.04
SEM±	0.24	0.46	0.36	0.50	0.15	0.16	0.49
ANOVA, P>F							
NPK Level	**	**	**	**	**	**	**
Variety	**	**	**	**	**	**	**
NPK level × Variety	**	*	NS	NS	**	NS	NS

Mean values (*P ≤ 0.05, **P ≤ 0.01, NS = Non significant); Data expressed as Mean of triplicate measurements (n = 3); #T0 = 0:0:0, T1 = 80:30:60, T2 = 120:60:80 and T3 = 150:70:100

The effect of NPK level on curd diameter, curd height and stalk length was reported to be significant. Application of treatment T2 resulted in maximum values of curd diameter (11.64 cm), curd height (8.54 cm) and stalk length (2.71 cm), whereas treatment T0 (control) resulted in the lowest value of curd diameter (10.06 cm), curd height (7.67 cm) and stalk length (2.11 cm). However, the interaction of levels of NPK and variety was recorded to be non-significant for these traits. The highest curd yield (10.92 kg/ plot) was recorded from treatment T2 followed by treatment T3 (9.43 kg/ plot). Among the interaction of NPK levels and variety highest curd yield was found from Pusa Snowball K-1 with treatment T2 and the lowest curd yield was found from Pusa Himjyoti with treatment T0 (control). Study of quality attributes of cauliflower in terms of ascorbic acid and β-carotene content were significantly influenced due to NPK levels as well as varietal difference (Table 3). The ascorbic acid as well as β-carotene content showed a decreasing trend irrespective of variety with increasing nitrogen level. Treatment T0 (control) had the highest ascorbic acid (57.24 mg/ 100 g) and β-carotene (0.30 µg/100 g) as compared to other treatment groups due to effect of NPK level alone. However, there was no significant interaction effect of NPK level and variety was noted. Study also suggested that there could be inverse relation between increasing NPK levels and ascorbic acid content. This might be due to the higher doses of NPK fertilizers, which reduced the dry matter content resulting in less ascorbic acid and β-carotene contents. These results are in conformity with the findings of Roni *et al*. (10) who have also recorded

Table 2. Effect of different NPK combinations on yield contributing characters of cauliflower.

Treatment#	Gross plant wt. (kg)	Net curd wt. (g)	Curd dia. (cm)	Curd height (cm)	Stalk length (cm)	Curd yield/ plot (kg)
NPK level						
T0 (control)	1.21[a]	390.56[a]	10.06[a]	7.67[a]	2.11[a]	7.69[a]
T1	1.27[b]	419.95[b]	10.36[a]	7.92[b]	2.22[a]	8.65[b]
T2	1.41[d]	497.45[d]	11.64[b]	8.54[d]	2.71[b]	10.92[d]
T3	1.33[c]	455.77[c]	10.39[a]	8.14[c]	2.23[a]	9.43[c]
SEM ±	0.01	2.61	0.13	0.06	0.05	0.11
Variety						
V1 (Pusa Snowball K-1)	1.37[b]	553.45[b]	12.17[b]	9.72[b]	2.80[b]	11.50[b]
V2 (Pusa Himjyoti)	1.24[a]	328.39[a]	9.05[a]	6.41[a]	1.83[a]	6.85[a]
SEM ±	0.01	1.85	0.09	0.04	0.04	0.08
NPK level × Variety						
V1T0	1.26	455.90[c]	11.47	9.32	2.58	8.96[c]
V1T1	1.32	516.10[d]	12.01	9.61	2.78	10.67[d]
V1T2	1.50	659.00[f]	13.33	10.12	3.25	14.29[f]
V1T3	1.41	582.80[e]	11.88	9.84	2.60	12.06[e]
V2T0	1.16	325.13[ab]	8.65	6.03	1.63	6.43[a]
V2T1	1.22	323.80[a]	8.70	6.23	1.66	6.64[a]
V2T2	1.32	335.90[b]	9.95	6.95	2.16	7.55[b]
V2T3	1.25	328.73[ab]	8.90	6.45	1.85	6.80[a]
SEM ±	0.02	3.69	0.18	0.08	0.07	0.15
ANOVA, P>F						
NPK Level	**	**	**	**	**	**
Variety	**	**	**	**	**	**
NPK level × Variety	NS	**	NS	NS	NS	**

Mean values (*P<0.05, **P<0.01, NS = Non-significant); Data expressed as mean of triplicate measurements (n = 3); #T0 = 0:0:0, T1 = 80:30:60, T2 = 120:60:80 and T3 = 150:70:100

reduction in ascorbic acid and β-carotene contents with higher doses of NPK in broccoli.

It is evident from the data presented in Table 4 that the application of different levels of NPK significantly influenced the N P and K content in soil. The maximum nitrogen content (112.69 kg ha^{-1}) in soil was recorded under T3 treatment, which was statistically at par with T2, whereas, minimum nitrogen content in soil (98.47 kg ha^{-1}) was recorded under control (T0). The maximum phosphorus content in soil (18.34 kg ha^{-1}) at harvest was recorded under 150:70:100 dose of NPK (T3), which was significantly superior to T1 and T2, whereas, the minimum phosphorus content in soil (14.14 kg ha^{-1}) was recorded under control (T0). Similar results were obtained for potassium content in soil. The maximum potassium content in soil (206.89 kg ha^{-1}) was recorded under 150:70:100 dose of NPK (T3). Results obtained are in line with the earlier findings (Merentola *et al*., 7; Tekasangla *et al*., 14) on cabbage. It can also be concluded from the present study that Pusa Snowball K-1 with NPK application of 120:60:80 could be suitable combination for cultivation in the regions for higher productivity and profitability.

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Table 3. Effect of different NPK combinations on ascorbic acid and β-carotene contents of cauliflower.

Treatment[#]	Ascorbic acid (mg/100 g)	β-carotene (µg/100 g)
NPK level		
T0 (control)	57.24[d]	0.30[d]
T1	54.65[c]	0.27[c]
T2	52.11[b]	0.24[b]
T3	49.93[a]	0.22[a]
SEM ±	0.29	0.01
Variety		
V1 (Pusa Snowball K-1)	44.09[a]	0.20[a]
V2 (Pusa Himjyoti)	62.88[b]	0.30[b]
SEM ±	0.21	1.85
NPK level × variety		
V1T0	47.69	0.24
V1T1	45.03	0.21
V1T2	42.89	0.18
V1T3	40.75	0.16
V2T0	66.79	0.35
V2T1	64.27	0.33
V2T2	61.33	0.30
V2T3	59.12	0.28
SEM ±	0.41	0.01
ANOVA, P>F		
NPK level	**	**
Variety	**	**
NPK level × variety	NS	NS

Data expressed as Mean ± SE of triplicate measurements (n=3); Mean in the same column bearing different superscripts vary significantly (P<0.05); [#]T0 = 0:0:0, T1 = 80:30:60, T2 = 120:60:80 and T3 = 150:70:100

Table 4. Effect of different NPK fertilization on available N, P_2O_5 and K_2O content in soil after harvest.

Treatment[#]	Available N (before)	Available N (after harvest)	Available P (before)	Available P (after harvest)	Available K_2O (before)	Available K_2O (after harvest)
T0 (control)	117.01	98.47[a] ± 2.61	11.04	14.14[a] ± 0.34	152.42	148.72[a] ± 7.93
T1	118.54	99.72[a] ± 1.25	11.83	16.53[b] ± 0.07	154.61	169.21[a] ± 9.20
T2	121.22	108.50[b] ± 1.92	12.08	16.84[b] ± 0.07	154.50	169.94[a] ± 8.86
T3	124.5	112.69[b] ± 1.11	13.25	18.35[c] ± 0.09	156.91	206.89[b] ± 5.96
P value		0.001		0.000		0.006

Data expressed as Mean ± SE of triplicate measurements (n=3); Mean in the same column bearing different superscripts vary significantly (P<0.05); [#]T0 = 0:0:0, T1 = 80:30:60, T2 = 120:60:80 and T3 = 150:70:100

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Evaluation of *kharif* onion varieties and transplanting time for production under North-Western mid Himalayan region

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ABSTRACT

In order to boost onion production during *kharif*, it was considered imperative to test onion varieties for their performance and assess the effect of planting time on its production. Four onion varieties, *viz.* N-53, Nasik Red, Agrifound Dark Red (AFDR) and Agrifound Light Red (AFLR) were transplanted on five dates separated at ten day intervals starting from 15th July to 25th August at Research Farm of Krishi Vigyan Kendra, Chamba for two consecutive seasons (2014 & 2015). There was a significant effect of varieties, transplanting dates and their interaction on bulb diameter, bulb weight and yield. The maximum bulb diameter (5.52 cm) and highest bulb weight (58.65 g) among cultivars was noticed in cultivar Agrifound Dark Red. The yield of onion was significantly affected both by variety and transplanting time. The highest average yield (184.98 q/ha) was observed in variety FDR. The highest bulb yield among transplanting dates was recorded on fourth transplanting date D_4 (15th August). AFDR transplanted around second fortnight of August produced the highest bulb yield.

Key words: Bulb yield, varieties, transplanting time, *kharif* onion.

INTRODUCTION

Onion (*Allium cepa* L.) is the most important commercial vegetable crops on account of its value for local consumption and exportation commodity. In India, it occupies an area of 10,52,000 ha with a total production of 1,68,13,000 MT and an average yield of 16.0 MT/ha (NHB, 8). The *rabi* season crop of onion is harvested in April-May, while *kharif* onion and late *kharif* crop of onion is available in the market in October to December and January to February, respectively. The major portion of *rabi* season crop is stored throughout the country. This stored material is available for domestic markets as well as for export from May to October. There is critical gap in supply of onion from October to December in the country and as a result the prices shoot up. A good harvest in *kharif* season can bridge the gap between demand and supply of onion during this dearth period. *Kharif* onion plays an important role in the supply and price stabilization of onion in India. The *kharif* season onion provides the opportunities of export of *rabi* onion. Further, production of onion during *kharif* season offers a good alternative to the farmers for obtaining higher returns. Therefore, an experiment was conducted during 2014-15 to assess the effect of varieties and planting time on *kharif* onion production under subtropical conditions of Himachal Pradesh.

MATERIALS AND METHODS

Four cultivars of onion, *viz.* N-53, Nasik Red, Agrifound Dark Red (AFDR) and Agrifound Light Red (AFLR) were transplanted on five dates separated by ten day interval starting from 15th July to 25th August at Research Farm of Krishi Vigyan Kendra (Dr YSPUH&F), Chamba, Himachal Pradesh, for two consecutive seasons (2014 & 2015). The experimental site was located at an altitude of 1,050 m above mean sea level with mean minimum and maximum temperature ranges between 12.31° to 25.3°C and average humidity remains around 63.91% (Fig. 1). The soils are well drained sandy loam with pH range of 5.8-6.5. The total rainfall during the growing seasons was 91.0 and 101 cm, respectively. The experiment was laid out in randomized block design with three replications for each treatment. Healthy seedlings were transplanted on raised beds at a spacing of 15 cm x 10 cm in plots of 3.0 x 3.0 m^2. All the observations pertaining to traits, *viz*. plant height (cm), neck thickness (cm), bulb diameter (cm), weight of bulb (g), days for harvesting, TSS and yield (q/ ha) were taken by randomly selecting 20 healthy plants from each plot. Recommended package of practices was adopted to raise the crop successfully. Data obtained during the two years were pooled and analyzed as per the standard procedure (Gomez and Gomez, 4).

RESULTS AND DISCUSSION

Perusal of data pooled over two years (2014 and 2015) presented in Tables 1-7 revealed significant effect of varieties, planting time and their interaction

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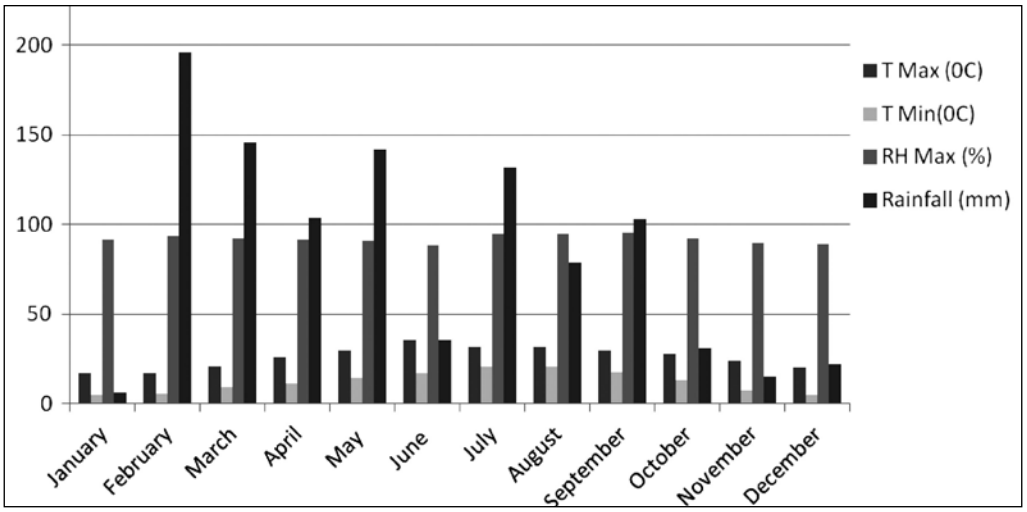

Fig. 1. Average meteorological data of experimental site (2014-15).

on different growth and bulb parameters of *kharif* onion. There was a significant difference in the plant height among the varieties during both the years. Plant height was measured as the plants reached maturity stage. The maximum plant height (52.19 cm) was observed in variety N-53 followed by variety AFDR (51.52 cm). Among transplanting dates, the maximum plant height (55.45 cm) was recorded on first transplanting date, D_1 *i.e.* on 15th July, which was statistically at par with second transplanting date D_2, *i.e.* on 25th July. A continuous decrease in plant height was observed with delay in transplanting. Dev *et al.*

Table 1. Effect of variety and date of transplanting on plant height (cm) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	51.46	49.15	45.25	44.21	41.29	46.27
V_2 (N-53)	58.35	55.90	50.92	50.76	45.05	52.19
V_3 (AFDR)	57.88	54.21	52.18	48.19	45.14	51.52
V_4 (Nasik Red)	54.11	52.59	48.20	47.38	46.32	49.72
	55.45	52.96	49.13	47.63	45.05	
$CD_{0.05}$	Variety		6.14			
	Date		4.32			
	Variety × Date		11.23			

Table 2. Effect of variety and date of transplanting on neck diameter (cm) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	0.84	0.87	0.92	0.98	0.90	0.90
V_2 (N-53)	0.88	0.95	1.25	1.14	1.10	1.06
V_3 (AFDR)	0.96	0.98	1.00	1.04	0.95	0.99
V_4 (Nasik Red)	0.80	0.82	0.81	0.85	0.77	0.81
	0.87	0.91	0.99	1.00	0.93	
$CD_{0.05}$	Variety		0.08			
	Date		0.07			
	Variety × Date		0.12			

Table 3. Effect of variety and date of transplanting on bulb diameter (cm) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	4.84	5.06	5.11	5.18	5.00	5.03
V_2 (N-53)	5.14	5.19	5.22	5.32	5.09	5.19
V_3 (AFDR)	5.38	5.42	5.56	5.95	5.29	5.52
V_4 (Nasik Red)	4.98	5.08	5.13	5.29	5.10	5.11
	5.09	5.18	5.26	5.44	5.12	
$CD_{0.05}$	Variety		0.15			
	Date		0.29			
	Variety × Date		0.45			

Table 4. Effect of variety and date of transplanting on bulb weight (g) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	40.12	42.92	44.36	50.61	41.54	43.91
V_2 (N-53)	49.26	52.11	54.45	57.90	50.33	52.81
V_3 (AFDR)	53.21	57.24	61.00	63.36	58.45	58.65
V_4 (Nasik Red)	47.32	49.46	52.29	53.76	49.17	50.40
	47.23	50.18	52.77	56.16	49.62	
$CD_{0.05}$	Variety		4.98			
	Date		6.21			
	Variety × Date		14.57			

Table 5. Effect of variety and date of transplanting on TSS (°B) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	9.25	10.50	11.00	11.66	10.83	10.65
V_2 (N-53)	9.50	10.80	10.15	11.00	9.90	10.27
V_3 (AFDR)	10.80	11.60	12.50	13.00	11.80	11.94
V_4 (Nasik Red)	9.15	9.90	10.00	11.00	10.50	10.11
	9.76	10.70	10.91	11.66	10.75	
$CD_{0.05}$	Variety		NS			
	Date		0.88			
	Variety × Date		NS			

Table 6. Effect of variety and date of transplanting on days to harvest (days) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	134	128	126	120	114	124.4
V_2 (N-53)	140	137	134	130	128	133.8
V_3 (AFDR)	139	133	129	125	120	129.2
V_4 (Nasik Red)	145	139	136	131	123	134.4
	139.5	134.25	131.25	126.5	121.25	
$CD_{0.05}$	Variety		5.10			
	Date		11.77			
	Variety × Date		23.65			

Table 7. Effect of variety and date of transplanting on total yield (q/ha) in onion.

Variety	Transplanting date					
	D_1 (15th July)	D_2 (25th July)	D_3 (5th Aug)	D_4 (15th Aug)	D_5 (25th Aug)	Mean
V_1 (AFLR)	142.2	153.3	160.1	169.7	154.2	155.90
V_2 (N-53)	153.7	161.5	172.3	180.5	165.9	166.77
V_3 (AFDR)	175.8	179.4	188.3	197.6	183.8	184.98
V_4 (Nasik Red)	154.1	163.4	167.3	177.9	166.2	165.78
	156.45	164.40	172.00	181.42	167.52	
$CD_{0.05}$	Variety		8.45			
	Date		18.55			
	Variety × Date		29.48			

(2) and Gautam *et al.* (3) also reported a significant effect of transplanting dates on plant height in *kharif* onion. The effect of variety x date interaction for this trait was also found to be significant with maximum plant height (58.35 cm) in combination V_2D_1 (N-53 and 15th July) and minimum value (41.29 cm) in combination V_1D_5 (AFLR x 25th August).

Neck thickness of sampled plants was measured with the help of Vernier callipers. Onion with thin neck diameter store better than those having thick diameter, thus neck diameter in onion is an important character to indicate bulb storage ability (Tripathi and Lawande, 10). The highest neck diameter (1.06 cm) was observed in variety N-53, which was statistically at par with AFDR (0.99 cm). The minimum neck diameter (0.81 cm) was noticed in variety Nasik Red. Among the transplanting dates maximum value (1.00 cm) for neck diameter was recorded on date D_4 (15th August), while the minimum value (0.87 cm) was observed on date D_1 (15th July). The interaction effect of variety x date was also significant for neck diameter having highest value (1.14 cm) in combination V_2D_4 (N-53 x 15th Aug.) and minimum value (0.77 cm) in combination V_4D_5 (Nasik Red x 25th Aug.). Maximum bulb diameter (5.52 cm) was recorded in variety AFDR, which was significantly higher than all other varieties under study, while the lowest bulb diameter (5.03 cm) was noticed in variety AFLR. Among transplanting dates the highest bulb diameter (5.44 cm) was observed on date D_4 (15th Aug.), which was statistically at par with transplanting dates D_3 (5th Aug.) and D_2 (25th July). A significant effect of variety x date interaction was also observed for this trait with maximum value (5.95 cm) in combination V_3D_4 (AFDR x 15th Aug.).

The highest bulb weight (58.65 g) was recorded in variety AFDR. All other varieties under study were found to have significantly lower bulb weight in comparison to AFDR and the lowest bulb weight (43.91 g) was observed in variety AFLR. The bulb weight among transplanting dates correspond to the bulb diameter and highest weight (56.16 g) was observed on date D_4 (15th Aug.). The variety x date interaction effect was observed to be highest (63.36 g) in combination V_3D_4 (AFDR x 15th Aug.). The highest TSS content (11.66°B) was observed on transplanting date D_4 (15th Aug.). The effect of variety and variety x date interaction effects were found to be non-significant for this trait.

The lowest number of days (124.40) to harvest was observed in variety AFLR, which was significantly lower than Nasik Red (134.80 days). Among the transplanting dates lowest number of days to harvest (121.25) was recorded on the last transplanting date (25th Aug.), while the longest duration to harvest (139.50 days) was noticed when the transplanting was done on 15th July (D_1). The interaction effect of variety and date was also found to be significant with maximum days to harvest (145.00) in combination V_4D_1 (Nasik Red x 15th July) and minimum (114.00 days) in combination V_1D_5 (AFLR x 25th Aug.).

The cultivars AFDR recorded the maximum average yield (184.98 q/ha), which was significantly higher than all other varieties. Bhagchandani *et al.* (1) had reported better performance of this cultivar in *kharif* season. The lowest yield (155.90 q/ha) was recoded in variety AFLR. Among transplanting dates, highest bulb yield (181.42 q/ha) was observed on date D_4 (15th Aug.) followed by transplanting date D_3 (172.00 q/ha). Further delay in planting of setts towards September and earlier in July resulted into decline in bulb production. It was marked that higher yield manifested by mid August planting was accompanied by higher sprouting of setts and better growth of the plants. This was corroborated by Neerja *et al.* (7), who indicated that enhanced crop growth rate of onion resulted in efficient metabolism, thereby increased the sink capacity. Higher metabolism, greater photosynthates mobilization and better source sink relationship helped to produce higher yield by planting in mid August. Sharma *et al.* (9)

and Kandil *et al*. (5) and Mohanty and Prusty (6) also advocated the role of transplanting dates on growth and yield of onion. Interaction effect of variety x date was also found to be significant with maximum value (197.6 q/ha) in treatment combination V_3D_4 (AFDR x 15th Aug.).

In the present studies it was found that maximum values for yield contributing traits like bulb weight, bulb diameter and total yield were obtained for cultivar Agrifound Dark Red. All the varieties produced maximum yield when transplanted on 15th August (D_4). Thus it can be summarized that Agrifound Dark Red is the best suited variety for *kharif* onion production under subtropical conditions of Himachal Pradesh and it should be transplanted around second fortnight of August for getting higher yield.

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Variability induction in Ox-eye daisy (*Leucanthemum vulgare* Lam.) using gamma rays

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ABSTRACT

The present investigation was aimed to study hormesis, morphological and biochemical variability attributes associated with mutation and purification of novel mutants in Ox-eye daisy. The seeds of *Leucanthemum vulgare* were exposed to 20, 40, 60, 80 and 100 Gy doses of gamma rays (source ^{60}Co). These irradiated seeds were used to raise seedlings and planted in combination with seedlings from non-irradiated seeds (control) in randomized block design. Low doses of gamma irradiation resulted in hormesis and evoked encouraging novelties, whereas, the higher doses elicited higher degree of abnormalities and consequently mortality. The M_2 seeds were seeded to observe new characters and mutations in population in every treatment. The minimum plant survival was 51.60% at 100 Gy gamma rays treatment, which significantly differed from all other treatments. The maximum plant survival (99.67%) was observed in non-irradiated control. It was observed that plant survival significantly declined with the increase in the dose of gamma irradiation. Plants raised from irradiated seeds showed significant delay in flowering over the control. The earliest blooms were observed in control (108.03 days), while the maximum days to bloom (118.30 days) were recorded with 100 Gy treatment. Three promising mutants, *viz*., Spatulate type (L_1) at 40 Gy, Quilled-spatulate type (L_2) at 60 Gy and Quilled type (L_3) at 60 Gy gamma irradiation treatment were labelled, screened and checked for stability of characters in M_2 and M_3 generations. The seeds of M_2 and M_3 generations were raised for observation for variation in morphological characters and stability mutants in every generation.

Key words: *Leucanthemum vulgare*, gamma rays, irradiation, mutation, Ox-eye daisy.

INTRODUCTION

Leucanthemum vulgare Lam. [*Chrysanthemum leucanthemum* Linn.] commonly known as Ox-eye daisy or white weed is a native to Europe and North Asia. It is a leafy, vigorously growing herbaceous, non-woody perennial plant. The species is strictly cross-pollinated due to presence of self-incompatibility. It can be cultivated as a decorative plant in the garden flower beds or as pot plant. Application of mutation breeding has significant role in creating novel genotypes in ornamental crops, like the usually high heterozygosity of the plants that permits direct detection of mutations within the irradiated material, with the intention of improvement in visible characteristics (Broertjes, 3).

Genetically modified ornamental plants do not find market in Europe due to their low acceptance amongst consumers and the ambiguous legal situation. As very little research work has been carried out on *Leucanthemum vulgare*, the current study was therefore aimed to study hormesis, morphological and biochemical variability attributes accompanied with mutation, creation and purification of novel mutants.

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MATERIALS AND METHODS

The experiment was conducted at Model Floriculture Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand during 2010 to 2012. The experimental material comprised of the seeds of *Leucanthemum vulgare*. The seeds of the parental line were procured from Thompson & Morgan, Great Britain and were exposed to gamma rays (source ^{60}Co) at 20, 40, 60, 80 and 100 Gy doses at gamma chamber facility of the CSIR-National Botanical Research Institute, Lucknow. The gamma ray-irradiated seeds together with the control (non-irradiated seeds) were seeded on raised nursery beds and transplanted in experimental field in randomized block design with three replications. The plot size was 180 × 100 cm^2 with 12 seedlings/plot with plant spacing of 50 × 30 cm^2. All the recommended package of practices were followed throughout the growing period. Morphological and biochemical parameters were recorded for 13 traits from randomly chosen three plants per treatment per replication. The chlorophyll content (*a*, *b* and total chl) of the leaves was estimated as proposed by Hiscox and Israelstam (9). Visual observations on totally different characters were prepared and the

plants showing modification in type of flowers, florets, etc. were critically determined and any abnormality determined within the plants of M_1 generation in the treatments was labelled, screened and recorded. At maturity, every mutant plant was separately harvested and also the seeds were labelled for sowing in the subsequent generations.

The M_2 and M_3 seeds together with the control (non-irradiated seeds) were seeded on raised nursery beds and transplanted in experimental field in randomized block design with three replications, with a plot size of 180 × 100 cm^2 (12 plants per plot) with spacing of 50 × 30 cm^2, following the recommended package of practices. M_2 mutants were sporadically observed right after germination and were labelled for further observations. Any abnormality or variation detected in the plants of M_2 generation was screened and tagged for subsequent observations in M_3 generation. At maturity, every mutant plants were individually harvested and the seeds were labelled and screened, for sowing in the subsequent generation (M_3) and checked for the stability of the characters. The data generated were subjected to the statistical analysis in accordance with the procedure outlined by Gomez and Gomez (7).

The genomic DNA was extracted by using the CTAB method (Doyle and Doyle, 6) with slight alterations. PCR amplification was performed with random decamer primers. Band sharing data was analyzed to get genetic similarities based on Jaccard's similarity coefficient among the isolates by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc, version 2.2) (Rohlf, 16). UPGMA (Unweighted Pair Group Method using Arithmetical Averages) algorithm was used to determine the genetic relationship of the parent and also the mutants generated in *L. vulgare*.

RESULTS AND DISCUSSION

The observations made on several un-irradiated plants of *L. vulgare* clearly revealed that normal plants grew to a mean plant height of 51.0 cm with plant spread of 38.17 cm (E-W) and 35.17 cm (N-S). The leaves were numerous, green, lacking punctate glandular hairs. Basal leaves were 9.15 cm long, 4.63 cm wide and had 22.62 cm^2 leaf area. Leaves were also long, petiolate with linear or oval cuneately narrow lamina, obtusely toothed, less often shallow lobed. Lower leaves were spatulate, short petiolate, but the upper leaves were sessile, gradually smaller and less divided. Each plant had solitary capitula (25) with ray florets (36) and disc florets (339.17). The diameter of the capitula was 6.87 cm, weighing 1.32 g and disc was 2.05 cm across, borne on a thin (0.475 cm) and long peduncle. Involucre was glabrous with involucre bracts of light-colour or brownish colour with membranous border. The weight of ray florets was 9.20 mg and that of disc florets was 0.92 mg. The ray florets were 2.77 cm long and 0.86 cm broad borne on the flower head, which was 2.10 cm high (Table 2).

Data pertaining to the effect of gamma irradiation on vegetative growth characters, biochemical content and abnormalities are presented in Table 1. The perusal of the data presented in Table 1 and Fig. 1 revealed that the minimum plant survival (51.60%)

Table 1. Effect of gamma irradiation on different characters of *Leucanthemum vulgare* and its mutants.

Trait	Control	Gamma irradiation (Gy)					CD at 5%
		20	40	60	80	100	
Plant survival (%)	99.67	94.67	85.20	76.90	64.83	51.60	7.16
Plant abnormality (%)	0.00	4.11	7.40	12.46	15.33	20.67	1.64
Plant height (cm)	46.33	45.13	42.50	40.87	39.53	36.67	6.12
Plant spread (E-W) (cm)	37.67	34.00	35.00	32.67	31.57	31.20	NS*
Plant spread (N-S) (cm)	34.00	33.13	32.47	31.00	29.83	28.30	3.02
Leaf length (cm)	8.97	8.83	8.47	8.4	7.73	7.67	0.69
Leaf width (cm)	4.53	4.13	4.05	3.82	3.53	3.3	NS
Days to flowering	108.03	110.80	111.57	113.97	117.63	118.30	3.54
Chlorophyll *a* (Chl *a*)	1.782	1.83	1.84	1.599	1.852	1.927	0.07
Chlorophyll *b* (Chl *b*)	0.515	0.531	0.55	0.552	0.564	0.583	0.03
Total chlorophyll (Chl)	2.282	2.345	2.373	2.134	2.399	2.493	0.08
Abnormal leaf (%)	0.00	3.80	7.30	11.87	15.53	21.93	1.28
Abnormal flower (%)	0.00	6.47	11.23	13.56	16.13	18.4	1.57

NS = non-significant; * = significance at 5% level.

Table 2. Morphological characters of *L. vulgare* and its mutants developed through gamma irradiation.

Trait	Original genotype	Mutants		
		L_1	L_2	L_3
		40 Gy	60 Gy	40 Gy
Plant height (cm)	51.00	39.33	34.67	32.33
Plant spread (E-W) (cm)	38.17	37.33	33.17	32.67
Plant spread (N-S) (cm)	35.17	34.50	33.50	31.50
Leaf length (cm)	9.15	9.77	9.62	8.52
Leaf width (cm)	4.63	3.52	3.35	3.60
Leaf area (cm^2)	22.62	18.39	17.23	16.40
No. of flowers/plant	25.33	21.50	23.00	24.00
Flower dia. (cm)	6.87	7.18	5.83	7.15
Disc dia. (cm)	2.05	2.90	2.47	2.60
No. of ray florets	36.00	39.00	34.00	33.67
No of disc florets	339.17	488.07	415.14	437.58
Head weight (g)	1.32	1.38	1.12	1.37
Ray floret weight (mg)	9.20	10.33	7.55	8.54
Disc floret weight (mg)	0.92	0.91	0.95	1.09
Ray floret length (cm)	2.77	2.80	2.37	2.30
Ray floret width (cm)	0.86	0.43	0.34	0.31
Head height (cm)	2.10	3.04	3.10	3.13
Flower form	Single	Semi-double	Single	Single
Shape of ray florets	Ligulate	Spatulate	Quilled-spatulate	Quilled

at 100 Gy gamma rays treatment was found, which significantly differed from all other treatments. The maximum plant survival (99.67%) was observed in non-irradiated control. It was observed that plant survival significantly declined with the increase in the dose of gamma irradiation. Reduction in plant survival after exposure to gamma rays has been explained to be due to disturbances of auxin synthesis, chromosomal aberration (Gunckel and Sparrow, 8). Similar results were also observed by Kapoor *et al.* (12) in *Chrysanthemum paludosum*. The plant height decreased from 46.33 cm in control with the increase in dose of gamma irradiation to 36.67 cm with 100 Gy treatment. Reduction in plant height was observed with increase in the dose of gamma rays irradiation, which may be due to the fact that inactivation of auxin and decrease in auxin content with increase in radiation doses was responsible for reduction in plant height (Banerji and Datta, 2). Earlier, Misra *et al.* (15) observed reduction in plant height due to gamma irradiation in *C. morifolium*.

Plants arisen from irradiated seeds had significant delay in flowering compared to control. The earliest blooms (108.03 days) were observed in control, while the maximum days to bloom (118.30 days) were recorded with 100 Gy treatment. The delay in bud initiation ultimately resulted in late blooming, which may be due to reduction in the rate of various physiological processes and inhibition of growth and the plant remained in juvenile stage and thus unable to differentiate flower heads due to gamma irradiation. Due to irradiation, many biosynthetic pathways are altered, which are directly and indirectly associated with the flowering physiology (Mahure *et al.*, 14). These results also corroborate with the finding of Dilta *et al.* (5) in *C. morifolium*.

The maximum leaf length (8.97 cm) was observed in control, while minimum (7.67 cm) was in 100 Gy treatments. Significant reduction in size of the leaf with the increasing doses of gamma irradiation was observed. This may be attributed to poor growth of plants due to radiation damage. The chlorophyll content was also influenced significantly by various gamma ray treatments. Increase in the chlorophyll content (*a*, *b* and total chl.) was evidenced with the increase in the doses of gamma irradiation. Datta (4) and Kapoor *et al.* (12) also observed similar results with incite in chlorophyll content as the

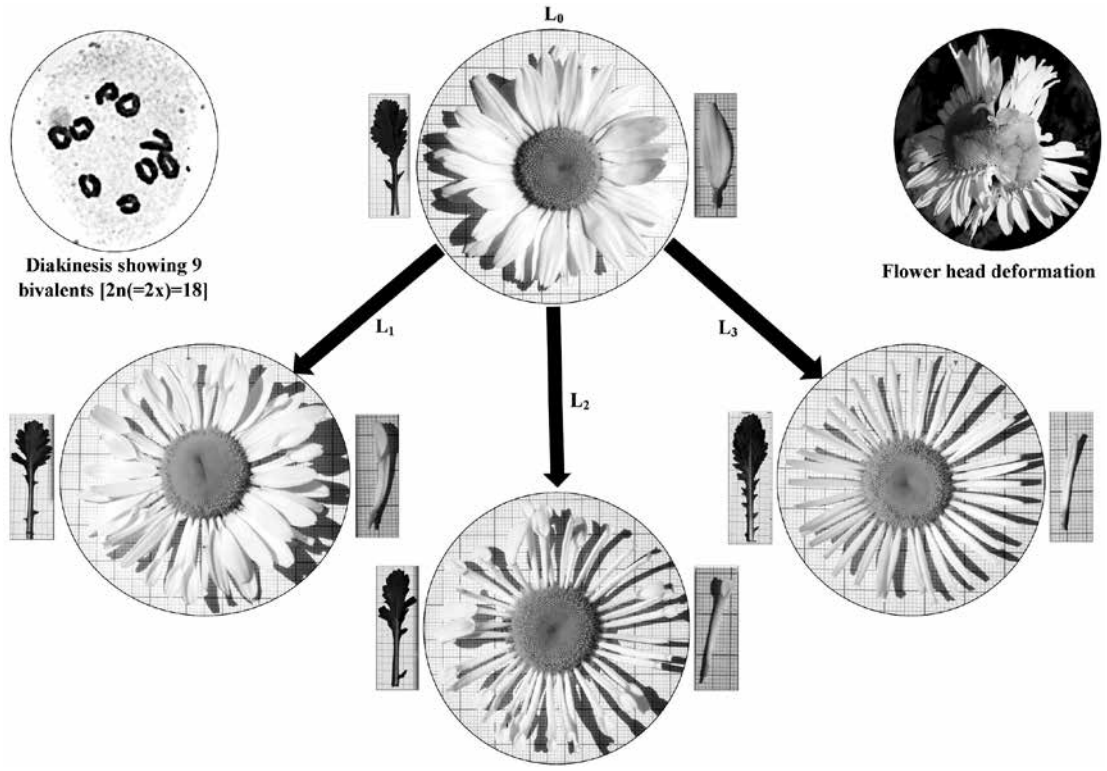

Fig. 1. Capitula of *Leucanthemum vulgare* (L_0) and its gamma irradiation induced mutants (L_1, L_2 and L_3)

gamma irradiation dose increased and reported that its basic cause may be associated with the physiological disturbances, change in enzyme activity and breakage of metabolites with the increase in irradiation doses.

The per cent abnormal plants increased significantly with the increase in gamma rays treatment over the control. Among the different gamma rays treatments, maximum deformed plants (20.67%) were recorded with 100 Gy treatment and none in control. This significant induction of abnormalities might be due to radiation damage of the irradiated plants particularly chromosomal breakage, which causes physiological, morphological and cytological disturbances. Misra *et al.* (15) also recorded similar trends in chrysanthemum variety Pooja. Per cent abnormal leaves increased significantly with the increasing gamma ray irradiation doses compared to control. The different types of leaf abnormalities recorded included change in leaf shape and size, margins, apex, fission and fusion after irradiation. There were no dose specific abnormalities in leaves. Earlier, Banerji and Datta (1) also observed similar results in *Dendranthema* cv. Surekha. Significant increase in plants with flower head fasciation/ asymmetrical flower heads due to irradiation was noted, which was not specific to the doses. Flower heads became fasciated in different forms (Fig. 1). The formation of fascinated heads after irradiation was also observed by Kapoor *et al.* (11). These abnormalities are genotype dependent and damage within the organism may be due damage to plant parts (Datta, 4).

Visual observations on traits were made and the plants showing change in form of flowers, florets *etc*. (Fig. 2 a-d), were critically observed and the type of forms other than normal ones were tagged and recorded. The plants were also observed for chimera formation, which could be maintained through vegetative propagation or through tissue culture techniques. The change in flower form was also recorded by Kumari *et al.* (13) on *C. morifolium*.

Three mutants, *viz*., Spatulate type (L_1) at 40 Gy, Quilled-Spatulate type (L_2) and Quilled type (L_3) at 60 Gy were screened, tagged and checked for the stability of the characters. The observations were recorded on morphological characters of the mutants developed after gamma irradiation in M_1, M_2 and M_3 generations and the pooled mean values are presented in Table 2 and Fig. 2. Mutation in flower head shape/ size has also been reported earlier in annual chrysanthemum (Jain *et al*., 10). Some of the prominent mutants identified were as listed below.

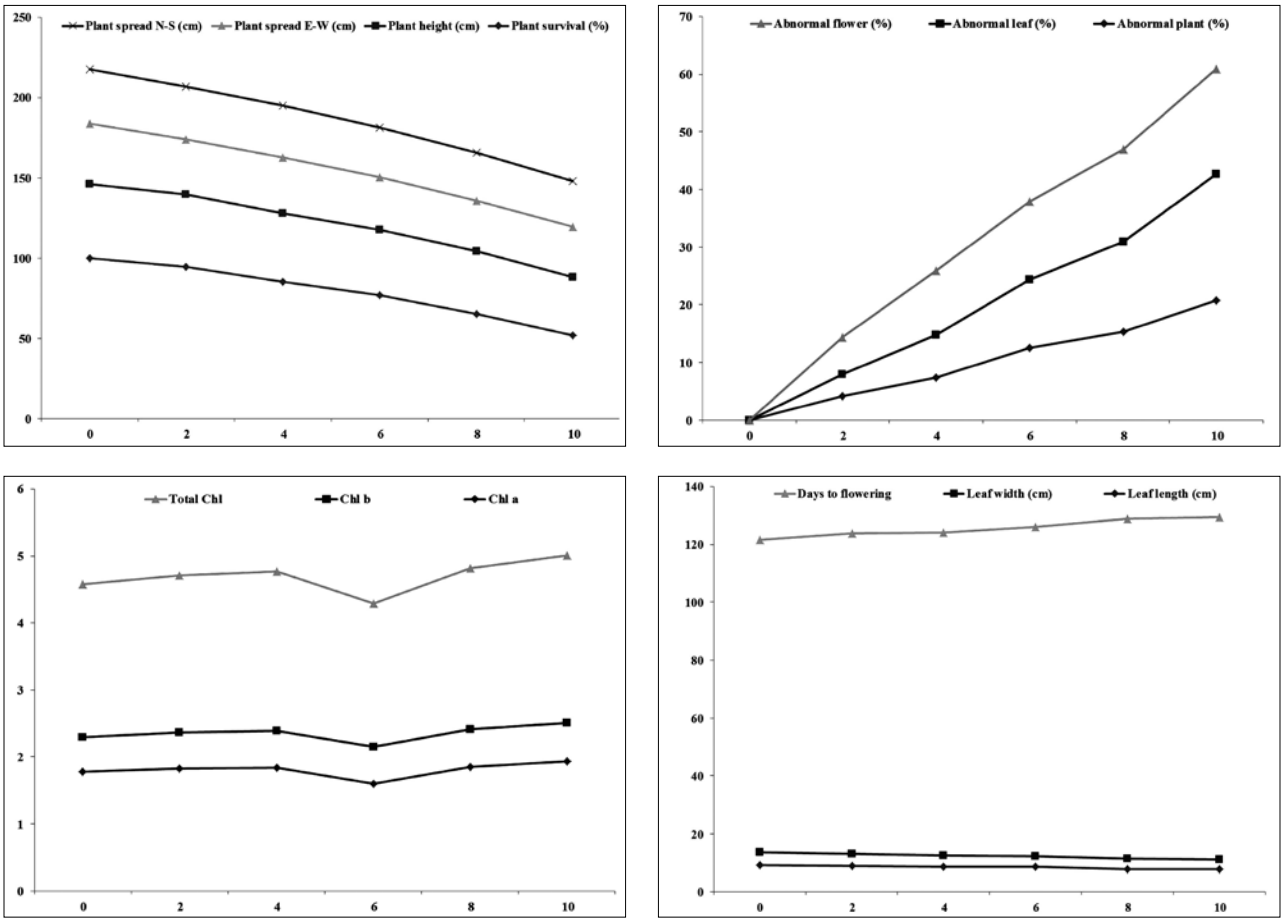

Fig. 2 **(a)** Effect of gamma irradiation on vegetative characters, **(b)** Effect of gamma irradiation on per cent abnormalities, **(c)** Effect of gamma irradiation on chlorophyll content, **(d)** Effect of gamma irradiation on days to flowering and leaf size.

A mutant L_1 (spatulate type) was selected after treatment with 40 Gy gamma rays irradiation treatment and was found significantly different from mother plants, *i.e.* dwarf and less spreading. The leaves were long, while width and area were lesser and the number of flowers per plant was marginally lesser. Flowers were of bigger size, flower head diameter, disc diameter were more with ray florets and disc florets. The flower head and ray floret weights were more, while disc floret weight was marginally less. The ray floret length was marginally more, while width was lesser but the flower had more flower head height. Change was recorded in flower form to semi-double, along with the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to spatulate (Table 2).

The traits of the mutant L_2 (Quilled-Spatulate type) is shown in Table 2. It is a dwarf mutant developed with 60 Gy gamma rays irradiation. The plant spread was also reduced in both the directions. The leaf length was though more, while leaf width and leaf area were lesser. The number of flowers per plant reduced marginally, but the flowers were smaller in size, more disc diameter and lesser number of ray florets of lesser weight. The disc florets were more in number (415.14) and weight (0.95 mg). The length and width of the ray florets were lesser than the original species. The flower had more flower head height. No change was recorded in flower form, except for the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to quilled-spatulate.

A third potential mutant L_3 (Quilled type) was developed with 40 Gy gamma rays irradiation treatment and differed in several traits from the original plants, *viz*., plants were very dwarf with lesser plant spread. The leaf length (8.52 cm), leaf width (3.60 cm) and leaf area (16.40 cm^2) were less and the number of flowers per plant also got reduced. Mutant L_3 had slightly bigger flower with higher flower head

diameter, disc diameter and lower number of ray florets and more number of disc florets. The flower head weight was more, while ray florets weight was lesser and disc florets weight was more. The ray floret size was lesser and flower head height was more than that of the original. No change was recorded in flower form, except for the change in the shape of the ray florets from ligulate with laciniate shape of tip and keeled upper surface to quilled (Table 2).

PCR amplification of DNA with random primers of which LC-94 and LC-86 showed sufficient polymorphism (93.33 to 90.91%) with an average polymorphic percentage of 92.12%. A total number of 26 loci were amplified (Fig. 3). This gave an average of 13 loci per primer. Polymorphic information content (PIC) value was 0.57 for primer LC-94 and 0.59 for primer LC-86 with an average of 0.58 for both the primers (Table 3).

The dendrogram generated using SAHN cluster analysis and UPGMA method illustrated in Fig. 4 and the matrix of the Jaccard's similarity coefficient of the mutants of *L. vulgare* based on RAPD markers (Table 4) reveal that the dendrogram separated the original species of *L. vulgare* and its three mutants into two major clusters A and B, at the demarcation of approximately 37% genetic similarity. Cluster A consisted of the original species and its 2 mutants, while the cluster B had only mutant L_3. Cluster A was further categorized into two sub-clusters I and II, at the demarcation of approximately 58% genetic similarity. Sub-cluster I had the original species and its mutants L_1 with approximately 68% genetic similarity. Sub-cluster II had only one mutant L_2.

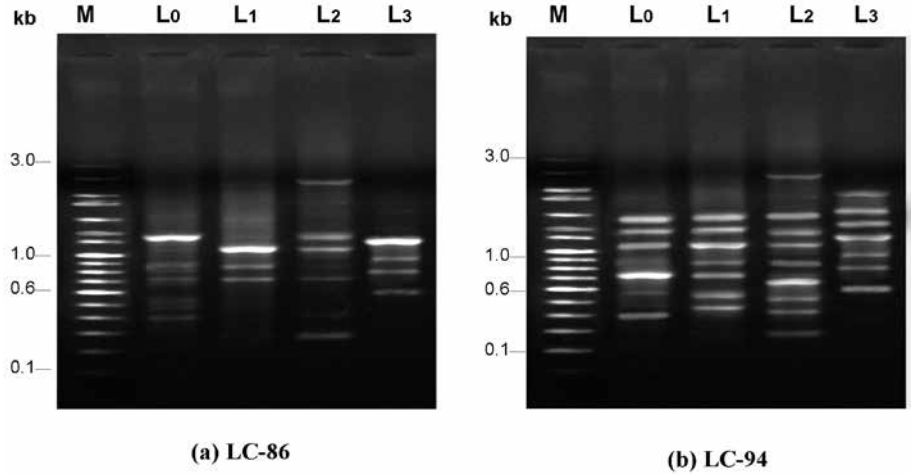

Fig. 3 Molecular diversity generated among *Leucanthemum vulgare* (L_0) and its three mutants (L_1, L_2 and L_3) by RAPD primer LC-86 and LC-94.

Table 3. Characterization of mutants of *Leucanthemum vulgare* using RAPD primers.

Code	Primer sequence (5′ to 3′)	% GC	MMB	PMB	Poly (%)	PIC	H_i	Rp	D	D_L
LC-94	'GTCGCCGTCA'	70	1	14	93.33	0.57	0.30	8	0.63	0.37
LC-86	'GTTGCGATCC'	60	1	10	90.91	0.59	0.33	7	0.66	0.38
Av.			1	12	92.12	0.58	0.31	7.5	0.65	0.37

MMB = monomorphic bands, PMB = polymorphic bands, % Poly = Per cent polymorphism, PIC = Polymorphic Information content, H_i = Average expected gene diversity, Rp = Resolving power, D = Discrimination power, D_L = Discriminating power.

Table 4. Jaccard's similarity coefficient of *L. vulgare* and its mutants based on RAPD markers.

Genotype	*L. vulgare*	L_1	L_2	L_3
L. vulgare	1.000			
L_1	0.667	1.000		
L_2	0.482	0.667	1.000	
L_3	0.407	0.444	0.259	1

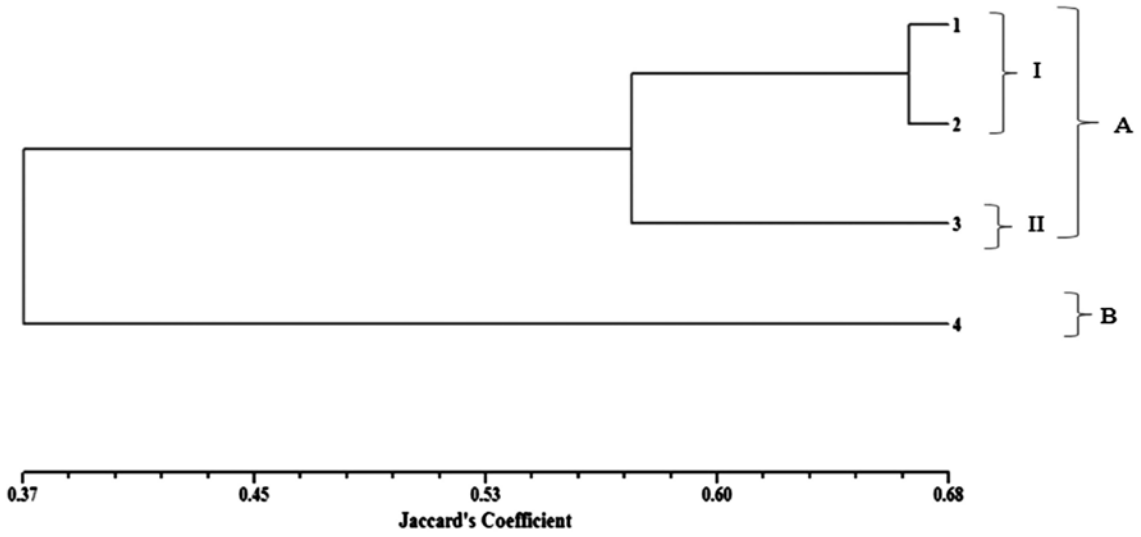

Fig. 4 Dendrogram depicting the classification of *Leucanthemum vulgare* and its three mutants based on RAPD. 1. *L. vulgare*, 2 to 3. Mutants of *L. vulgare* L_1 to L_3.

From the present investigation, it has been empirically perceived that in addition to change in flower shape, cogent changes in some morphological characters had occurred in the mutants. Gamma irradiation induced new flower shape appearance mutants, screened in the present investigation may find very advantageous in future practical breeding programmes and can also be used directly for cultivation.

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Effect of foliar application of zinc and iron on growth, flowering and post-harvest life in lilium cv. Navona

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ABSTRACT

A research was conducted India to evaluate the necessity of micronutrients such as zinc and iron on growth, flowering and postharvest attributes in Asiatic lilium cv. Navona. The experiment comprised nine treatments, *viz*., $ZnSO_4$ 0.2%, $ZnSO_4$ 0.4%, $FeSO_4$ 0.2%, $FeSO_4$ 0.4%, $ZnSO_4$ 0.2% + $FeSO_4$ 0.2%, $ZnSO_4$ 0.2% + $FeSO_4$ 0.4%, $ZnSO_4$ 0.4% + $FeSO_4$ 0.2%, $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% and control (distilled water) in a randomized block design with three replications. Significant effect was observed with the application of $ZnSO_4$ 0.4% + $FeSO_4$ 0.4%, which increased No. of leaves/ plant, stem diameter, plant height, fresh and dry weight of leaves/ plant, leaf area, chlorophyll content, No. of flower buds/ plant, flower stalk length, pedicel length, diameter of 1st flower, longevity of 1st, 2nd and 3rd flower, No. of buds opened, No. of buds opened at a time in vase, stem weight on 1st, 3rd and 5th day, and weight of stem after withering. Early flower colour show and days to opening of 1st bud were exhibited with $ZnSO_4$ 0.2% + $FeSO_4$ 0.4%, which was statistically at par with $ZnSO_4$ 0.2% + $FeSO_4$ 0.4% treatment. In general both individual and combined doses of zinc sulphate and iron sulphate gave significant results over control.

Key words: Flowering, iron, lilium, post-harvest life, vegetative growth, zinc.

INTRODUCTION

Lilium spp. are bulbous flowering plants which have originated in the south-western and Himalayan Asia. Asiatic lilies (*Lilium bulbiferum*) have been used as cut flowers, pot and garden plants for centuries. Major producers of lilies are China followed by Kenya and Japan (Hanks, 4). The yield and quality of horticultural crops are enriched with application of micronutrients in balanced ratios (Eskandari, 2). Zinc is most likely micronutrient, which is deficit in Indian soils. It is an important structural component and regulatory co-factor of several enzymes. Zinc deficient plants exhibit reduced rate of protein synthesis and is known to maintain structural integrity of ribosomes. Iron is fourth abundant element on earth, but its non-availability to plants can be attributed to low solubility of its minerals (Eskandari, 2). Iron plays important role in oxidation reduction reactions. It is required for enzyme activities in electron transport chain, chlorophyll synthesis and maintenance of chloroplast structure. It is also required at active site of glutamyl-tRNA reductase, which is needed for formation of 5-aminolevulinic acid, a precursor of chlorophyll (Kumar and Soll, 7). Iron also regulates respiration and photosynthesis.

Deficiency of micronutrients has been globally reported, and about one-third of world's agricultural soils are devoid of these because of injudicious use of phosphatic fertilizers (Mousavi, 11). Giving a thought to all above, this study was undertaken to evaluate the effect of zinc and iron on growth, flowering and postharvest aspects of lilium cv. Navona.

MATERIALS AND METHODS

The study has been conducted on Asiatic hybrid lily cv. Navona. This lily is worldwide popular owing to its numerous buds and bright white six-petaled flowers. For planting, healthy and disease-free bulbs were selected and planted in 27 plots, each of size 1.00 m × 0.8 m at a spacing of 25 cm × 20 cm. Planting of bulbs was carried out in the month of October. The experiment was conducted inside a polyhouse at Horticulture Research Farm, IAS, BHU, Varanasi, whereas postharvest study was exercised at the Postharvest Lab of Department of Horticulture. Plants were subjected to foliar application of zinc sulphate and iron sulphate at concentrations of 0.2 and 0.4%, individually and in combinations (Table 1). The experiment was conducted in randomized block design having three replications. Spraying was done twice at 30 and 45 days after planting. Cultural operations such as weeding, irrigation, staking and plant protection measures were undertaken as and when required.

Observations for growth, flowering and postharvest parameters were investigated. Record of growth specifications like No. of leaves/ plant, stem

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diameter, plant height, fresh weight of leaves/ plant, dry weight of leaves/ plant, leaf area and chlorophyll content was ventured. Flowering traits like No. of buds/ plant, flower stalk length, pedicel length, days to flower colour show, days to opening of 1st bud and diameter of 1st flower were examined. Postharvest parameters comprising of longevity of 1st, 2nd and 3rd flower, No. of buds opened, No. of buds opened at a time, weight of stem on 1st, 3rd day and 5th day, weight of stem after withering and vase-life were inspected in lab. Lilium stems were cut in a slant for postharvest study when the 1st bud was fully opened. After harvesting flower stems were placed in bucket containing water then brought from polyhouse to postharvest lab. One re-cut was given to all the flower stems then kept in a vase solution of 2% sucrose + 200 ppm 8-HQC. The observations recorded were subjected to statistical analysis.

RESULTS AND DISCUSSION

The effect of zinc and iron sprays on growth and flowering parameters has been presented in Table 1. The vegetative parameters like No. of leaves/ plant, stem diameter, plant height, fresh weight of leaves/ plant, dry weight of leaves/ plant, leaf area and chlorophyll content were significantly influenced due to the treatments. Plants treated with $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% produced maximum No. of leaves/ plant (57.00), stem diameter (12.26 mm), plant height (52.00 cm), fresh weight of leaves/plant (8.33 g), dry weight of leaves/ plant (0.98 g) and leaf area (306.31 cm^2). This treatment was observed to be significant to all other treatments for No. of leaves/ plant. For stem diameter it produced conspicuously results over $ZnSO_4$ 0.2%, $FeSO_4$ 0.2%, $FeSO_4$ 0.4%, $ZnSO_4$ 0.2% + $FeSO_4$ 0.2% and $ZnSO_4$ 0.4% + $FeSO_4$ 0.2%. For plant height, same treatment accorded significant effect compared to $ZnSO_4$ 0.4% and $ZnSO_4$ 0.4% + $FeSO_4$ 0.2%. $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% gave at *par* result for maximum leaf area with $ZnSO_4$ 0.4% and $FeSO_4$ 0.4%. Fresh and dry weight of leaves/plant influenced by ZnSO4 0.4% + FeSO4 0.4% were at *par* with $ZnSO_4$ and $FeSO_4$ at 0.4%. The results are in agreement with the study for combined application of zinc and iron on lilium cv. Tresor (Hembrom and Singh, 5). Chlorophyll content (73.33) was increased with $ZnSO_4$ 0.4% treatment which was at par with $ZnSO_4$ 0.4% + $FeSO_4$ 0.4%. Similar observations were made by Singh *et al.* (13) in lilium. Memon *et al.* (8) also described significance of zinc application in promoting the growth of phlox. Zinc is a constituent of metallo-enzymes, which is obligatory for many physiological reactions in plants (Uchida, 15). The enzyme carbonic anhydrase is peculiarly activated by zinc, which raises CO_2 volume in chloroplast thereby increasing carboxylation rate of the enzyme RuBisCO where CO_2 is converted into organic carbon sugars through photosynthesis. Severe inadequacy of iron has detrimental effect on cell division and therefore reduces growth of leaves (Mohamed and Aly, 9). It acts as an activator for several biochemical processes such as respiration, photosynthesis and symbiotic nitrogen fixation. These results validate the substantial role of zinc and iron in accumulation of photo-assimilates for growth and development of plants.

The aftermath of foliar spray of zinc and iron examined for flowering characters were found to exhibit significant influence (Table 2). Application of $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% improved No. of flower buds/ plant (4.67), length of flower stalk (15.53 cm), length of pedicel (9.10 cm) and diameter of 1st flower (15.50 cm). Days to flower colour show (47.00) and

Table 1. Effect of foliar application of zinc and iron on growth parameters in lilium cv. Novana.

Treatment	No. of leaves/ plant	Stem dia. (mm)	Plant height (cm)	Fresh wt. of leaves/ plant (g)	Dry wt. of leaves/ plant (g)	Leaf area (cm^2)	Chlorophyll content (SPAD value)
Control (distilled water)	54.00	11.56	44.00	4.13	0.58	194.66	39.67
$ZnSO_4$ 0.2%	47.00	10.67	48.67	4.17	0.55	168.24	42.33
$ZnSO_4$ 0.4%	50.33	11.22	36.33	7.45	0.94	275.47	73.33
$FeSO_4$ 0.2%	50.00	10.37	45.33	4.99	0.63	194.43	51.67
$FeSO_4$ 0.4%	49.67	9.96	47.67	6.15	0.82	279.82	61.33
$ZnSO_4$ 0.2% + $FeSO_4$ 0.2%	50.67	11.56	45.67	4.99	0.62	207.78	53.00
$ZnSO_4$ 0.2% + $FeSO_4$ 0.4%	43.33	10.46	46.33	5.47	0.62	208.34	54.33
$ZnSO_4$ 0.4% + $FeSO_4$ 0.2%	49.33	10.63	43.00	5.40	0.66	224.03	56.33
$ZnSO_4$ 0.4% + $FeSO_4$ 0.4%	57.00	12.26	52.00	8.33	0.98	306.31	68.33
CD at 5%	2.75	1.15	6.82	2.45	0.18	73.57	8.48

Table 2. Effect of foliar application of zinc and iron on flowering parameters in lilium cv. Novana.

Treatment	No. of buds/ plant	Flower stalk length (cm)	Pedicel length (cm)	Days to flower colour show	Days to opening of 1st bud	Diameter of 1st flower (cm)
Control (distilled water)	3.00	12.23	8.13	54.00	55.00	14.50
$ZnSO_4$ 0.2%	3.67	15.20	8.23	49.00	51.33	15.00
$ZnSO_4$ 0.4%	2.50	10.05	7.10	55.00	56.50	14.87
$FeSO_4$ 0.2%	2.33	11.37	8.23	52.67	54.33	15.17
$FeSO_4$ 0.4%	3.00	9.50	7.27	51.67	53.33	14.90
$ZnSO_4$ 0.2% + $FeSO_4$ 0.2%	3.33	12.87	7.87	49.00	51.00	13.87
$ZnSO_4$ 0.2% + $FeSO_4$ 0.4%	3.00	15.20	8.60	47.00	49.67	15.30
$ZnSO_4$ 0.4% + $FeSO_4$ 0.2%	3.00	12.07	8.63	47.00	50.33	14.37
$ZnSO_4$ 0.4% + $FeSO_4$ 0.4%	4.67	15.53	9.10	48.33	50.67	15.50
CD at 5%	1.23	3.84	1.23	3.82	4.21	1.20

days to opening of 1st bud (49.67) were noticed to be early with treatment $ZnSO_4$ 0.2% + $FeSO_4$ 0.4%. Treatment combination of zinc and iron gave exceptional outcomes over $ZnSO_4$ 0.4%, $FeSO_4$ 0.2% and $FeSO_4$ 0.4%. The results were in accord with findings of Hembrom and Singh (5) in lilium. Singh *et al.* (14) also reported beneficial effects of zinc on flowering parameters in gladiolus cv. Pink Friendship. Nasiri and Najafi (12) also reported role of zinc and iron for better flowering in chamomile. Zinc is required in synthesis of tryptophan which is precursor of indole acetic acid (IAA) that helps in axillary growth. It also enact in synthesis of RNA and protein (Moroney, 10). Iron governs reduction of nitrates and sulphates which are imperative for proper development and reproduction of plants (Eskandari, 2).

The impact of foliar spray of zinc and iron on post-harvest quality of lilium are enlisted in Table 3. Postharvest quality determined by longevity of 1st, 2nd and 3rd flower and vase-life has been illustrated in Fig. 1 and 2, respectively. Plants treated with $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% produced spectacular results for all the quality parameters. $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% sprayed plants had the highest longevity of 2nd flower (9.33 days), which was at par with $FeSO_4$ 0.4% and $ZnSO_4$ 0.2% + $FeSO_4$ 0.2% (Fig. 1). Same treatment was observed to be significant to $ZnSO_4$ 0.2%, $ZnSO_4$ 0.4% and $FeSO_4$ 0.4% for longevity of 3rd flower (9.00 days). Higher dose combination of zinc and iron, *i.e.* $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% displayed highest No. of buds opened (4.33) and No. of buds opened at a time (3.33) in vase which was at par with $ZnSO_4$ 0.2% and $ZnSO_4$ 0.2% + $FeSO_4$ 0.2%. Weight of stem on 1st (31.91 g), 3rd (29.48 g) and 5th (23.71) day in vase solution was recorded to be highest in the treatment $ZnSO_4$ 0.4% + $FeSO_4$ 0.4%. Pre-harvest

Table 3. Effect of foliar application of zinc and iron on post-harvest life in lilium cv. Novana.

Treatment	No. of buds opened	No. of buds opened at a time	Stem wt. (g)			Stem wt. (g)
			1st day	3rd day	5th day	
Control (distilled water)	3.00	2.67	23.31	21.61	18.30	7.30
$ZnSO_4$ 0.2%	3.33	3.00	29.55	26.81	23.56	9.43
$ZnSO_4$ 0.4%	2.50	2.50	24.66	23.52	20.68	5.36
$FeSO_4$ 0.2%	2.33	2.33	21.18	19.25	16.93	6.98
$FeSO_4$ 0.4%	2.67	2.33	21.70	20.18	18.06	7.49
$ZnSO_4$ 0.2% + $FeSO_4$ 0.2%	3.33	3.00	26.65	24.82	22.08	8.85
$ZnSO_4$ 0.2% + $FeSO_4$ 0.4%	3.00	3.00	25.07	21.56	19.39	9.31
$ZnSO_4$ 0.4% + $FeSO_4$ 0.2%	3.00	2.67	25.19	21.86	19.74	8.39
$ZnSO_4$ 0.4% + $FeSO_4$ 0.4%	4.33	3.33	31.91	29.48	23.71	10.79
CD at 5%	1.30	0.49	4.53	4.05	3.36	1.85

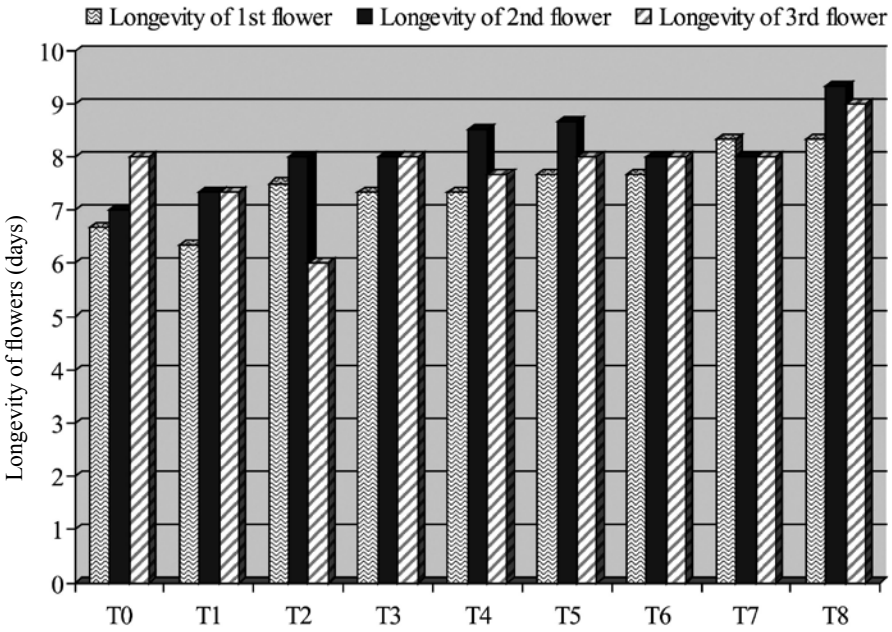

Fig. 1. Longevity of lilium flowers as affected by different treatments of zinc and iron. T0 = control (distilled water), T1 = $ZnSO_4$ 0.2%, T2 = $ZnSO_4$ 0.4%, T3= $FeSO_4$ 0.2%, T4 = $FeSO_4$ 0.4%, T5 = $ZnSO_4$ 0.2% + $FeSO_4$ 0.2%, T6 = $ZnSO_4$ 0.2% + $FeSO_4$ 0.4%, T7 = $ZnSO_4$ 0.4% + $FeSO_4$ 0.2%, and T8 = $ZnSO_4$ 0.4% + $FeSO_4$ 0.4%.

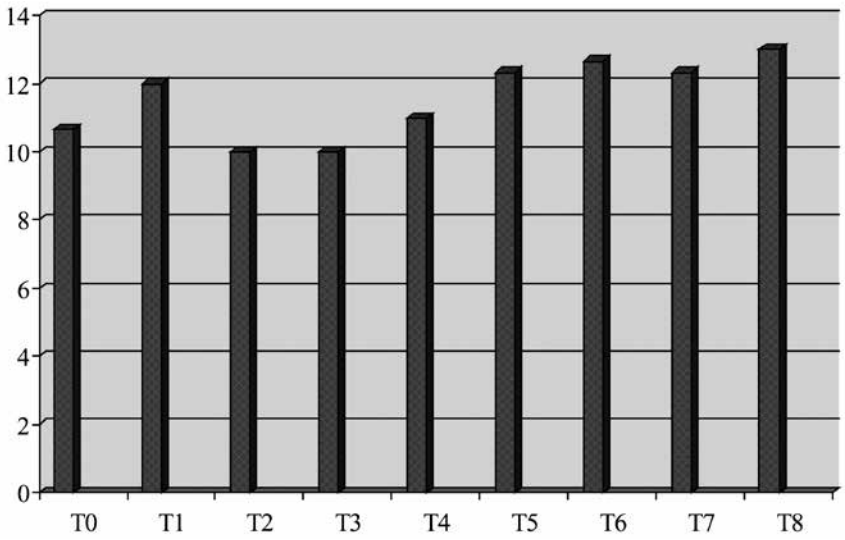

Fig. 2. Vase-life of lilium as affected by different treatments of zinc and iron. T0 = control (distilled water), T1 = $ZnSO_4$ 0.2%, T2 = $ZnSO_4$ 0.4%, T3 = $FeSO_4$ 0.2%, T4 = $FeSO_4$ 0.4%, T5 = $ZnSO_4$ 0.2% + $FeSO_4$ 0.2%, T6 = $ZnSO_4$ 0.2% + $FeSO_4$ 0.4%, T7 = $ZnSO_4$ 0.4% + $FeSO_4$ 0.2%, and T8 = $ZnSO_4$ 0.4% + $FeSO_4$ 0.4%.

treatment of $ZnSO_4$ 0.4% + $FeSO_4$ 0.4% also led to increase in the weight of stem after withering (10.79 g) and enhanced vase-life (13.00 days), which was at par with treatments $ZnSO_4$ 0.2% and $ZnSO_4$ 0.2% + $FeSO_4$ 0.4% (Fig. 2). Singh *et al.* (13) also observed cumulative effect of zinc and iron on various post-harvest parameters in lilium. Fahad *et al.* (3) reported application of zinc and iron to improve vase-life and other postharvest parameters of gladiolus. Similar findings were also cited by Chopde *et al.* (1) in gladiolus and Karuppaiah (6) in chrysanthemum. Zinc has been reported to increase permeability of plasma membranes and stabilizes the bio-membranes. It also determines structural orientation of macromolecules

present within membranes and controls the membrane integrity. Therefore, zinc aids in proper absorption and translocation of vase solution and helps in maintaining turgidity and integrity of cells so that flowers can be kept in vase for longer duration. This study has demonstrated the positive effect of micronutrients like zinc and iron on growth and development of lilium.

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Evaluation of perennial chrysanthemum cultivars under sub-humid southern plains and Aravali hills of Rajasthan

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ABSTRACT

An investigation was carried out with eleven cultivars of *Dendrenthema grandiflora* L. at Udaipur, Rajasthan to evaluate the cultivars for floral and relative economic parameters. The experiment was laid out in randomized block design with three replications, eleven cultivars by planting 25 plants/ replication and data were recorded on five plants. The data were analyzed by analysis of variance. The maximum plant height (44.21 cm), leaves plant^{-1} (185.87), plant spread from North-South (27.31 cm) and East-West direction (28.34 cm), sprays plant^{-1} (16.73), days to end of flowering (145.33 days), flower duration (63.47 days), freshness of flower on the plant under open field (17.53 days), flowers plant^{-1} (66.86), ten-flower weight (37.30 g), ray floret flower^{-1} (187.60), spray length (34.56 cm), disc floret flower^{-1} (209.27), flower weight plant^{-1} (249.52 g), gross, net returns and B:C ratio were recorded in 'UHF CRY-77'. Minimum days to first bud initiation (71.07 days), first flower bud opening (84.42 days), complete flower opening (92.8 days) were recorded in 'Autumn Joy'. The maximum flower diameter (9.68 cm) recorded in cultivar in cv. Garden Beauty, respectively. On the basis of two years pooled data for vegetative, floral and relative economics parameters cv. UHF CRY-77 for cut flowers followed by 'Jaya' were found best for cut spray purpose and recommended for sub-humid southern plain and Aravalli hills of Rajasthan.

Key words: Cut flower, chrysanthemum, plant spread, flowers plant^{-1}, ray florets.

INTRODUCTION

Chrysanthemum often called mums or this genus (*Chrysanthemum*) contains 30 species a plant belongs to family Asteraceae. *Dendranthema grandiflora* Tzevlrev has basic chromosome number χ = 9 and which is hexaploid in nature, *i.e.* 2n = 54. It is native from Asia and North-Eastern Europe. Chrysanthemum words derived from Greek word '*Chryos*' means golden and '*anthos*' means flower. It is also known as 'Queen of the East' in English, 'Guldaudi' in Hindi and wide variation showed by its large number of cultivars in respect of growth habit, size, colours and shape of bloom make the chrysanthemum suitable for various purposes like cut flower, loose flower, standard and pot mum production. Besides this certain species like *Chrysanthemum cineraiifolium* is grown in temperate regions for making an insecticide called pyrethrum and *C. coccineum* is called 'Painted Daisy' is also grown in temperate countries from seeds. Ryori Giku is a yellow flowering culinary type, which is eaten as delicacy in Japan after frying. However, scanty research works are available on hence, the present investigation was carried out to evaluate perennial chrysanthemum (*D. grandiflora* Tzevelrev) cultivars under sub-humid southern plains and Aravalli hills of Rajasthan.

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MATERIALS AND METHODS

The experiment was conducted at AICRP on Floriculture, Horticulture Farm, RCA Campus, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan during July 2010-April 2012. This is situated at 24º35′ N latitude and 24º42′ E longitude at an altitude of 579.5 m above mean sea level. The region falls under agro-climatic zone IV A-sub humid southern plain and Aravalli hills of Rajasthan. The experiment was conducted on clayey loam soil with pH 8.4 and EC 0.54 dS/m under irrigated conditions. The cultivars used, namely, 'Ajina Purple' (T_1), 'Anmol-F_1' (T_2), 'Autun Joy' (T_3), 'Garden Beauty' (T_4), 'Jaya' (T_5), 'PAU-A-43' (T_6), 'PAU-B-107' (T_7), 'PAU-D-1' (T_8), 'UHFS Chry-83' (T_9), 'UHFS Chry-77' (T_{10}) and 'Winter Queen' (T_{11}). The well-decomposed farm yard manure @ 35 t ha^{-1} was incorporated in all plots 4 week prior to planting. A basal fertilizer dose comprising @ 150 kg N and 150 kg P_2O_5 ha^{-1} at planting time and remaining 150 kg of N was applied at 30 days after planting as suggested by Chawla *et al.* (3). Uniform cultural practices were adopted during the experiment. The terminal rooted cutting was planted at 30 cm row to row and 30 cm from plant to plant on well prepared bed during July, 2010 and 2011 with bed size 1.5 × 1.5 m^2 on flood irrigation system in three replications and 11 varieties used as a treatment in randomized block design. The

observations were recorded on various parameters, *viz*. plant height, number of leaves, plant spread, number of spray, days to first bud initiation, days to first flower bud opening, days to complete flower opening, days to end of flowering, flowering duration, freshness of the flower on the plant under field, flower colour, flower diameter, flowers plant^{-1}, weight of ten flowers, ray floret flower^{-1}, spray length, disc floret flower^{-1}, flower yield plant^{-1}, cost of production, spray yield, suckers, gross returns, net returns ha^{-1} and B:C ratio. The data were recorded on five plants and all the mean value of the recorded data were statistically analyzed as per the method suggested by Gomez and Gomez (7) at 5% level of significance.

RESULTS AND DISCUSSION

Out of eleven cultivars evaluated for their vegetative, floral and economic parameters the pooled data revealed in Table 1 showed significant difference for plant height. The maximum plant height was recorded in cv. UHFS CHRY-77 (44.21 cm), whereas, it was minimum in 'Autumn Joy' (17.87 cm). This variation in plant height among various cultivars may be due to the hereditary traits and prevailing environmental conditions, which resulted in varied growth rate. Similar results were recorded by Rao and Partap (15) who found that plant height ranges from cv. Punjab Gold (25.80 cm) to Neelima (57.33 cm) in chrysanthemum.

Whereas, pooled data revealed that there was a significant difference in number of leaves plant^{-1} among all the cultivars at the stage of the first bud initiation. The maximum leaves plant^{-1}, *i.e.* 185.87 was recorded in cv. UHFS CHRY-77 at time of first bud initiation. The variation in number of leaves plant^{-1} among different cultivars might be due to the distinguished varietal inherent genetic makeup of a particular cultivar as a result, variations in phenotypic expression were expected to occur. Hence, wide variations for vegetative characters were observed.

However, pooled data indicated for maximum plant spread from North-South (27.31 cm) and east-west direction (28.34 cm) were obtained in cv. UHFS CHRY-77. Whereas, minimum north-south (11.69 cm) and east west (12.07 cm) plant spread were observed in 'UHFS CHRY-83', while rest of the cultivar came in between the range. This plant spread increase was mainly due to production of increased number of branches and wider angles from point of origin. Greater plant spread shows better vegetative growth of plant. Present findings are in conformity with the findings of Peddi *et al.* (12) who reported range of earlier cv. (18.47 cm) 'Basanthi' to (34.77 cm) 'Raichur' in chrysanthemum showing much variation.

The pooled data revealed that there was a significant difference among all the cultivars with respect to number of spray produced plant^{-1}. The maximum number of sprays plant^{-1} were recorded in cv. UHFS CHRY-77 (16.73), which was statistically at *par* with cv. Jaya (15.33), while minimum number of sprays plant^{-1} were noted in cv. UHFS CHRY-83 (5.93). Present findings for number of sprays

Table 1. Vegetative and floral parameters in perennial chrysanthemum under sub-humid southern plains and Aravalli hills of Rajasthan.

Cultivar	Plant height (cm)	No. of leaves plant^{-1}	Plant spread North-South (cm)	Plant spread East-West (cm)	Spray plant^{-1}	First bud initiation (days)	Flower bud opening (days)	Complete flower bud opened (days)
Ajina Purple	20.83	100.98	17.47	16.52	8.60	79.42	99.02	105.68
Anmol F-1	21.54	61.93	25.55	26.33	9.27	96.87	111.92	118.02
Autman Joy	17.87	76.00	14.51	15.39	8.53	71.07	84.42	92.80
Garden Beauty	35.15	45.63	24.45	23.48	9.20	79.78	98.57	105.47
Jaya	37.93	79.40	21.45	21.47	15.33	79.87	101.93	109.20
PAU A-43	25.91	129.77	26.33	25.90	14.60	92.07	110.20	115.93
PAU B-107	25.24	41.12	24.28	23.22	11.67	80.05	99.48	107.02
PAU D-1	34.45	153.53	26.87	26.93	12.13	79.93	96.73	105.00
UHFS CRY-83	21.61	29.53	11.69	12.07	5.93	80.27	99.80	106.60
UHFS CRY-77	44.21	185.87	27.31	28.34	16.73	78.53	95.33	101.73
Winter Queen	35.23	173.80	25.12	24.01	10.00	80.00	99.93	107.00
$CD_{0.05}$	2.22	5.60	2.06	1.60	1.23	5.20	6.25	4.57

plant^{-1} are in conformity with the findings reported by Gaikwad and Dumbre-Patil (6) that range from cv. (11.88), Mountaineer to (20.16), Indira in chrysanthemum and Kumar *et al*. (8) in dahlia cv. Jyotsna (11.67).

Whereas, pooled data indicate that days to first bud initiation was earlier in cv. Autumn Joy (71.07 days), followed by UHFS CHRY-77 (78.53 days), whereas it was very late in Anmol F_1 (96.87 days). Similar findings are reported by Swaroop *et al.* (16), which ranges from 80.66 days (Yellow Bangla) to 108.33 days (Thai Chin Queen) in chrysanthemum. The variation in number of days to first bud initiation was primarily due to the different genetic constitution of various cultivars under prevailing environmental conditions during the period of crop growth.

Moreover, pooled data indicated days to first flower bud opening ranged from a minimum in 84.42 days (Autumn Joy) to a maximum in 111.92 days (Anmol F_1) days after transplanting. Present findings are in conformity with the findings of Peddi *et al.* (12) as reported minimum days to first flower bud initiation observed in cv. CO-1 (63.46 days) to maximum in cv. Raichur (97.67 days), Swaroop *et al.* (16) found first bud initiation of 83.50 days in cv. Thai Chin Queen, Rao and Pratap (15) reported the range from 77.00 days in cv. Ravikiran to 86.00 days in cv. Yellow Gold in chrysanthemum and Kumar *et al.* (8) found cv. NT Pompon with bud initiation of 81.60 days in dahlia. This variation in number of days to first flower bud opening in various cultivars of chrysanthemum may be due to different genetic makeup and prevailing environmental conditions.

Although, pooled data revealed that maximum days to complete flower opening after transplanting were recorded 118.02 days in cv. Anmol F_1 followed by PAU A-43 (115.93 days), whereas, minimum in Autumn Joy (92.8 days) . The present findings are in close conformity with the results of Mishra *et al*. (11) in dahlia. Similar findings were also noted by Kumar *et al*. (8) in dahlia, *i.e.* 113.57 to 136.07 days and Rao and Pratap (15) in chrysanthemum. This variation may be attributed because of varied genetic makeup of different cultivars along with prevailing environmental conditions.

Whereas, pooled data are presented in Table 2 showed significant differences among different cultivars with respect to days to end of flowering under study. The longest duration taken to end of flowering was noted in cv. UHFS CRY-77 (145.33 days) followed by Jaya (145.30 days), and Ajina Purple (144.62 days) were statistically at par, while it was shortest in 'Autumn Joy' (129.13 days). This variation in days to end of flowering among various cultivars may be due to the hereditary traits and prevailing environmental conditions, which resulted in varied growth rate. In chrysanthemum similar findings are reported by Peddi *et al.* (12), *i.e.* which range from 102.33 (Co-1) to 151.0 days (Raichur).

However, pooled data indicated highly significant variation for flower duration which ranges from a minimum in 'PAU A-43' (48.23 days) to maximum in 'UHFS CHRY-77' (63.47 days). Present results are in close conformity with the findings obtained by Deepa and Chezhiyan (4) showing range of 47.50 days (Acc1) to 89.50 days (Acc3). Earlier, Swaroop *et al.*

Table 2. Floral parameters in chrysanthemum under sub-humid southern plains and Aravalli hills of Rajasthan.

Cultivar	End of flowering (days)	Flower duration (days)	Freshness of flower (days)	Flower plant^{-1}	Flower dia. (cm)	Ten-flower wt. (g)	Ray florets flower^{-1}	Spray length (cm)	Disc floret flower^{-1}
Ajina Purple	144.62	49.40	14.87	28.47	4.09	9.18	92.48	12.68	62.30
Anmol F-1	141.13	56.33	15.33	31.95	3.99	8.79	28.80	14.99	139.47
Autumn Joy	129.13	49.28	16.13	25.96	6.53	21.74	131.73	12.46	29.80
Garden Beauty	136.74	48.75	15.28	32.23	9.68	17.43	49.05	25.92	171.13
Jaya	145.30	60.15	16.98	59.74	6.01	32.63	170.13	26.52	90.87
PAU A-43	139.60	48.23	14.13	52.76	6.73	10.77	50.73	18.81	147.33
PAU B-107	139.03	49.87	14.73	40.76	6.05	28.27	148.33	18.67	28.40
PAU D-1	139.78	53.90	14.40	43.04	6.25	30.96	154.00	24.95	60.07
UHFS CRY-83	137.77	48.40	12.53	15.62	3.70	9.92	182.40	14.89	12.07
UHFS CRY-77	145.33	63.47	17.53	66.86	7.18	37.30	187.60	34.56	209.27
Winter Queen	144.00	51.27	12.47	34.43	8.87	8.44	72.33	25.92	115.13
$CD_{0.05}$	7.21	3.35	1.28	7.30	0.57	1.72	11.19	1.56	8.26

(16) recorded 56.83 days in cv. Flirt in chrysanthemum and Kumar *et al.* (8) in 90.73 days (NT Pompon) in dahlia. This variation in flower duration among various chrysanthemum cultivars may be due to different genetic makeup, which might be further modified by the prevailing environmental conditions.

Moreover, the pooled data revealed that freshness of flower on the plant under open field conditions ranged from a minimum in cv. Winter Queen (12.47 days) to maximum in cv. UHFS CHRY-77 (17.53 days). This variation in chrysanthemum cultivars may be due to different genetic makeup of cultivar, which is affected by the prevailing environmental condition, ultimately, which affects physiological processes of the plant like cell turgidity, water loss through evaporation, transpiration and breakdown of the reserve food material, which reduces the freshness of the flower under field conditions. These results are in conformity with the findings of Mishra *et al*. (11) as reported the longevity of flower under open field condition ranges from 13.32 (Vigour) to 14.41 days (Kenya) in dahlia; and Kumari *et al*. (10) reported shelf-life in gerbera cv. Balance (10.11 days) at 25ºC ambient temperature and (15.30 days) at 18ºC temperature, respectively.

Whereas, the pooled data revealed that there are significant differences for number of flowers plant^{-1} among all the chrysanthemum cultivars during experimentation. The maximum number of flowers plant^{-1} was recorded in cv. UHFS CHRY-77 (66.86), followed by Jaya (59.74), while minimum was observed in 'UHFS CHRY-83' (15.62). The variation in number of flowers plant^{-1} may be due to genetic variability among the different chrysanthemum cultivars, which were tested under this trial. Another probable reason for this variation in number of flowers plant^{-1} may be due to effect of environmental condition prevailing during field trial. Similar variation for number of flowers plant^{-1} were reported by Puneeta *et al*. (14) in cv. Suneel (66.33) to cv. Paris White (301), Dilta *et al.* (5) in cv. Glance (65.67 flower / plant) in chrysanthemum.

Although, the pooled data showed that flower diameter differed significantly and maximum flower diameter was recorded in cv. Garden Beauty (9.68 cm), while minimum in cv. UHFS CHRY-83 (3.70 cm). Similar findings were obtained by Poonam and Kumar (13) in cv. Garden Beauty (9.97 cm); Rao and Pratap (15) in cv. Silper (5.83 cm) in chrysanthemum. It may be concluded that variation in flower diameter is mainly due to genetic makeup, which might have been further modified by the prevailing environmental conditions.

However, pooled data revealed that maximum ten flower weight was recorded in cv. UHFS CRY-77 (37.30 g), while, minimum in Winter Queen (8.44 g). Variation in flower weight might be due to different genetic makeup of the different cultivars. The present findings are in conformity with the results reported by Poonam and Kumar (13) reported range in cv. Sadhbhawna (10.62 g) to Ratlam Selection (46.00 g); and Swaroop *et al.* (16) in cv. Pink Cloud (8.5 g) to Thai Chin Queen (70.6 g) in chrysanthemum.

Moreover, pooled data result shows that maximum ray florets flower^{-1} was recorded in cultivar UHFS CRY-77 (187.60), while minimum in 'Anmol F_1' (28.80). The variation in number of ray florets flower^{-1} among different cultivars might be due to the distinguished varietal inherent genetic makeup of a particular cultivar. Similarly wider variations for ray florets flower^{-1} are observed by Swaroop *et al.* (16) that range from 90.00 to 308.66 in cvs. Snow Ball to Flirt; Baskaran *et al.* (1) in chrysanthemum cv. Nilima (253.2).

However, pooled data revealed that there was highly significant difference among all the cultivar for the spray length. The maximum spray length was recorded in 'UHFS CHRY-77' (34.56 cm), while minimum was in cv. Autumn Joy (12.46 cm). This variation in spray length might be due to the distinguished genetic makeup among various cultivars, which were used for investigation. Similar findings are recorded by Rao and Pratap (15) for spray length (22.50 cm) in cv. Neelima; Poonam and Kumar (13) reported the range from 22.30 cm in cv. Punjab Anuradha to 58.73 cm in cv. Garden Beauty. Peddi *et al*. (12) too obtained similar variation in chrysanthemum cultivars.

Although, pooled data results shows that maximum numbers of disc florets flower^{-1} were recorded in cultivar UHFS CHRY-77 (209.27), whereas minimum in cultivar UHFS CRY-83 (12.07). The variations in number of disc florets were primarily due to the different genetic constitution of various cultivars under study. Hence, wide variations for yield contributing characters were observed. Similar results are found by Kumar *et al.* (9) in cv. Basanti disc florets (160.00) produced in chrysanthemum.

Whereas, the pooled data in Table 3 indicate highly significant differences among different chrysanthemum cultivars for flower yield per plant. The flower weight plant^{-1} ranged from minimum in cv. UHFS CHRY-83 (15.59 g) to maximum in cv. UHFS CHRY-77 (249.52 g). The different genetic makeup contributed different growth and yield attributing character for flower weight plant^{-1} in cultivars of chrysanthemum. Thus, the cultivar UHFS CHRY-77 was found to be high yielder followed by cultivars Jaya and PAU D-1. Similar findings were also noted by Peddi *et al.* (12) on chrysanthemum.

The variation in flower colour among chrysanthemum cultivars (Table 3) is mainly due to genetic makeup and colouring pigments present in

Table 3. Relative economic parameters in chrysanthemum under sub-humid southern plains and Aravalli hills of Rajasthan.

Cultivar	Flower yield plant^{-1} (g)	Flower colour as par R.H.S Colour Chart	Spray ha^{-1} (No.)	Suckers ha^{-1} (No.) (Rs.)	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	B:C ratio
Ajina Purple	26.03	Red purple 61(A)	955555.5	505555.51	651944.40	284661.30	0.78
Anmol F-1	28.08	Yellow Group 9(A)	1029999.9	438888.85	676222.16	308939.06	0.84
Autumn Joy	56.51	Red purple 62(D)	947777.7	472222.18	639333.28	272050.18	0.74
Garden Beauty	56.13	Red purple 71(C)	1022222.1	405555.52	663611.04	296327.94	0.81
Jaya	195.95	Red Group 46(A)	1703333.2	399999.96	1036833.25	669550.15	1.82
PAU A-43	56.84	Yellow Group 8(A)	1622222.1	116666.66	921388.82	554105.72	1.51
PAU B-107	115.13	White Group NN 155(D)	1296666.5	516666.62	842333.23	475050.13	1.29
PAU D-1	133.10	White Group NN 155(D)	1347777.6	522222.17	871833.22	504550.12	1.37
UHFS CRY-83	15.59	Yellow Group 6(A)	658888.8	116666.66	391555.51	24272.41	0.07
UHFS CRY-77	249.52	White Group NN 155(C)	1858888.7	516666.62	1151555.44	784272.34	2.14
Winter Queen	29.13	Red purple 70(C)	1111111.0	522222.17	741666.59	374383.49	1.02
$CD_{0.05}$	10.92	-	-	-	-	-	-

Estimated total cost of cultivation was Rs. 3,67,283.10 ha^{-1} selling price for each spray @ Rs. 0.55 and suckers @ Rs. 0.25.

a particular genotype. Red colour flower is due to anthocyanin pigment in cultivar Jaya, yellow colour flower is due to chalcones and aurones as colouring matter exist in cultivars UHFS CRY-83, PAU-A-43 and Anmol F_1. The white colour of flower is due to flavonols and carotenoid pigment exist in 'UHFS CHRY-77', 'PAU B-107' and 'PAU D-1', while purplish colour in 'Ajina Purple', 'Winter Queen', 'Autumn Joy' and 'Garden Beauty' is due to cyanidin pigment as reported by Bhattacharjee (2). These findings are in close conformity with the results obtained by Kumar *et al.* (8) in various dahlia cultivars.

Relative economics were calculated for different cultivars under study (Table 3) revealed that cultivar 'UHFS CHRY-77' was found best with a gross income of Rs. 11,51,555.44 ha^{-1} on the basis of number of cut spray 18,58,888.7 and number of suckers 5,16,666.62 obtained ha^{-1}. The selling price for each cut spray and per suckers were Rs 0.55 and 0.25, respectively. Hence, the maximum net income of Rs. 7,84,272.34 ha^{-1} was obtained from cv. 'UHFS CHRY-77' while the cost of cultivation was Rs. 3,67,283.10 ha^{-1}. Among various cultivars the net returns / Rs investment (B: C ratio) was maximum, *i.e.* Rs 2.14 in cultivar UHFS CHRY-77, while it was minimum in 'UHFS CHRY-83', *i.e.*, Rs. 0.07.

On the basis of two year experimentation (2010-12) pooled data it was concluded that cv. UHFS CHRY-77 was found best for maximum flower duration, freshness of the flower, number of flowers plant^{-1}, flower weight plant^{-1} and B: C ratio, therefore, recommended for cut flower production for sub- humid southern plains and Aravalli hills of Rajasthan.

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Shelf-life extension of pear with coatings under ambient and super market conditions

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ABSTRACT

Pear fruits of cv. Punjab Beauty were harvested at firm mature stage. The fruits were coated with different food grade Nipro Fresh SS-40T™ and SS-50™ coatings. After coating, the fruits were packed in corrugated fibre board cartons and stored under two different temperature conditions, *i.e.* under super-market conditions (20-22°C and 80-85% RH), and under ambient conditions (30-32°C and 70-80% RH). The fruits were evaluated for various quality attributes periodically. The data revealed that both the coatings proved effective in minimizing weight and firmness loss and maintained quality attributes as evident from higher total soluble solids (TSS), minimum microbial decay and optimum organoleptic quality. These coatings helped in delaying the ripening process of fruits and the one coated with Nipro Fresh SS-40™ and SS-50™ can be stored for 12 days under ambient conditions and 15 days under super market conditions. On the other hand, the control fruits maintained their storage life for 6 and 9 days under both the conditions, respectively.

Key words: Fruit coatings, pear, quality, storage conditions.

INTRODUCTION

Pear is an important fruit of sub-tropical India growing primarily in north-west parts. The important pear cultivars grown are Patharnakh, Punjab Nakh, Punjab Gold, Punjab Nectar, Punjab Beauty, Punjab Soft, Nijisseiki *etc*. Punjab Beauty is a semi-soft cultivar, preferred by the farmers, traders and consumers due to its juicy pulp and crisp texture. The harvesting of fruits of this cultivar start in the third week of July that continues up to the mid of August. Generally, this period coincides with high rainfall and high temperature, which interferes with post-harvest quality and marketability of fruits. Therefore, the farmers are forced to sell their produce during this period sometimes at a throw away price due to lack of knowledge about post-harvest handling practices that leads to glut in the market, resulting in huge post-harvest losses. The fruits have a natural wax coating, which develops during the maturation and ripening processes. However, during handling of the fruits the natural wax gets destroyed, as a result, bruising occurs during packing and transport operation. Therefore, the application of commercial food grade waxes is important to replace this loss during post-harvest period. Coating or waxing reduce shriveling, wilting and respiration rate of fruits and enhances the gloss and cosmetic appearance of fruits (El-Anany *et al.*, 5). The use of food grade wax coating on fruits is safe, and approved for application on fresh fruits and vegetables (PFA, 11).

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The concept of super market is coming up in the country and many leading corporate sectors have opened their outlets in various cities, where different types of fruits and vegetables are displayed after coatings and packaging that has an added advantage of maintaining freshness and produce quality. Therefore, the present investigation was planned to study the effect of coatings on the shelf-life and quality of pear fruits under ambient (30-32°C; 70-80% RH) as well as super-market (20-22°C; 80-85% RH) conditions.

MATERIALS AND METHODS

The 'Punjab Beauty' pear fruits of uniform size, disease and bruise-free were picked randomly from all the four directions of the plants at physiological mature stage and shifted to laboratory. The fruits were then sorted, graded and washed in chlorine solution (100 ppm). Thereafter, fruits were divided into requisite lots for further handling. In the present studies, two commercial formulations, *viz*. Nipro Fresh SS-40 T™ and SS-50™ were used for application on pear fruits. These coatings were procured from Nipro Technologies Limited, Panchkula, Haryana. The coatings were applied on fruit surface manually with a piece of foam pad. The coated and control fruits were then stored under two temperature conditions, *i.e.*, under super-market (20-22°C; 80-85% RH) and under ambient (30-32°C; 70-80% RH). The various physico-chemical parameters of fruits were recorded at three day intervals up to 15 day for

ambient conditions and up to 18 day for super market conditions. The physiological loss in weight (PLW) was calculated on initial weight basis and expressed in per cent. The fruit firmness was measured with the help of a penetrometer (Model FT-327, USA) using 8 mm stainless steel probe and expressed in terms of pounds force pressure (lb force). The overall organoleptic rating of the fruits was done by a panel of ten judges on the basis of external appearance of fruits; texture, taste, and flavour by making use of a 9-point hedonic scale (Amerine *et al.*, 1).

The total soluble solids (TSS) of the fruit juice were determined using a hand refractometer. The titratable acidity was estimated by titrating the known volume of juice against N/10 NaOH using phenolphthalein as an indicator. The decay percentage of treated and untreated fruits was calculated as the number of decayed fruit divided by initial number of all fruits multiplied by hundred (El-Anany *et al.*, 5). Pectin methyl esterase activity was determined as per method described by Mahadevan and Sridhar (8). There were four replications for each treatment and each replication comprised of 25 fruits. The experiment was laid-out in completely randomized design and analyzed for variance by using SAS (V9.3, SAS Institute INC, and Cary NC, USA) package.

RESULTS AND DISCUSSION

It was noticed that SS-40 T coated fruits registered the lowest average PLW (3.07%) followed by SS-50 coated fruits (3.14%) under ambient conditions (Table 1). The PLW in SS-40 T™ and SS-50™ coated fruits, ranged between 0.45 to 6.95 and 0.52 to 7.10 per cent from 3 to 15 days of storage as compared to control where PLW was found to be the highest (9.31%) and ranged between 3.88 to 14.22 per cent. However, under super market conditions, the lowest mean PLW (3.37%) was observed in fruits coated with SS-40T closely followed by SS-50™ coated fruits (3.55%) (Table 2). On the other hand, the highest mean PLW (6.60%) was observed in control fruits. In pear fruits, permissible limit of weight loss is 6% to maintain the market acceptability (Singh *et al.*, 13). Keeping in view the acceptable level of PLW, it can be visualized from the data that under ambient conditions (28-30°C), the SS-40T™ and SS-50™ coated fruits can be stored for 12 days. On the other hand under super market conditions (18-20°C), the desirable weight loss was noticed up to 15 days in SS-40T™ and SS-50™ coated fruits. The control fruits maintained acceptable weight loss for market acceptability only up to 6 and 9 days, respectively under both the storage conditions. The application of coatings have been reported to play an important role in lowering the weight loss of mango (Baloch and Bibi, 2).

The fruit firmness followed a declining trend commensurate with advancement in storage period. The fruits coated with SS-40™ T maintained the highest average firmness (13.1 lb force), followed by SS-50™ (12.9 lb force) under ambient conditions (Table 1). The control fruits registered the lowest mean firmness (10.62 lb force). In case of super market conditions, the highest average firmness was recorded with SS-40T (13.4 lb force), closely followed by SS-50 (13.1 lb force). The control fruits registered firmness of 11.4 lb force (Table 2). The soft pear fruits attain best eating quality at 10 lb force firmness. Considering this value as cut off limit for firmness, it was observed that SS-40T™ and SS-50™ coated fruits could be stored for 12 and 15 days, respectively at ambient and super market conditions, however for control fruits it was only up to 6 and 9 days of storage, respectively. Softening of fruits is caused either by breakdown of insoluble proto-pectins into soluble pectin or by hydrolysis of starch. The loss of pectin substances in the middle lamella of the cell wall is perhaps the key steps in the ripening process that leads to the loss of cell wall integrity thus cause loss of firmness and softening (Solomos and Laties, 14). The coating of fruits with SS-40T™ and SS-50™ resulted in higher fruit firmness, under both the storage conditions, which might be due to reduction in moisture loss and respiratory activity, thus maintained the turgidity of the cells. Applications of coatings have been reported to play an important role in maintaining the fruit firmness in apple (Bishnoi *et al.*, 3) and Kinnow (Mahajan *et al.*, 9).

The decay of pear fruits increased with storage period under both the storage conditions. However, coated fruits recorded minimum rotting (3%) under ambient conditions after 12 days of storage (Table 1) and 2-3% decay under super market conditions after 15 days of storage (Table 2). The control fruits registered high decay (18 and 9%) after 12 and 15 days under ambient and super market conditions, respectively. The coatings play an important role in the reducing water loss of produce and thereby responsible for lowering the spoilage of fruits. The present study confirms the results of Bishnoi *et al.* (3 & 4) who noticed that terpenoidal oligomer coating retarded the growth of microorganisms in case of stored apple and sweet lime fruits.

The maximum sensory score was shown by fruits coated with SS-40T (7.0) followed by SS-50 (6.9) under ambient conditions (Table 1). However, control fruits registered the minimum sensory score (6.1). The sensory score of coated fruits increased gradually up to 12 days in case of SS-40T and SS-50

Table 1. Effect of coatings on physico-chemical quality and enzymatic changes of pear under ambient conditions.

Treatment	Storage period (days)					Mean
	3	6	9	12	15	
PLW (%)						
Nipro Fresh SS 40-T	0.45	1.35	1.80	4.80	6.95	3.07
Nipro Fresh SS 50	0.52	1.40	1.92	4.78	7.10	3.14
Control	3.88	5.62	10.34	12.50	14.22	9.31
Mean	1.62	2.79	4.69	7.36	9.42	
$CD_{0.05}$	Treatment (T) = 0.87 Storage (S) = 0.60 T × S = 1.80					
Firmness (lb force)						
Nipro Fresh SS 40-T	17.00	14.90	13.45	11.00	9.20	13.11
Nipro Fresh SS 50	16.80	14.60	13.20	10.90	9.00	12.90
Control	15.60	11.60	10.20	9.00	6.70	10.62
Mean	16.47	13.70	12.28	10.30	8.30	
$CD_{0.05}$	Treatment (T) = 0.70 Storage (S) = 0.90 T × S = 1.50					
Decay (%)						
Nipro Fresh SS 40-T	0	0	0	3	5	2
Nipro Fresh SS 50	0	0	0	3	7	2
Control	0	5	12	18	25	12
Mean	0	2	4	8	12	
$CD_{0.05}$	Treatment (T) = 0.50 Storage (S) = 0.62 T × S = 0.90					
TSS (%)						
Nipro Fresh SS 40-T	11.57	12.00	12.77	13.40	11.57	12.26
Nipro Fresh SS 50	11.60	12.00	12.60	13.30	11.40	12.18
Control	11.90	12.73	13.03	10.03	9.00	11.34
Mean	11.69	12.24	12.80	12.24	10.66	
$CD_{0.05}$	Treatment (T) = 0.2 Storage (S) = 0.4 T × S = 0.5					
Acidity (%)						
Nipro Fresh SS 40-T	0.35	0.30	0.27	0.24	0.22	0.28
Nipro Fresh SS 50	0.33	0.31	0.29	0.25	0.20	0.28
Control	0.32	0.28	0.27	0.25	0.23	0.27
Mean	0.33	0.30	0.28	0.25	0.22	
$CD_{0.05}$	Treatment (T) = NS Storage (S) = 0. 02 T × S = NS					
Sensory quality						
Nipro Fresh SS 40-T	6.7	7.0	7.2	7.5	6.8	7.0
Nipro Fresh SS 50	6.7	7.0	7.0	7.2	6.5	6.9
Control	6.2	7.0	6.5	6.0	5.0	6.1
Mean	6.5	7.0	6.9	6.9	6.1	
$CD_{0.05}$	Treatment (T) = 0.3 Storage (S) = 0.2 T × S = 0.6					

(7.5 and 7.2) and thereafter decreased. In contrast, for control fruits, the sensory score increased up to 6 days of storage (7.0) and thereafter declined at faster rate. Under super market conditions, the sensory quality gradually increased in SS-40T and SS-50 coated fruits up to 15 days (7.5 and 7.3) and then declined. However, the control fruits recorded the highest sensory score of 7.0 after 9 days of storage but thereafter a sudden decline in sensory quality was noticed and fruits registered a score of 5.7 after 18 days of storage (Table 2). In the present investigation, it was noticed that pear fruits coated

Table 2. Effect of coatings on physico-chemical quality and enzymatic changes of pear under super-market conditions.

Treatment	Storage period (days)						Mean
	3	6	9	12	15	18	
PLW (%)							
Nipro Fresh SS 40-T	0.35	1.20	1.65	4.35	5.50	7.18	3.37
Nipro Fresh SS 50	0.42	1.36	1.80	4.60	5.78	7.35	3.55
Control	3.70	4.20	5.40	7.50	9.00	9.80	6.60
Mean	1.49	2.25	2.95	5.48	6.76	8.11	
$CD_{0.05}$	Treatment (T) = 0.65 Storage (S) = 0.52 T × S = 1.20						
Firmness (lb force)							
Nipro Fresh SS 40-T	17.8	16.2	14.4	12.0	10.8	9.2	13.4
Nipro Fresh SS 50	17.4	15.7	14.0	11.8	10.4	9.0	13.1
Control	16.6	14.0	11.2	9.6	8.9	8.0	11.4
Mean	17.3	15.3	13.2	11.1	10.0	8.7	
$CD_{0.05}$	Treatment (T) = 0.50 Storage (S) = 0.46 T × S = 1.12						
Decay (%)							
Nipro Fresh SS 40-T	0	0	0	0	2	3	1
Nipro Fresh SS 50	0	0	0	0	3	3	1
Control	0	0	0	5	9	15	5
Mean	0	0	0	2	5	7	
$CD_{0.05}$	Treatment (T) = 0.3 Storage (S) = 0.2 T × S = 0.8						
TSS (%)							
Nipro Fresh SS 40-T	11.30	12.20	12.80	13.15	13.30	11.00	12.29
Nipro Fresh SS 50	11.20	12.00	12.70	13.00	13.20	10.80	12.15
Control	11.70	12.45	13.10	11.90	11.40	10.00	11.76
Mean	11.40	12.22	12.87	12.68	12.63	10.60	
$CD_{0.05}$	Treatment (T) = 0.3 Storage (S) = 0.5 T × S = 0.6						
Acidity (%)							
Nipro Fresh SS 40-T	0.38	0.35	0.33	0.30	0.28	0.25	0.32
Nipro Fresh SS 50	0.40	0.36	0.32	0.30	0.26	0.22	0.31
Control	0.35	0.31	0.29	0.26	0.22	0.20	0.27
Mean	0.38	0.34	0.31	0.29	0.25	0.22	
$CD_{0.05}$	Treatment (T) = NS Storage (S) = 0.04 T × S = NS						
Sensory quality							
Nipro Fresh SS 40-T	6.8	7.0	7.0	7.2	7.5	6.7	7.0
Nipro Fresh SS 50	6.5	7.0	7.0	7.0	7.3	6.5	6.9
Control	6.0	6.8	7.0	6.5	6.0	5.7	6.3
Mean	6.4	6.9	7.0	6.9	6.9	6.3	
$CD_{0.05}$	Treatment (T) = 0.2 Storage (S) = 0.4 T × S = 0.5						

with SS-40T and SS-50 under both the storage conditions developed better sensory quality, which might be due to partial modifications as result of coatings, which also resulted in development of the acceptable flavour. Earlier, Gol *et al.* (7) noticed that carambola fruits coated with edible coating improved the organoleptic quality and consumer acceptability without the development of off-flavour.

The fruits coated with SS-40T registered the maximum average TSS content (12.26%), followed by SS-50 coated fruits (12.18%) under ambient conditions (Table 1). The control fruits recorded the

lowest average TSS (11.34%). It was further observed that in SS-40 T and SS-50 coated fruits, the TSS content increased slowly and steadily up to 12 days and thereafter declined. On the other hand, control fruits recorded a rise in TSS content up to 6 days and then started to decline at a faster rate. Under super market conditions, SS-40T and SS-50 coated fruits registered an increase in TSS (13.30%) content up to 15 days (Table 2). In control fruits, the TSS content increased up to 9 days (13.10%) and then a sudden decline was noticed. The increase in TSS during storage may possibly be due to breakdown of complex organic metabolites into simple molecules or due to hydrolysis of starch into sugars. The delayed increase in TSS over a longer period of time in coated pear fruits under both the storage conditions might be attributed that coating retard ripening and senescence processes and simultaneously delayed the conversion of starch into sugars. A delayed and smaller increase in TSS as seen in the present study has also been reported in *Aloe vera* gel coated sweet cherry (Martinez *et al*., 10). The acidity of pear fruits showed a linear decline irrespective of different treatments as the storage period advanced under both the storage conditions (Tables 1 & 2). However, non-significant differences were observed between coated and non-coated (control) fruits. The decrease in titratable acids during storage might be attributed to utilization of organic acid in pyruvate decarboxylation reaction occurring during the ripening process of fruits (Pool *et al*., 12).

The coatings significantly influenced the PME activity in pear fruits. It was observed that both the coatings minimized the enzyme activity in pear fruits under both the storage conditions as compared to control. Under ambient conditions, the PME activity in fruits coated with SS-40T and SS-50 increased up to 12 days of storage and declined thereafter. On the other hand control fruits recorded maximum activity up to 6 days of storage and afterwards a sharp decline was noticed (Fig. 1). Under SMC, the SS-40 T and SS-50 coated fruits recorded increase in PME activity upto 15 days of storage and thereafter declined. The control fruits recorded an increase in PME activity up to 9 days of storage and after that declined at a much faster rate (Fig. 2). The lower and delayed increase in PME activity in coated fruits, *viz.* 12th day under ambient conditions and 15th day under SMC as against 6th and 9th day in control fruits under both the storage conditions, respectively. This effect might be related to slower respiratory activity due to the creation of modified atmospheric conditions by the coatings. Edible coatings are presented as an excellent way to preserve the quality of fruits by maintaining the firmness and consumer acceptability of fruits which is probably due to suppressing the fruit softening enzyme activities (Wijewardane and Gularea, 15). Gol and Rao (6) reported that zein or

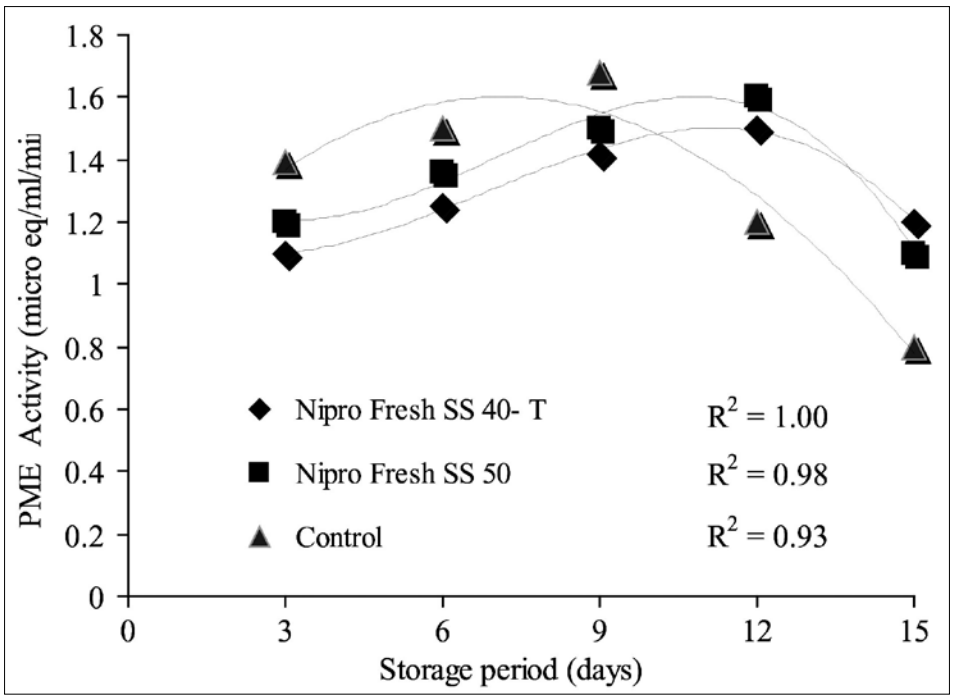

Fig. 1. Effect of coatings on PME activity of pear during storage under ambient conditions.

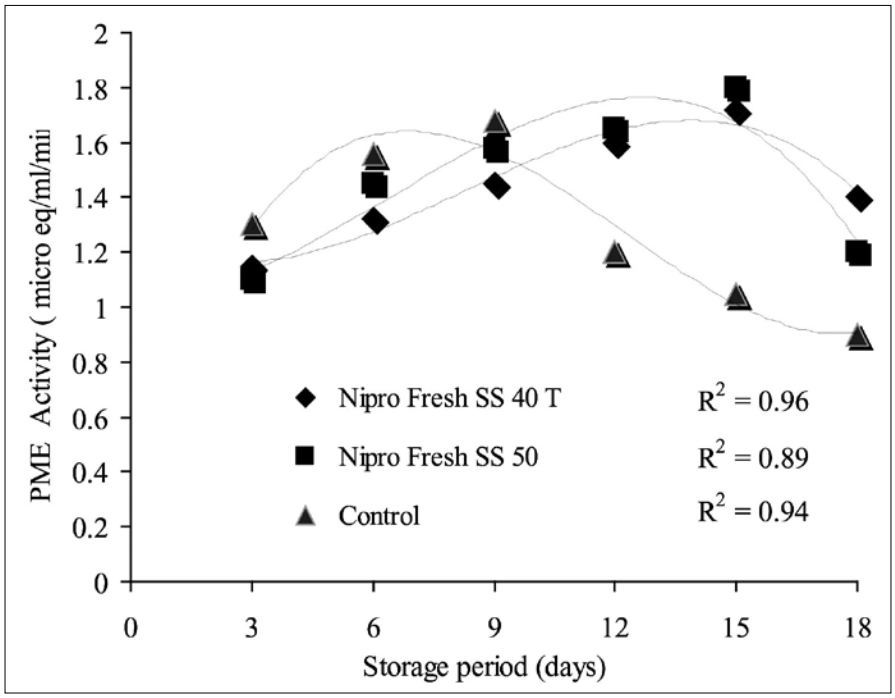

Fig. 2. Effect of coatings on PME activity of pear during storage under super market conditions.

gelatin coatings delayed the ripening of mango fruits by suppressing the activity of softening enzymes such as polygalacturonase, pectin methyl esterase, cellulase and β-galactosidase.

It can be concluded from the present studies that both the coatings, *i.e.*, Nipro Fresh SS-40T and SS-50 were equally effective in extending the storage-life of pear fruits for 12 days under ambient conditions and 15 days under super market conditions. On the other hand, the control fruits maintained their storage-life for 6 and 9 days under the two marketing conditions, respectively.

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Short communication

Variability in physico-chemical characters of mango genotypes collected from Kuttanad tracts of Kerala

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ABSTRACT

An extensive survey was conducted during 2011-13 to identify the elite mango (*Mangifera indica* L.) types among its natural population existing in Kuttanad tracts of Kerala. The ripe fruits of 28 selected genotypes were analyzed for physical and quality parameters. There was significant variation among the collections. Individual fruits ranged from 60.17-920.03 g, pulp weight 40.13-780.98 g, fruit length 8.32-19.20 cm, fruit diameter 14.29-31.28 cm, fruit volume 58.65-918.75 cc, and specific gravity 1.00-1.05 g/cc. Similarly, the chemical parameters also varied significantly. The TSS varied from 9.83-21.0°Brix, ascorbic acid 2.40-91.33 mg/100 g, acidity 0.16-0.92% and total sugars 7.43-51.70%. Wide range of variability in physico-chemical parameters amongst suggested that superior genotypes could be selected for commercial cultivation based on their usage.

Key words: Mango, physico-chemical characters, seedling variability.

Kerala has mango cultivation over an area of 74.44 thousand hectares with an annual production of 441.43 thousand tonne with productivity of 5.93 t ha^{-1} (FIB, 3). Mango is an important fruit tree crop grown in Kuttanad tracts of Kerala as mixed crop in homesteads along with coconut. Mango is also planted along the highways as shade trees in many parts of Kuttanad. Detailed information on these indigenous mango varieties/ clones grown in Kuttanad tracts are lacking. Since most of these trees are seedling in origin a lot of variability exist in fruit quality. Hence, an attempt was made to study the natural variations in the genotypes.

This study was conducted in the Horticulture department of Regional Agriculture Research Station, Kumarakom under Kerala Agriculture University during 2010-14. A survey was conducted in 33 *panchayats* of Alappuzha, Kottayam and Pathanamthitta districts coming under the Kuttanad tracts. Only seedling progenies were included in this study and tagged for identification. The fully matured fruits were collected from each trees and were ripened in laboratory under ambient conditions. The sample size in each genotype was five fruits. Organoleptic study was also conducted. The physical, morphological and biochemical characters of the fruits were recorded. The data on physical parameters like fruit weight, pulp weight, peel weight, stone weight were recorded with the help of an electronic balance. The chemical parameters were analyzed following the standard procedures. Total soluble solids (TSS) was measured with the help of a hand refractometer (AOAC, 2). Reducing sugars, non reducing, total sugars and titratable acidity were also estimated (Ranganna, 7). Ascorbic acid content was estimated following the methods of Sadasivam and Manickam (8). Analysis of variance (ANOVA) using SPSS version 19 was performed to ascertain the differences in fruit parameters among different mango selections.

A significant variation in physico-chemical characters of fruits was observed among 28 mango selections surveyed. The information related to these parameters enables to select the superior chance seedling clones, which could have different uses (Simi *et al*., 12; Radha and Manjula, 5; Radha and Nair, 6; Satyavati *et al*., 10).

The results of this study showed considerable variability in fruit characters (Table 1). Highest variability was observed for pulp weight and minimum for specific gravity. The fruits of selection AKM-3 showed superiority in average fruit weight (920.03 g) and pulp weight (780.98 g), whereas the selections AKM-8 (60.17 g) possessed lowest fruit weight (Table 2). The peel weight was also highest in AKM-3 (69.64 g). The stone weight ranged from 9.80 to 82.41 g and was maximum in AKM-3 followed by AMM-1. The maximum fruit length was observed in KKM-2, while maximum fruit diameter was in AKM-3. The variation in length and diameter of fruits in mango varieties was also earlier observed by Sharma *et al.* (11) and Singh *et al*. (13).

Result of the qualitative analysis of mango fruits indicated that the selection KKM-3 (21.0°Brix) (Table 3) showed superiority for TSS followed by KKM-2, AKM-

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Table 1. Physico-chemical variations in fruits of mango selections from Kuttanad tracts of Kerala.

Parameter	Mean	CV (%)	SD	Range	
				Min.	Max.
Fruit wt. (g)	274.63	63.81	175.24	60.17	920.03
Stone w(. g)	40.54	46.60	18.89	9.80	82.41
Pulp wt. (g)	209.51	72.73	152.38	40.13	780.98
Peel w(. g)	25.61	55.88	14.31	8.36	69.64
Fruit length (cm)	13.11	21.82	2.86	8.32	19.20
Fruit dia. (cm)	22.24	21.31	4.74	14.29	31.28
Fruit vol. (cc)	273.14	64.14	175.18	58.65	918.75
Specific gr. (g/cc)	1.01	1.00	0.01	1.00	1.05
TSS (°Brix)	16.27	18.93	3.08	9.80	21.00
Total sugars (%)	31.26	43.95	13.74	7.43	51.70
Reducing sugar (%)	4.01	34.91	1.40	1.79	8.14
Acidity (%)	0.40	52.50	0.21	0.16	0.92
Ascorbic acid (mg/100 g)	28.09	93.34	26.22	2.40	91.33

CV = Coefficient of variation, SD = Standard deviation

Table 2. Variability in physical characteristics of fruits among mango selections.

Genotype	Fruit wt. (g)	Peel wt. (g)	Pulp wt. (g)	Seed wt. (g)	Fruit length (cm)	Fruit dia. (cm)	Fruit vol. (cc)	Sp gr. (g/ cc)
APM-1	196.68	11.23	146.59	38.50	12.13	21.66	196.59	1.00
APM-2	95.30	10.50	70.53	19.70	8.60	17.91	95.01	1.00
AMM-1	480.25	36.17	358.97	82.41	16.20	25.15	477.05	1.01
KPM-1	70.50	11.64	46.36	11.91	9.50	15.36	70.15	1.00
AKM-1	214.07	13.88	167.08	31.91	14.08	22.67	213.78	1.00
AKM-2	250.07	20.98	184.32	44.34	11.40	19.56	249.61	1.00
AKM-3	920.03	69.64	780.98	69.42	14.54	31.28	918.75	1.01
AKM-4	380.01	31.26	317.40	31.69	16.27	28.66	377.35	1.01
AKM-5	200.06	28.28	136.10	44.68	12.03	14.29	199.26	1.00
AKM-6	420.35	38.24	329.26	52.21	15.07	25.98	419.97	1.00
AKM-7	380.03	17.48	310.83	51.65	14.73	23.03	379.71	1.00
AKM-8	60.17	8.36	40.13	11.56	8.32	14.73	58.65	1.01
AKM-9	220.07	15.79	168.75	35.40	12.5	22.50	216.43	1.02
AKM-10	220.09	20.95	184.73	44.39	15.25	22.40	219.43	1.00
AKM-11	80.02	10.43	59.80	9.80	9.53	16.10	78.40	1.01
KKM-1	303.33	18.36	240.02	40.90	11.57	17.02	298.10	1.02
KKM-2	480.20	57.04	384.54	48.24	19.20	29.10	479.68	1.00
AKM-12	399.93	36.33	363.40	18.36	17.60	24.60	399.35	1.00
AKM-13	300.12	35.9	214.50	49.53	14.1	26.13	299.38	1.00
KKM-3	340.08	27.77	256.00	56.25	14.19	26.04	339.57	1.00
KKM-4	140.11	15.50	102.00	22.56	12.20	19.60	139.25	1.01
AKM-14	220.01	23.98	151.74	44.40	12.17	22.50	219.10	1.00

Contd...

Table 2 Contd...

Genotype	Fruit wt. (g)	Peel wt. (g)	Pulp wt. (g)	Seed wt. (g)	Fruit length (cm)	Fruit dia. (cm)	Fruit vol. (cc)	Sp gr. (g/ cc)
AKM-15	240.03	34.4	139.21	67.04	11.17	22.13	229.08	1.05
AKM-16	339.89	27.71	257.48	56.27	14.43	25.96	339.25	1.00
KKM-5	140.01	19.00	106.00	15.50	12.07	19.50	139.44	1.00
KKM-6	120.16	17.10	67.13	35.97	9.63	17.46	119.35	1.01
KAM-1	159.66	20.06	67.88	34.25	10.67	20.93	158.65	1.01
AKM-17	318.33	39.03	214.52	66.24	18.01	30.50	317.53	1.01
$CD_{0.05}$	12.47	1.63	1.53	1.63	0.61	0.70	12.70	0.009

Table 3. Variability in chemical characteristics of different mango selections.

Genotype	TSS (°Brix)	Total sugars (%)	Red. sugar (%)	Ascorbic acid (mg/100 g)	Acidity (%)
APM-1	18.30	48.85	3.04	44.00	0.19
APM-2	14.10	51.70	5.50	91.33	0.24
AMM-1	13.80	7.43	3.68	4.00	0.51
KNM-1	11.30	10.70	3.43	7.53	0.54
AKM-1	17.50	49.96	3.25	18.00	0.42
AKM-2	15.90	38.28	3.69	60.33	0.51
AKM-3	17.40	33.03	4.79	8.40	0.21
AKM-4	11.20	26.11	4.18	2.40	0.92
AKM-5	11.00	17.80	2.70	2.50	0.46
AKM-6	9.80	14.80	1.79	7.46	0.26
AKM-7	18.30	47.67	3.86	7.12	0.49
AKM-8	18.30	18.24	2.56	11.00	0.26
AKM-9	17.10	46.26	3.86	76.00	0.33
AKM-10	19.10	32.93	2.68	74.47	0.51
AKM-11	18.00	48.07	4.64	42.52	0.22
KKM-1	19.00	20.10	2.08	80.83	0.28
KKM-2	19.20	28.20	8.14	27.45	0.26
AKM-12	19.10	32.89	3.96	8.50	0.34
AKM-13	18.00	36.72	2.95	8.50	0.30
KKM-3	21.00	32.55	5.49	25.85	0.22
KKM-4	16.00	39.06	4.67	17.50	0.23
AKM-14	18.00	36.98	4.49	22.05	0.84
AKM-15	12.40	14.43	3.38	42.07	0.33
AKM-16	12.40	12.19	5.33	13.05	0.82
KKM-5	16.00	39.11	4.60	17.72	0.23
KKM-6	18.00	49.04	3.21	8.01	0.16
KAM-1	17.50	15.68	3.26	25.84	0.73
AKM-17	17.60	26.46	6.97	32.07	0.35
$CD_{0.05}$	0.30	2.29	0.44	4.70	0.06

10, AKM-12 and KKM-1. In our study TSS value ranged between 9.8° to 21.0°Brix (Table 1). Earlier, Sathyavathi *et al*. (10) reported that TSS of fruits of local types of Kerala varied from 10° to 24°Brix. Similarly, Salvi and Gunjate (9) also reported significant variation for pulp TSS in different mango genotypes at Vengurle. The range of TSS existing in the indigenous genotypes were comparable with the commercial mango varieties. Furthermore, they were also found free from most of the pests and diseases commonly found in commercial varieties of Kerala. The total sugars were found to be high in APM-2 and was on par with AKM-1. The highest value for reducing sugar was obtained for KKM-2. The ascorbic acid content was highest in AMP-2.

All these physico-chemical parameters of mango fruits may be given due consideration to operate selection procedure for identifying the promising selections for table purpose. According to Navprem *et al.* (4) the variability existing in the germplasm can be exploited for strategic future mango improvement programmes. The results of the present experiment indicate that many of indigenous mango types in low land of Kuttanad met the standard parameters for considering the fruit to be of high quality. These selections were propagated though soft-wood grafting and evaluated on large scale before their commercial release.

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Short communication

Effect of training system and in row spacing on yield and fruit quality of peach in the sub-tropical regions

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ABSTRACT

Information on the use of different training systems in peach under the sub-tropics is inadequate. Trees of Shan-i-Punjab peach were planted at two spacings, *viz.*, 5 m x 2 m and 5 m x 3 m and were trained to four training systems, *viz.*, Y shaped, Hedge row, Espalier and V trellis. The fruits harvested from Espalier and V trellis trained trees were superior in fruit quality in terms of fruit size, weight, colour and TSS as compared to fruit harvested from other training systems. Fruit yield was found to be maximum from V trellis trained trees. Trees planted at 5 m x 3 m gave higher fruit yield and better quality fruits as compared to 5 m x 2 m planted trees irrespective of training systems.

Key words: Fruit quality, peach, spacing, training system.

Peach is the third most widely cultivated fruit after apple and pear in the temperate zone of India. Its cultivation is gaining popularity in the north Indian sub-tropics due to higher returns on unit area basis and availability of suitable low chilling cultivars. Considerable research work on high density planting using different training systems in peach has been reported in the temperate parts of the world, but only few studies seem to have been conducted in the subtropical climate. Information on the effects of different training systems and spacings on yield and fruit quality are not well documented in the sub-tropical climate of north India. Therefore, present study was undertaken at PAU, Ludhiana during 2014 and 2015. Peach trees of cv. Shan-i-Punjab were planted in January 2011 at two spacings, viz., 5 m x 2 m and 5 m x 3 m and were trained to four training systems, *viz.*, Y shaped, Hedge row, Espalier and V trellis. There were four replications and each replication consisted of two trees in a randomized block design. Trees were pruned every year in winter and it consisted of a combination of heading back and selective thinning out of fruitful branches. Observations on fruit size, weight, firmness, total soluble solids, acidity, total sugars and yield were recorded as per the standard methods. Fruit colour was estimated with the help of colour meter (Colour Flex, Hunter Lab, USA) and expressed as *L*, *a* and *b* values. The data was analyzed using statistical SAS software.

Data in Table 1 show that maximum mean fruit size over a two year period was found in trees trained to Espailer system (5.96 cm length and 5.68 cm dia.), which was significantly higher than the trees trained to other systems. It was followed by fruit size recorded in V trellis (5.78 cm length and 5.44 cm dia.) trees. Minimum fruit size was recorded in Hedge row trees (5.42 cm in length and 5.07 cm dia.). Mean fruit weight was also found to be maximum (91.85 g) in Espailer trained trees followed by V trellis trees (89.42 g) and minimum (85.04 g) in Hedge row trees. More fruit size and weight in Espalier and V trellis trained trees was apparently due to better radiation interception and distribution within the tree canopy. The data further shows that spacings also affected fruit size and weight significantly. Trees planted at wider spacings (5 m x 3 m) recorded higher fruit size and weight as compared to closely planted trees (5 m x 2 m), irrespective of training system. This may be due to more competition for metabolites and water at closer spacings. Earlier, McDermott and Sherman (10) reported that upright and compact canopy interfered with light penetration during critical periods of fruit development resulting in smaller sized fruits.

Data in Table 2 show that maximum "*a*" value was recorded in fruits harvested from Espailer trained trees system (25.50) and minimum from Hedge row trees (20.57). The maximum "*L*" and "*b*" values were found in fruits of Hedge row (57.85 and 28.79, respectively) trained trees and minimum in Espailer (48.77 and 21.51, respectively) trained trees. More redness and low brightness and greenness in Espailer and V trellis trained fruits may be due to canopy architecture, which did not allow the light to fall on ground and allows maximum light to penetrate even in inner parts of the tree canopy as compared

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Table 1. Effect of training systems and spacings on fruit size and fruit weight of peach cv. Shan-i-Punjab

Training system	Spacing (m)	Fruit length (cm)			Fruit dia. (cm)			Fruit wt. (g)		
		2014	2015	Mean	2014	2015	Mean	2014	2015	Mean
Y-shaped	5 x 2	5.54	5.60	5.57	5.19	5.27	5.23	86.78	87.82	87.30
	5 x 3	5.68	5.74	5.71	5.28	5.40	5.34	87.84	88.76	88.30
	Mean	5.61^{c}	5.67^{c}	5.64^{c}	5.23^{c}	5.34^{c}	5.29^{c}	87.31^{c}	88.29^{c}	87.80^{c}
Hedge row	5 x 2	5.22	5.43	5.33	4.92	5.06	4.99	84.84	84.39	84.62
	5 x 3	5.44	5.60	5.52	5.06	5.23	5.15	85.59	85.36	85.47
	Mean	5.33^{d}	5.52^{d}	5.42^{d}	4.99^{d}	5.14^{d}	5.07^{d}	85.35^{d}	84.74^{d}	85.04^{d}
Espailer	5 x 2	5.87	5.95	5.91	5.58	5.72	5.65	91.28	91.47	91.37
	5 x 3	5.98	6.07	6.02	5.61	5.82	5.71	92.23	92.44	92.33
	Mean	5.92^{a}	6.01^{a}	5.96^{a}	5.59^{a}	5.77^{a}	5.68^{a}	91.75^{a}	91.95^{a}	91.85^{a}
V trellis	5 x 2	5.69	5.76	5.72	5.32	5.45	5.39	88.66	89.34	89.00
	5 x 3	5.81	5.88	5.84	5.44	5.56	5.50	89.72	89.97	89.84
	Mean	5.75^{b}	5.82^{b}	5.78^{b}	5.38^{b}	5.51^{b}	5.44^{b}	89.19^{b}	89.66^{b}	89.42^{b}
Spacing mean	5 x 2	5.60^{b}	5.70^{b}	5.65^{b}	5.25^{b}	5.38^{b}	5.31^{b}	87.93^{b}	88.22^{b}	88.07^{b}
	5 x 3	5.73^{a}	5.82^{a}	5.77^{a}	5.35^{a}	5.50^{a}	5.42^{a}	88.87^{a}	89.10^{a}	88.98^{a}
$LSD_{0.05}$	Training system	0.05	0.04	0.05	0.06	0.04	0.06	0.47	0.29	0.32
	Spacing	0.03	0.03	0.03	0.04	0.03	0.02	0.33	0.20	0.23
	TS x spacing	0.07	0.06	0.07	0.09	0.06	0.08	0.66	0.41	0.46

Table 2. Effect of training systems and spacing on fruit colour, firmness and yield of peach cv. Shan-i-Punjab.

Training system	Spacing (m)	Fruit colour			Fruit firmness (kg/cm^2)			Fruit yield (kg/tree)		
		L	*a*	*b*	2014	2015	Mean	2014	2015	Mean
Y-shaped	5 x 2	55.69	23.20	25.62	6.10	6.11	6.10	19.07	12.25	15.66
	5 x 3	53.31	23.70	25.08	5.97	6.03	6.00	21.02	15.09	18.06
	Mean	54.50^{b}	23.45^{c}	25.35^{b}	6.03^{b}	6.07^{c}	6.05^{b}	20.04^{b}	13.67^{b}	16.86^{b}
Hedge row	5 x 2	59.27	20.68	28.49	6.63	6.52	6.57	17.12	10.03	13.57
	5 x 3	56.42	20.46	29.10	6.41	6.33	6.37	19.33	12.01	15.67
	Mean	57.85^{a}	20.57^{d}	28.79^{a}	6.52^{a}	6.42^{a}	6.47^{a}	18.22^{d}	11.02^{d}	14.62^{d}
Espailer	5 x 2	50.86	24.78	21.74	5.93	5.74	5.84	18.68	11.03	14.86
	5 x 3	44.67	26.23	21.28	5.81	5.61	5.71	20.01	14.06	17.04
	Mean	48.77^{d}	25.50^{a}	21.51^{d}	5.87^{c}	5.68^{d}	5.77^{c}	19.35^{c}	12.55^{c}	15.95^{c}
V trellis	5 x 2	51.88	24.30	22.86	6.03	6.27	6.15	22.04	17.01	19.52
	5 x 3	48.03	24.45	22.04	5.93	6.14	6.03	24.99	18.89	21.94
	Mean	49.96^{c}	24.37^{b}	22.45^{c}	5.98^{b}	6.20^{b}	6.09^{b}	23.51^{a}	17.95^{a}	20.73^{a}
Spacing mean	5 x 2	54.43^{a}	23.24^{b}	24.68^{a}	6.17^{a}	6.16^{a}	6.17^{a}	19.23^{b}	12.58^{b}	15.90^{b}
	5 x 3	50.61^{b}	23.71^{a}	24.37^{b}	6.03^{b}	6.03^{b}	6.03^{b}	21.34^{a}	15.01^{a}	18.17^{a}
$LSD_{0.05}$	Training system	1.09	0.43	0.22	0.07	0.07	0.05	0.27	0.20	0.16
	Spacing	0.77	0.30	0.15	0.05	0.05	0.03	0.19	0.14	0.11
	TS x spacing	1.54	0.61	0.31	0.10	0.11	0.07	0.38	0.28	0.23

to other training systems. Heinicke (7) found that fruits which received less than 30% of full sunlight were less coloured, had less dry matter and sugars as compared to fruits which received full sunlight in apple. Decrease in fruit colour in a three row system in apples due to poor illumination has also been reported by various workers (Loreti and Massai, 9; Keppel, 8). Data further shows that maximum mean "*a*" values were found in fruits of 5 m x 3 m planted trees and maximum "*L*" and "*b*" values were observed in fruits in the trees planted at 5 m x 2 m spacing. Mika *et al*. (11) reported that mutual shading of densely planted trees, insufficient illumination and tree competition lead to lower percentage of good coloured fruits in apple. McDermott and Sherman (10) also observed that trees in high density orchards contained less coloured fruits than those from standard spaced trees.

Maximum mean firmness was recorded in fruits harvested from Hedge row (6.47 kg/ cm^2) trees and minimum (5.77 kg/ cm^2) in fruits of Espailer trained trees. Higher firmness in Hedge row fruits was apparently due to reduction in receiving radiant energy, which may attribute to delay in ripening. The reason of low firmness in Espailer fruit might be due to earliness in maturity, since firmness has been reported to decrease with the advancement of fruit maturity in fruit crops. Similar findings were observed by Deell (5) in apple. Maximum mean fruit firmness (6.17 kg/ cm^2) was recorded in 5 m x 2 m planted trees as compared to those planted at 5 m x 3 m (6.03 kg/ cm^2) irrespective of the training systems. Lower firmness in widely spaced plants may be due to higher radiation penetration and canopy temperature recorded in such plants, which advanced maturity.

Data in Table 3 show that fruits, which were harvested from Espalier trees had significantly higher mean total soluble solids (12.21%) and total sugars (7.49%) as compared to the fruits harvested from other training systems. Minimum total soluble solids (11.05%) and total sugars (6.96%) were recorded in fruit harvested from Hedge row trees. The total soluble solids and total sugars content in fruit harvested from V trellis and Y shaped trees were found to be statistically at par. The mean acid content of the fruits harvested from Espailer, V trellis and Y shaped system were found to be statistically at par and significantly lower than those from Hedge row trees. Higher total soluble solids and lower acidity in the fruit harvested from Espalier and V trellis trained trees was apparently due to more exposure of fruits to sunlight, which helps in the degradation of malic acid. These results are in close conformity with those of other workers (Cortell and Kennedy, 4; Ristic *et al.*, 12) who also found that fruits in exposed portion

Table 3. Effect of training systems and spacings on TSS, acidity and total sugars of peach cv. Shan-i-Punjab.

Training system	Spacing (m)	TSS (%)			Acidity (%)			Total sugars (%)		Mean
		2014	2015	Mean	2014	2015	Mean	2014	2015	
Y shaped	5 x 2	11.63	11.78	11.70	0.75	0.73	0.74	7.11	7.33	7.22
	5 x 3	11.73	11.92	11.83	0.74	0.72	0.73	7.38	7.45	7.42
	Mean	11.67^{b}	11.84^{b}	11.75^{b}	0.75^{a}	0.73^{bc}	0.73^{b}	7.25^{b}	7.39^{c}	7.32^{c}
Hedge row	5 x 2	10.89	11.02	10.96	0.78	0.76	0.77	6.87	6.94	6.90
	5 x 3	11.09	11.2	11.15	0.77	0.75	0.76	6.98	7.06	7.02
	Mean	10.99^{c}	11.11^{c}	11.05^{c}	0.77^{a}	0.76^{a}	0.77^{a}	6.92^{c}	7.00^{d}	6.96^{d}
Espailer	5 x 2	12.11	12.19	12.15	0.74	0.71	0.73	7.34	7.42	7.38
	5 x 3	12.21	12.32	12.27	0.73	0.71	0.72	7.54	7.64	7.59
	Mean	12.16^{a}	12.26^{a}	12.21^{a}	0.74^{b}	0.71^{c}	0.72^{b}	7.44^{a}	7.53^{a}	7.49^{a}
V trellis	5 x 2	11.71	11.91	11.81	0.76	0.72	0.74	7.26	7.37	7.31
	5 x 3	11.86	12.05	11.95	0.74	0.72	0.73	7.45	7.53	7.49
	Mean	11.78^{b}	11.98^{b}	11.88^{b}	0.75^{a}	0.72^{c}	0.73^{b}	7.36^{a}	7.45^{b}	7.40^{b}
Spacing mean	5 x 2	11.58^{a}	11.72^{a}	11.65^{a}	0.76^{a}	0.73^{a}	0.75^{a}	7.14^{b}	7.26^{b}	7.20^{b}
	5 x 3	11.72^{a}	11.87^{a}	11.80^{a}	0.75^{a}	0.73^{a}	0.74^{a}	7.34^{a}	7.42^{a}	7.38^{a}
LSD 0.05	Training system	0.18	0.24	0.18	0.02	0.02	0.02	0.08	0.05	0.06
	Spacing	0.13	0.17	0.13	0.01	0.01	0.01	0.06	0.03	0.04
	TS x Spacing	0.26	0.34	0.26	0.03	0.03	0.02	0.12	0.07	0.08

of canopy exhibit higher concentration of sugars as compared to shaded fruits. Robinson (13) and Erez (6) also found that greater light interception with angled canopy improved fruit quality. Data further shows that spacings did not affect total soluble solids, total sugars and acid content significantly. These results are in line with those of Bargioni *et al.* (1) who found no appreciable effects of planting density on soluble solids content or acids in peach and nectarine fruits.

Maximum mean fruit yield/ tree of 20.73 kg, over a two year period, was recorded in trees trained to V trellis, which was significantly more than the trees trained to Y shaped, Espalier and Hedge row systems where yield of 16.86, 15.95 and 14.62 kg/ tree, respectively were recorded. The general effect of the training system on fruit yield/ tree was true for year wise effects in 2014 and 2015. Highest fruit yield in V trellis system was due to higher shoot number and canopy volume. Lower yield in Espailer trained trees was due to heavy pruning done to restrict the trees for intersecting with each other as a result of which these trees had lower shoot number and canopy volume. Spacing also affected the fruit trees significantly. Highest fruit yield (18.17 kg) was recorded in 5 m x 3 m planted trees, which was significantly more than the trees planted at 5 m x 2 m (15.90 kg). This may be due to the reason that trees planted at closer spacings had to compete with each other for light, water and nutrients as a result of which yield decreased (Mika *et al*., 11). These results are in agreement with those of Cepoiu and Muravi (3) who reported that wider spacings were helpful in increasing yield due to higher tree volume and reduced competition for metabolites among plants. From these studies, it is concluded that V trellis training system was found to be better for growing high density peaches in terms of yield and fruit quality under sub-tropics of north India.

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Short communication

Fertilizer-use efficiency, nutrient uptake and water requirement of capsicum under fertigation in open field conditions

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ABSTRACT

Three experiments were conducted on capsicum during 2012-2014 at Dr YS Parmar University of Horticulture & Forestry, Solan, Himachal Pradesh, wherein four fertigation levels were tried. The experiment comprising eight treatment combinations in randomized block design was replicated four times. Humic acid significantly increased plant height (9%), leaf area index (10%), nutrient uptake, fruit yield (8%) and fertilizer-use efficiency (29%) over control. Fruit quality was also significantly influenced by humic acid application. Interaction of fertigation and humic acid was statistically significant. Further, crop response was comparable between combined fertigation with 80 percent recommended dose and fertigation with 100 percent recommended dose with and without humic acid. It is therefore concluded that the efficacy of fertigation can further be increased by humic substances.

Key words: Capsicum, fertigation, humic acid, nutrient uptake, water requirement.

In Himachal Pradesh, capsicum it is generally grown under rainfed condition and covered almost 50 percent of state's area and production. In the recent past, monsoon distribution pattern in the state has become highly erratic. In 2009-10, all the twelve districts were declared as drought hit. Undulating topography, steep slopes and shallow soils with poor retentivity of water and nutrient further aggravate the problems. Under such conditions, use of drip irrigation becomes imperative to produce good quality crop as drip irrigation can be used to maximize water-use efficiency and production (Spehia *et al*., 6). Furthermore, in the era of intensive agriculture, the use of chemical fertilizers has tremendously increased all over the world which can also be efficiently and effectively utilized by the plants through fertigation. Hence, fertigation can help in reduction of ground water pollution by indiscriminate use of fertilizers. In addition, humic acid (HA) can be used to lower the concentration of inorganic fertilizers. One of the functions of HA is the positive effect on promotion of root development (Trevisan *et al*., 9). Humic substances are recognized as a key component of soil fertility properties, since they control chemical and biological properties of the rhizosphere leading to higher soil moisture and nutrient content (Nardi *et al*., 2). Combined fertigation of chemical fertilizers and humic acid could, therefore, affect the productivity of crops in a different ways and hence the present investigation was carried out.

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A field experiment was conducted at the experimental farm of Precision Farming Development Centre, Department of Soil Science and Water Management, Dr YSPUH&F, Solan, Himachal Pradesh during 2012-14. Study area lies between 31°45'30'' N latitude and 77°25'30'' E longitude with an altitude of 1720 m above mean sea level. Experimental soil was moderately acidic loamy sand having 0.63 per cent organic carbon and EC 0.31 dS m^{-1}. Volumetric soil water content at field capacity and permanent wilting point was 25.8 and 8.4 per cent, respectively. Available N content at 0-15, 15-30 and 30-45 cm soil depth was 112.1, 102.5, 88.7 mg kg^{-1}. Such values of available P and K were 16.9, 11.9, 4.6 and 120.8, 110.0, 93.2 mg kg^{-1}, respectively. Capsicum cv. California Wonder was planted at 60 cm x 45 cm distance in open field conditions. Water was applied based upon pan evaporation (0.75 class 'A' pan evaporation), using the equation $V = \sum (Ep \times Kc \times Kp \times A \times N - Re \times A)$, where, V = is the volume of water required in litres; Ep = pan evaporation (mm day^{-1}); Kc = crop co-efficient; Kp = pan factor (0.75); A = Area of plot (9 m^2), Re = effective rainfall (mm) and N = number of days in a month. Crop factor (Kc) were taken as 0.68, 0.73, 0.81 and 0.87 for May, June, July and August, respectively, based on existing relative humidity, wind velocity and crop growth stage. Water requirement (WR) of capsicum during entire growth season is presented in Table 1.

Water soluble fertilizers-MAP (12:61:0), muriate of potash (60% K_2O) and urea (46% N) were used for fertigation. For humic acid, super potassium

Table 1. Water requirement (WR) of capsicum under open field fertigation.

Month	No. of days	Ep (mm/ day)	Kc	Kp	Re (mm)	ET. crop (mm)	Crop WR (mm)
May	15	6.5	0.7	0.8	16.8	49.0	65.8
June	30	4.9	0.7	0.8	11.2	80.3	91.5
July	31	3.7	0.8	0.8	15.3	69.1	84.4
August	31	2.7	0.9	0.8	35.7	53.6	89.3
Total							331.0

*excluding 5 cm water application in field preparation

f-humate (75% potassium humate and 15% fulvic acid) was used. The following six treatments, in triplicate, were tried in randomized block design. Treatments were T_1 = Drip fertigation with NPK (sole application) at recommended dose (RDF); F = control; T_2 = sole fertigation of NPK at 80% RD; T_3 = sole fertigation of NPK at 60% RD; T_4 = Fertigation of NPK and HA (combined application) at RD; T_5 = combined fertigation at 80 percent RD and T_6 = combined fertigation at 60 percent RD. Fertilizers and humic acid was applied as per recommendations. Drip system comprised inline emitters spaced at 50 cm distance having emitter's discharge rate 4 lph. Uniformity coefficient of the system was 90-92 percent and operating pressure was 1.16 kg cm^{-2}. Fertigation was done through venturi having suction rate of 1013 ml per min., at bi-weekly intervals starting from four leaf stage. Nutrient concentration in irrigation water was well within the prescribed limits.

Soil moisture was recorded after 72 h of irrigation/ fertigation at weekly interval using virtual soil moisture sensors. Observations were also made on plant height, leaf area index (LAI), number of fruits per plant, fruit weight and fruit yield. Plant N, P and K contents were determined as per standard methods. Nutrient uptake (NPK) was estimated by multiplying total NPK content in plant parts (root, shoot, leaf and fruit) with the respective dry weight of plant part. Available N, P and K contents of soil was also determined following standard procedures (Tandon, 8).

Soil moisture decreased with increasing soil depth (Fig. 1). Treatment received humic acid registered higher soil moisture as compared to sole fertigation. At 0-15 and 15-30 cm soil depth, moisture content in T_4 was 2.8 and 2.0 percent unit higher, respectively. Such an increase at 30-45 cm depth was only 0.5 percent unit. The HA may have increased the water retention property of soil especially in surface 0-30 cm soil depth because of its high content of hydrophilic groups. Higher soil moisture in combined fertigation may be attributed to addition of humic acid which added considerable organic matter in soil. Piccolo *et al.* (16) also reported that humic substances significantly improved moisture retention property of soil owing to the presence of hydrophilic functional groups.

Among different fertigation levels (Table 2), T_1 registered significantly maximum plant height (69.7 cm) over T_2 (62.6 cm) and T_3 (55.7 cm). Fruit yield was also significantly superior in T_1, which was 20 and 25 percent higher over T_2 and T_3, respectively. Higher growth parameters in T_1 can be attributed to higher leaf area. Leaf area index (LAI) is a measure of source size which directly contributes in chlorophyll synthesis and consequently in plant growth and yield. Significantly higher LAI was recorded in T_1 (2.9) compared to T_2 (2.6) and T_3 (2.3). Since, experimental soil was medium in available N, P and K content; therefore, higher responses were expected at higher fertigation levels. This resulted in higher LAI in T_1. Higher LAI at higher fertigation level has also been reported by Shedeed *et al.* (5). Increased fruit yield in T_1 may have resulted from higher average fruit weight (74.3 gm) over T_2 (70.4 g) and T_3 (65.9 g). Hebbar *et al*. (1) also reported more number of fruits, higher average fruit weight and consequently higher fruit yield of tomato at higher fertigation level. Similar trend was noticed in T_4, T_5 and T_6.

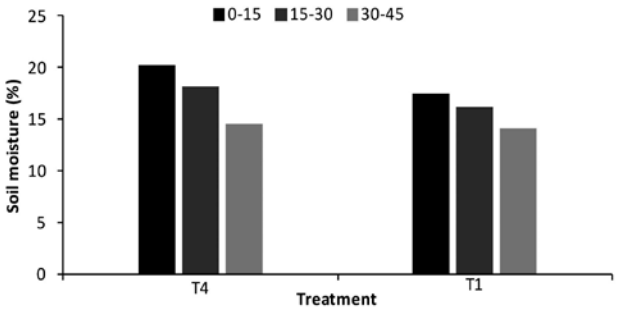

Fig. 1. Soil moisture content under combined and sole fertigation. Humic acid resulted in 2-3 percent higher moisture especially in 0-30 cm soil depth.

Table 2. Effect of different treatment on growth, yield and FUE of capsicum under open field fertigations (pooled data).

Treat.	Plant ht. (cm)	LAI	Fruits per plant	Fruit wt. (g)	Yield (kg plant^{-1})	FUE (kg kg^{-1})
T_1	69.7^{b}	2.8^{b}	14.8^{b}	78.0^{b}	1.2^{b}	119.3^{c}
T_2	62.6^{c}	2.5^{c}	13.5^{c}	70.4^{c}	1.0^{c}	128.9^{c}
T_3	55.7^{d}	2.2^{d}	12.7^{d}	62.9^{d}	0.8^{d}	151.2^{b}
T_4	75.8^{a}	3.1^{a}	16.9^{a}	86.1^{a}	1.4^{a}	154.2^{b}
T_5	68.7^{b}	2.8^{b}	15.1^{bc}	77.4^{b}	1.2^{b}	154.4^{b}
T_6	61.6^{c}	2.5^{c}	13.5^{c}	72.5^{b}	1.0^{c}	176.9^{a}
$CD_{0.05}$	5.5	0.2	1.3	7.2	0.1	13.3

Data in column followed by the same letters are not significantly different but statistically significant over other treatment combinations.

Furthermore, combined NPK fertilizer at all fertigation levels, in general, influenced the productivity of capsicum significantly over sole fertigation. Fruit yield was 17-27 percent higher under combined fertigation over sole application. Also, growth, yield and quality of capsicum were statistically at par between T_1 and T_5. These results could be attributed to the improvement of moisture retention and nutrient supply potentials of soil after humic acid application. This may have resulted in higher LAI and thereby, higher plant growth and yield under combined fertigation. Similar observations were made by Suganya and Sivasamy (7) and Selim *et al*. (4). This contention also gets support from higher nutrient uptake in combined fertigation of NPK and HA compared to NPK alone (Table 3).

Irrespective of the levels, fertigation with humic acid had significantly higher N uptake over sole application (Table 3). Similar trend in the uptake of P and K was noticed. The higher uptake was the result of significantly higher dry matter production at 120 days after transplanting (DAT) and higher nutrient contents in different plant parts. The increased N uptake was supposed to be due to the better use efficiency of applied N fertilizers in the presence of humic acid coupled with retarded nitrification process enabling the slow availability of applied N. The increase in P uptake may be due to the prevention of P fixation in the soil and the formation of humophospho complexes, which are easily assimilable by the plants. According to Samson and Visser (19), humic acid induced increase in permeability of bio-membranes for electrolytes accounted for increased uptake of K.

Treatment comprising T_1, T_2 and T_3 registered FUE to the tune of 119.3, 128.9 and 151.2 kg kg^{-1}, respectively. This implies higher FUE at lower fertigation level (Table 2). Higher FUE in treatments receiving HA may be attributed to improvement of moisture retention and nutrient supply potentials of soil. Hence, fertigation of chemical fertilizers and humic acid resulted in significantly higher growth and yield of capsicum over sole application of NPK. Use of humic acid alongwith inorganic fertilizers increased the fruit yield by 08% besides increasing fertilizer-use efficiency upto 29% over sole application of fertilizers.

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Table 3. Effect of different treatment on dry matter production, plant nutrient content and nutrient uptake of capsicum.

Treat.	Dry matter (mg plant^{-1})	Plant nutrient content (%)			Nutrient uptake (kg ha^{-1})		
		N	P	K	N	P	K
T_1	204.1^{a}	2.94^{b}	0.32^{c}	4.38^{b}	221.9^{b}	24.0^{c}	330.6^{b}
T_2	192.0^{b}	2.78^{c}	0.32^{c}	4.00^{c}	197.2^{c}	22.8^{c}	284.4^{c}
T_3	180.3^{c}	2.64^{d}	0.31^{c}	3.73^{d}	175.8^{d}	20.5^{d}	248.9^{d}
T_4	212.4^{a}	3.12^{a}	0.40^{a}	4.77^{a}	245.0^{a}	32.0^{a}	374.9^{a}
T_5	204.0^{ab}	2.95^{b}	0.37^{b}	4.40^{b}	222.9^{b}	28.0^{b}	332.2^{b}
T_6	190.4^{bc}	2.78^{c}	0.32^{c}	4.00^{c}	196.1^{c}	22.8^{c}	281.7^{c}
$CD_{0.05}$	13.6	0.13	0.02	0.25	18.4	2.2	20.4

Data in column followed by the same letters are not significantly different but statistically significant over other treatment combinations.

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Short communication

Evaluation of potato cultivars for phosphorus efficiency under Nilgiris conditions

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ABSTRACT

Field experiments were conducted at ICAR-Central Potato Research Station, Muthorai, the Nilgiris, Tamil Nadu for three years with seven potato varieties under four levels of phosphorus to evaluate its efficiency. Varieties Kufri Neelima and Kufri Swarna were found more P efficient because of their higher relative biomass production, tuber yield efficiency index, harvest index, agronomic use efficiency (AUE) and P uptake efficiency. These two varieties produced higher tuber yields under no P and at P_{max} of standard variety. The varieties Kufri Girdhari, Kufri Jyoti and Kufri Himalini proved P responsive as they responded well to P application and produced very low yields under no P application. Higher root biomass in these two efficient varieties could be the reason behind their higher P use efficiency as compared with other genotypes. The two P efficient potato varieties also happen to be resistant to potato cyst nematodes, which is very common and serious problem in the Nilgiris. The mechanism to have resistance against PCN, whose cysts emerge only in the presence of root exudates of susceptible potato varieties, could also have benefitted those cultivars to show more efficiency in native P utilization. Further investigations are required to find out the exact mechanism of P efficiency in these two PCN resistant genotypes.

Key words: Agronomic use efficiency, phosphorus use efficiency, potato cultivars, P uptake, tuber yield efficiency.

Most of the P (>80%) is obtained from nonrenewable sedimentary deposits. Phosphorus use efficiency is generally very low at <30% in potato. Cultivated soils contain good reserves of P and its availability to the plants is seized because of transformations to other forms depending upon soil pH. P is limiting because of its chemistry, *i.e.*, low solubility of phosphates and their rapid transformation to insoluble forms (Smil, 9). Al, Fe, Ca, K, and Mg can all react with fertilizer P and produce relatively insoluble compounds (Smil, 9). Potato is classified as “inefficient responder” to P application (Miyasaka and Habte, 8). Hence, the need to improve P use efficiency is more important in the future due to economic environmental, and mineral resource availability pressures.

Plants take up P in the form of orthophosphates which is a very small proportion in the soil when compared with the total P. But the P efficient plants or cultivars are supposed to have the ability to convert soil P into orthophosphates or other available forms through some sort of mechanism which may include higher root biomass, root/shoot ratio, root exudates which can dissolve the phosphates or some other mechanism of that sort. Lee *et al*. (7) reported that the cultivar adaptation to low-P stress growing conditions depends on various traits, such as mobilization of insoluble phosphates, utilization of limited bioavailable P sources, and P-uptake efficiency. An elite genotype that can adapt to P-limiting growing conditions needs to be excellent in each of the above traits. If such cultivars are identified and their mechanism is known then it becomes easier to breed varieties with higher P efficiency through improved biotechnological tools. Potato is widely grown in Nilgiris with large doses of P application under lateritic soil conditions. If P efficient cultivars are identified and recommended, it can avoid soil build up of P, thereby eutrophication of water bodies and environmental pollution. Hence, the present investigation was carried.

A field experiment was conducted at CPRS, Muthorai, the Nilgiris, Tamil Nadu during 2010 to 2012 by planting seven potato varieties under four different levels of P (0, 50, 100 and 150 kg P_2O_5 per hectare). The seven varieties tried were Kufri Swarna, K. Jyoti, K. Neelima, K. Girdhari, K. Shailja, K. Giriraj and K. Himalini, which differ in their maturity periods under Nilgiri conditions (equinox). The trials were planted during summer season (April to August) under rainfed conditions as the region receives good amount of (800 mm) rainfall during South-West monsoon. The plot size adopted was 2.4 x 2.0 m with four rows of potato having 10 plants in each row (at 60 x 20 cm spacing). Standard cultural practices were followed

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for inter-culture and harvesting by cutting the haulms 15 days before harvest. The soil type in experimental plot was sandy loam with high available N and P and medium at K. The soils of the Nilgiris are rich in P but the availability is very less because of the transformation of P to Fe and Al phosphates.

Per cent emergence of different varieties under four levels of P application was estimated after one month of planting. At 45 days, plant height, shoot number and number of leaves per plant were recorded by selecting five plants per plot at random. Shoot weight, root length and root biomass were estimated at 90 days after planting. Tuber number and yield was estimated in different size grades (<25, 26-50, 51-75 and >75 g) after separation. Tuber yields were recorded net plot wise in all the three years in four different size grades. The total biomass of plants were recorded at 90 days and the tuber yield at 120 days. Harvest index was calculated in all the varieties at different P levels. P uptake was estimated in different plant parts at 90 days after planting by drawing samples from five plants in each plot. Phosphorus content in tubers was estimated at harvest. Soil samples were analysed for nutrient status using standard procedures before and after the conduct of the experiment. The following nutrient efficiency indices like Tuber yield efficiency index, tuber harvest index, agronomic use efficiency (AUE), and phosphorus uptake efficiency (PUE) were estimated. The pooled data of three years was used to fit quadratic models for yield estimation in seven varieties and the *Pmax* was estimated for standard variety Kufri Jyoti using the procedure suggested by Govindakrishnan *et al.* (4). The yields of different varieties at *Pmax* were estimated using the quadratic equations developed.

Plant height, number of shoots per plant and leaf number was significantly affected by P application in potato varieties. The two varieties, namely, Kufri Swarna and Kufri Neelima performed better in terms of plant height, number of shoots and leaf number than rest of the varieties. Efficiency of the above two varieties in utilization of P could be witnessed from the initial stage itself as the plant growth parameters were superior in them (Table 1). Potato being a heavy feeder requires higher levels of nutrients from the initial stage itself. Non availability of required quantities of P might have caused imbalance in many of the treatments leading to reduction in growth and related parameters. Shortage of phosphate supply was found to increase mainly the ratio of root length per weight of plants (Fist 2; Jungk *et al*., 6; Trehan and Sharma, 10). The regulating mechanism is reported to be root cell elongation (Anuradha and Narayanan, 1).

The yield produced by Kufri Neelima and Kufri Swarna without P application was higher than all other cultivars even at their highest levels of P application except for Kufri Girdhari at 100 kg P that too it was higher than Kufri Swarna at zero level of P application. This indicates that these two varieties are highly efficient in utilization of native P under Nilgiri conditions. The varieties Kufri Swarna (31.3 t/ha) and Kufri Neelima (32.9 t/ha) produced very high yields

Table 1. Growth parameters, yield components and efficiency indices of potato varieties.

Variety	Pl. ht. (cm)	No. of shoots	No. of leaves	Yield/ net plot (kg)	Tuber No. / net plot	P content in tubers (%)	Plant P conc. (%)	P uptake in stems (kg/ ha)	Stem DMP (t/ ha)	Tuber DMP (t/ ha)	AUE
K. Jyoti	22.76	2.58	17.69	4.49	62	0.28	0.14	2.02	1.44	4.65	150.13
K. Swarna	33.53	3.05	23.12	6.64	65	0.29	0.10	2.21	2.21	6.91	222.98
K. Girdhari	24.97	2.87	18.31	4.83	65	0.29	0.13	2.02	1.56	5.03	162.13
K. Shailja	12.47	1.45	10.85	1.16	19	0.28	0.14	0.51	0.36	1.21	38.90
K. Himalini	22.73	2.41	15.20	3.58	68	0.29	0.14	1.57	1.12	3.73	120.39
K. Giriraj	18.01	2.26	14.43	2.78	42	0.29	0.10	0.90	0.90	2.90	93.42
K. Neelima	31.19	2.69	23.03	6.79	80	0.29	0.12	2.80	2.33	7.07	228.06
$LSD_{0.05}$	2.711	0.311	2.356	0.607	7	0.00					
P0	20.31	2.26	15.31	3.59	50	0.27	0.12	1.39	1.16	3.74	120.77
P50	24.99	2.64	18.43	4.59	61	0.29	0.13	1.99	1.53	4.78	154.19
P100	25.15	2.58	18.59	4.72	60	0.29	0.13	2.05	1.57	4.92	158.71
P150	24.21	2.40	17.75	4.37	58	0.30	0.14	2.10	1.50	4.55	146.84
$LSD_{0.05}$	2.050	0.235	1.781	0.462	5	0.00					

under no application of P and the varieties Kufri Jyoti, Kufri Himalini and Kufri Girdhari responded very well to the application of P at different levels (Tables 1, 2 & 3). Efficiency of the above two varieties in utilization of soil available P could result in increased tuber yield even at zero level of P application under acidic soil conditions of the Nilgiris.

This gives an indication about the efficiency of a particular cultivar to yield efficiently under non application and high level of application of a particular nutrient in comparison with other varieties. Tuber yield efficiency index was high in Kufri Swarna and Kufri Neelima as they could produce more yield at P deficient conditions. Among the seven cultivars tested Kufri Shailja and Kufri Giriraj were the least P efficient (Fig. 1). Varieties Kufri Girdhari, Kufri Himalini and Kufri Jyoti were intermediate and P responsive. This shows that the varieties Kufri Neelima and Kufri Swarna were highly effective in utilizing the native P in comparison with other varieties under testing.

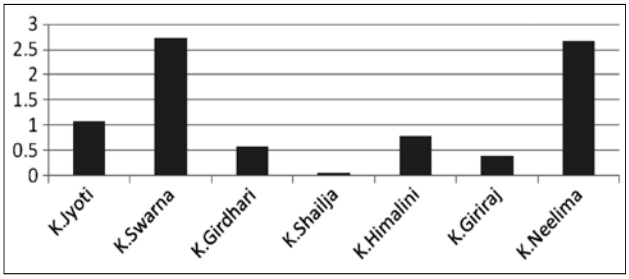
Harvest index represents conversion efficiency of vegetative source to economical part. The cultivars Kufri Neelima and Kufri Swarna recorded the highest values for harvest index indicating that they are the most P efficient and the HI increased with increase in P level upto 150 kg per hectare (Fig. 1). That means these two varieties are more efficient in converting source into economical parts. The two varieties Kufri Neelima (228) and Kufri Swarna (222) had shown higher Agronomic Use Efficiency (AUE) when compared with other varieties and Kufri Girdhari (162), Kufri Jyoti (150) and Kufri Himalini (120) showed moderate values for AUE. The least AUE values were recorded for K. Shailja (38) and K. Giriraj (93) (Table 1). Trehan and Sharma (10) reported that Kufri Pukhraj was the most N, P and K efficient cultivar among ten cultivars tested in the absence as well as

Table 2. Tuber yield per net plot (kg) in potato varieties.

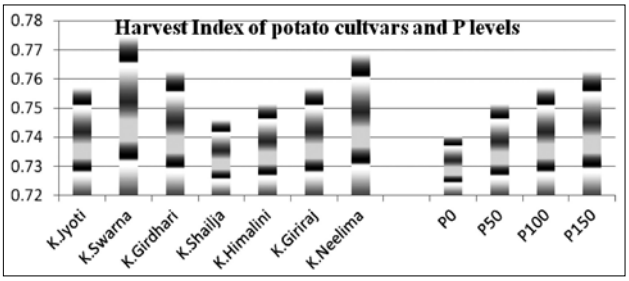
Variety	P0	P50	P100	P150
K. Jyoti	3.3783	4.6433	4.8133	5.0367
K. Swarna	6.0200	6.6583	6.7517	7.1100
K. Girdhari	3.4783	5.0267	6.2700	4.5283
K. Shailja	0.9483	1.4167	1.2467	1.0150
K. Himalini	2.5667	4.1450	3.8367	3.7850
K. Giriraj	2.4383	3.1733	3.0183	2.4900
K. Neelima	6.3250	7.0633	7.1317	6.6283

Table 3. ANOVA for tuber yield.

Source	DF	Type III SS	Mean square	F value	Pr>F
Rep (year)	3	2.4639107	0.8213036	0.73	0.5380
year	2	154.4015512	77.2007756	68.23	<.0001
variety	6	591.4661988	98.5776998	87.12	<.0001
P_level	3	32.1779833	10.7259944	9.48	<.0001



a. Tuber yield efficiency index



b. Harvest index

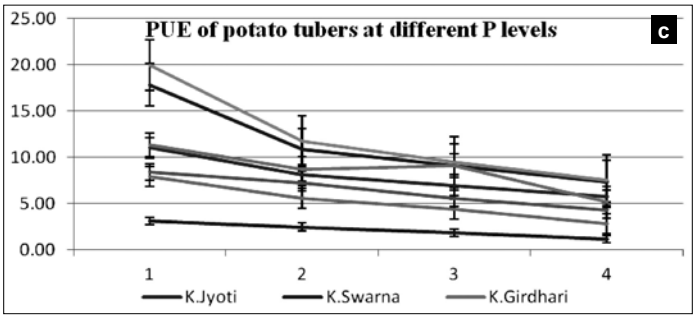


Fig. 1. Tuber yield efficiency, (a) harvest index (b) and phosphorus uptake efficiency (c) of potato cultivars under different P levels.

Table 4. Yield at economic optimum, P_{Max}, no P and dry root biomass of potato varieties.

Variety	Quadratic equation	Econ Opt (kg/ ha)	Yd at Econ opt P (t/ ha)	Yd at Max P of std variety (t/ ha)	Yd at no P (t/ ha)	Root dry wt. (g /plant)
K. Jyoti	$y = -0.0005x^2 + 0.135x + 17.895$ $R^2 = 0.8935$	132	27.4	27.0	17.9	1.72
K. Swarna	$y = -0.0005x^2 + 0.0884x + 31.14$ $R^2 = 0.7459$	85	35.0	40.2	31.1	3.07
K. Girdhari	$y = -0.0017x^2 + 0.3028x + 17.418$ $R^2 = 0.8539$	88	30.9	26.5	17.4	2.61
K. Shailja	$y = -0.0004x^2 + 0.055x + 5.0894$ $R^2 = 0.6745$	65	6.9	14.2	5.9	1.54
K. Himalini	$y = -0.0008x^2 + 0.1622x + 13.926$ $R^2 = 0.6822$	99	22.1	23.0	13.9	2.52
K. Giriraj	$y = -0.0007x^2 + 0.0987x + 12.834$ $R^2 = 0.4901$	68	16.3	21.9	12.8	1.98
K. Neelima	$y = -0.0006x^2 + 0.1072x + 32.968$ $R^2 = 0.9475$	87	37.7	42.1	32.9	3.14

(Econ opt = Economic optimum, Yd = Yield); P opt (kg/ ha) =- (cp-b) /2c; Cp: cost of P fertilizer per kg/ Price of potatoes per tonne = (37.5/12000) = 0.003125

presence of green manure. They also reported that the efficient cultivars gave higher tuber yield under N, P and K stress (*i.e.* with less dose of N, P and K fertilizer) and had higher Agronomic Use Efficiency (AUE) than less efficient cultivars.

The P uptake efficiency indices recorded higher values at no P application in Kufri Neelima and Kufri Swarna indicating their efficiency to convert native P to available forms. Other varieties showed very low uptake efficiency at no application and the values were lower at higher levels of P application (Fig. 1). The variation in phosphorus efficiency of different potato cultivars was due to both their capability to use absorbed P to produce potato tubers (PUE) and to their capacity to take up more P per unit soil. Trehan and Singh (12) reported that Kufri Pushkar is more P efficient than Kufri Pukhraj. Quadratic models were developed for all the cultivars and from them the optimum P dose was estimated (economic optimum). The tuber yield at economic optimum was 37.7 t/ha in Kufri Neelima and 35.0 t/ha in Kufri Swarna. The varieties next in order were Kufri Girdhari (30.9), Kufri Jyoti (27.4), Kufri Himalini, Kufri Giriraj (16.3) and Kufri Shailja (6.9) (Table 4). The P_{Max} for standard variety Kufri Jyoti (135 kg/ha) is estimated using the technique developed by Govindakrishnan *et al.* (4). The yields at P_{Max} and at no P also followed similar trend.

The root biomass (dry) produced in Kufri Neelima (3.14 g/plant) and Kufri Swarna (3.07 g/plant) were significantly higher on an average at all the levels of P application substantiating their efficiency in utilizing soil available and applied P resources. Further, these two varieties are resistant to PCN infection, which makes them maintain healthier roots without any cysts when compared with other varieties. This could also have been contributed for their better P use efficiency. Lee *et al.* (7) reported that 'Harley Blackwell' and 'Satina' cultivars of potato showed greater P mobilization ability in soils without supplemental P than the other five cultivars tested.

The ability to uptake more P from soil available level made the cultivars Kufri Neelima and Kufri Swarna more efficient in producing better tuber yields in comparison with other cultivars. Nutrient efficient plants are defined as those plants, which produce higher yields per unit of nutrient, supplied or absorbed than other plants (standards) under similar agro-ecological conditions (Trehan and Singh, 12). The main properties that affect the uptake of nutrients from soil are kinetics of ion absorption by roots, the size of root system and morphological root properties as reported by Jungk and Claassen (6). Earlier, Gahoonia (3) also reported that phosphate availability could be influenced by root induced changes of soil pH. Kufri Girdhari, K. Jyoti and K. Himalini responded greatly to the application of P and proved most P responsive varieties. Investigations are required to confirm the actual reasons for P efficiency.

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Short communication

An assessment of contract farming system for potato seed production in Punjab – A case study

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ABSTRACT

The study was conducted to assess the contract farming system of potato seed production in Punjab with a sample of 30 contract and 30 non-contract farmers from Jalandhar district of Punjab. The major motivating factors identified for participating in the contract farming were assured price, diversification, access to assured market, extension services etc. There was no participation of marginal and small farmers in contract farming. The average net income of contract farmers was about 12 per cent higher than non-contract farmers. The major constraints in potato seed contract farming were pest and disease attacks, non-availability of labor during peak period, difficulty in meeting quality requirement etc. The study suggests that government, non-governmental organizations and other related agencies should play an active role in the contractual arrangement.

Key words: Contract farming, constraints, motivational factors, potato farming.

As per Census 2011, 52 per cent of the workforce in India is engaged in agriculture. Unfortunately, majority of the farming communities in the country get very less remuneration for their hard work. Out of total value of the produce, farmer gets only 35 per cent and majority of the price hike is done by the intermediaries (Deloitte, 2). Tripathi *et al*. (13) argued that despite much of technological and economic advancement, the condition of the farming communities continues to be miserable and unstable due to uncertainty in crop yields and prices of produce. Hence, contract farming system, if carefully planned and executed, is a viable alternative farming system that provides a win-win situation for both farmers and processing firms as it ensures better prices of agricultural produce to the farmers and timely and consistent supply of quality raw-materials to the agro-based processing firms (Pandit *et al*., 8; Tripathi *et al*., 13). The Government of India in the XII Plan (2012-2017) also emphasizes the promotion of properly designed contractual farming arrangement, so that marginal and small farmers have the requisite technology and market access (Planning Commission, 9).

Many studies reported that contract farming provides many advantages to the farmers such as assured price, assured market, credit, technologies, inputs, extension services, risk sharing; augment income, employment generation and reducing the cost of production and transaction (Kumar *et al*., 5; Pandit *et al*., 8; Singh, 12). In spite of several advantages, farmers under contract farming also faced many problems like delay in payments, delay in the delivery of inputs (Nagaraj *et al*., 6), lack of remunerative price (Rampal and Gill, 10), lack of clear contract agreement (Pandit *et al*., 8), withdrawal of extension services, reneging on prices and procurement (Kumar *et al*., 4), *etc*. Presently, in developing countries including India there are very few studies related to potato contract farming system in general and potato seed contract farming system in particular. With this backdrop, the present study was undertaken.

Ex post facto research design was used for the study. The state of Punjab was selected purposively for the study, because it is a major player in the supply of potato seeds in India. Jalandhar district of Punjab, also known as potato seed hub was also selected purposively for the study (Indian Express, 3). Farmers of Jalandhar district who were under contract with PepsiCo's Frito-Lay for seed production were selected for the study. Frito-Lay Division undertakes contract farming for production of good quality seeds and processing potatoes for ensuring regular supply of raw materials to its three state-of-the-art plants in Punjab (Sangrur), West Bengal (Sankrail near Kolkata) and Ranjangaon near Pune in Maharashtra. At the first stage, company officials were contacted and a list of blocks with large area covering large number of farmers was obtained. Based on the list, two blocks, namely, Nakodar and Phillaur were purposively selected as higher number

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of contract farmers was concentrated in these blocks. Consequently, from each block 15 contract farmers and equal number of non-contract farmers were randomly selected for the study. Thus, a total of 60 farmers, comprising of 30 potato seed contract farmers and 30 non-contract farmers were studied.

Data was collected through personal interviews with the respondents during January 2015 and was analyzed with the help of simple descriptive statistics like frequencies, averages, percentages, *etc.* The t-test was used in order to know the significance of differences between variables/ items under contract and non-contract farming. For studying the motivating factors for participation in the contract arrangement, a set of 12 statements was framed to elicit the motivating factors of contract farmers. The farmers were asked to indicate the extent of their agreement to each statement on a five point continuum Likert-type scale (1-5 scale from strongly agree to strongly disagree). The weighted total score was arrived at by summing up the weightage of responses for each statement. Mode of operation was analyzed through an interview schedule containing the terms and condition that could possibly represent the mode of operation of contract farming like price settlement, nature and kind of contract, linkages, credit facility, *etc*. Comparative economic analysis was done by estimating the total cost of variable cultivation and net income generated under contract and non-contract farming using enterprise budget technique. The constraints of contract farming was measured based on the responses of farmers to a set of 13 statements. The respondents were asked to response to each statement using a 5-point response categories ranging from "very severe" to "least severe" (score ranging from 1 to 5). The weighted total score was obtained based on the summation of weightage of response of each respondent for each selected statement.

The motivating factors for participating in the contract farming were identified and presented in Table 1. It can be observed that "assured price" with a mean score of 4.57 was ranked as the first motivating factor followed by "access to assured market" with a score of 4.27. Similar findings were also observed by Rampal and Gill (10) in their study about contract farming in Punjab. "Diversification" was ranked third (4.23) by the respondents. Contract farming was adopted by the Government of Punjab as a tool to promote diversification in the state (Sharma and Singh, 11). "Access to extension services" having mean score of 4.10 was ranked fourth by the farmers. Frito-Lay hired one area manager and five agronomists for monitoring and providing technical services to the contract farmers in Jalandhar district. The experience of contract farming in India shows that there is significant saving in consumption of production inputs due to the introduction of improved technology and better extension services (Pandian *et al*., 7).

The practice of contract farming differs across regions, crops, firms and farmers and it also varies according to situation-specific variables making it difficult to generalize the concept (Singh, 12). PepsiCo's Frito-Lay division undertook contractual

Table 1. Ranks accorded to motivational factors for participating in potato seeds contract farming system among the contract farmers (n = 30).

Motivational factor	Frequency					WTS	WMS	Ranks
	SA	A	N	DA	SDA			
Assured price	17	13	0	0	0	137	4.57	I
Access to assured market	14	2	2	2	0	128	4.27	II
Diversification	14	12	1	3	0	127	4.23	III
Access to extension services	11	14	3	1	1	123	4.10	IV
Efficient transportation facilities	9	14	5	2	0	120	4.00	V
Access to appropriate technologies	7	18	4	0	1	120	4.00	V
Supplies of inputs by the company	7	13	8	2	0	115	3.83	VI
Favorable climate for potato	10	8	7	5	0	113	3.77	VII
Reduction in yield uncertainty	9	8	4	6	3	104	3.47	VIII
Access to loans or credit	4	12	8	4	2	102	3.40	IX
Inspired by other contract farmers	3	11	10	5	1	100	3.33	X
Just to have a try	1	6	12	6	5	82	2.73	XI

Rating Scale: Strongly agree (SA) = 5, Agree (A) = 4, Neutral (N) = 3, Disagree (DA) = 2, Strongly disagree (SDA) = 1; WTS = Weighted total score, WMS = Weighted mean score

agreement with more than 400 potato seed farmers in Punjab during 2014-15. Its purpose of contract farming in Jalandhar district of Punjab was solely for good quality seed production. Potato varieties produced by the company under contract potato seed farming in Jalandhar were ATL, FC1, FC3 and FC5. The company undertook contract farming with only those farmers who were willing to cultivate at least 5 acres of land for potato seeds production. Thus, there was no participation of marginal and small farmers. Kumar *et al*. (4) also observed that contract farming in Punjab was skewed towards medium and large farmers. The duration of contract was for one crop season (October to March) and the contract was renewed every year based on the loyalty and willingness of the farmers. The contractual agreement was a direct (bi-partite) agreement between the company and the farmers. The format of contractual agreement was a written agreement. The agreements were written in English and given to farmers. The company supplied seeds to the contracting farmers at Rs.10-12/ kg and bought back the produce (potato seed) at pre-agreed price and quality. The payment for purchasing of seeds by the contracting farmers was done in two installments; 60 per cent of the seed cost was paid by the farmers in advance and 40 per cent was deducted by the company at the time of procurement of the harvested produce. All technical advice and extension activities were provided free of cost. The company procured 100 per cent (full buy back) of the produce, which were under contractual agreement. The price was pre-agreed at Rs.10/ kg for A grade (28-35 mm seed size) and B grade (36-45 mm seed size), Rs. 8/ kg of C grade (46-55 mm seed size) and Rs. 5/ kg for D (>55 mm seed size) and Z grade (<28 mm seed size). The company paid the transportation cost as per the distance covered from the farms to the collection centre. The company also paid the cost of sorting and grading through third party arrangement. No compensation was paid to the farmers in case of crop damage.

The total variable cost of cultivation under contract farming and non contract farming was Rs. 63,412 and Rs. 56,160 per hectare, respectively (Table 2). The higher cost of cultivation under contract farming was mainly due to a higher expenditure on seeds. Majority of non-contract farmers adopted indigenous varieties developed by the ICAR-Central Potato Research Institute, Shimla like Kufri Pukhraj, Kufri Jyoti, Kufri Bahar *etc*., which were obtained locally at a relatively lower price. The average seed cost under contract farming was about Rs. 33,167 per hectare, which was nearly 23.21 per cent higher than that under non-contract farming (Rs. 25,467/ ha). Other cost components like expenditure on fertilizers, plant protection, irrigation, labour, *etc*. had no significant difference. This may be because these inputs were not supplied by the company and therefore the cost was more or less the same. Pandit *et al.* (8) and Tripathi *et al.* (13) also observed the higher cost of cultivation in contract farming than in non-contract farming.

Data in Table 3 indicated that the yield under non-contract farming was about 6 percent higher than that of contract farming. This could be due to the fact that the company encouraged the contract farmers to produce optimum seed size so that they would get higher price for the produce. However, the price received by the contract farmers was higher at Rs. 6.33 per kilogram as against Rs. 5.25 per kilogram of the non-contract farmers. The average net income of contract farmers was Rs. 73,468.94 per hectare, which was about 12 per cent higher than that of non-contract farmers (Rs. 64,259.25/ ha). Pandit *et al*. (8) and Tripathi *et al*. (13) also observed a better profitability of potato production under contract farming than non-contract farming.

Table 2. Variable cost of cultivation of potato seed contract farming *vis-à-vis* non-contract farming (n = 60).

Cost component (Rs./ha)	Contract (n_1 = 30)	Non-contract (n_2 = 30)	Difference	Increase/ decrease %
Seed cost	33166.67	25466.67	**	23.21
Fertilizers cost	7160.00	7950.00	NS	-9.94
Farm yard manure	883.33	683.33	NS	22.64
Plant protection	5016.67	5100.00	NS	-1.66
Irrigation	2136.67	2093.33	NS	2.02
Human labour#	11516.66	10833.33	NS	5.93
Hired machineries##	3883.33	4033.33	NS	-3.86
Tot. variable cost of cultivation	63412.67	56160.00	**	11.43

Note: # including hired and family labour, ##including cost of fuel, repairing cost, *etc*. ** Significant at 1 %, NS = Non-significant

Table 3. Economic profitability analysis of potato seed contract farming *vis-à-vis* non-contract farming (n = 60).

Particulars/ Items	Contract (n_1 = 30)	Non-contract (n_2 = 30)	Difference	Increase/ decrease (%)
Avg. yield (q/ha)	216.13	229.37	NS	-6.12
Avg. price (Rs./q)	633.33	525.00	*	17.10
Gross Income (Rs./ha)	136881.61	120419.25	NS	12.02
Net income (Rs./ha)	73468.94	64259.25	NS	12.53

*Significant at 5%, NS = Non-significant

The constraints in potato seed contract farming were analyzed and the results were presented in Table 4. The results revealed that "pest and disease attack" with a mean score of 3.37 was considered as the first major constraint in potato seed contract farming. Occurrence of late blight and viral diseases had been potential threats to potato seed cultivation in the study area. Arneja *et al*. (1) reported similar findings. Late blight disease in potato sometimes caused 80 to 100 per cent loss (Indian Express, 3). The second important constraint was "non-availability of labour during the peak period" with a mean score of 3.20. In Jalandhar, potato was grown by a large number of farmers and majority of labour were hired from outside the state. Pandit (8) reported that after the initiation of Mahatma Gandhi National Rural Employment Guarantee Scheme in the country, the problem of labor had aggravated. "Difficulty in meeting quality requirement" with a mean score of 3.13 was the third important problem. This might be due to the fact that the company fixed too many grades (A, B, C, D and Z) with price ranging from Rs. 5-10/ kg. The farmers expressed dissatisfaction of the pre-agreed price fixed by the company, especially for large sized potato, which could feed higher price in the open market. Thus, "price fixed by the company is lower than the prevailing price" was ranked fourth along with "absence of government's active role" with a mean score of 3.00. Singh (12) suggested that government needs to play an enabling role by legal provisions and institutional mechanisms, like helping farmers co-operatives and groups, to facilitate smooth functioning of contract system.

The contract farming of potato seed production was beneficial in the study area. Hence, it may be promoted in other states or regions which grow potato for seed purpose. The study suggests that the Govt./ NGOs/ VOs should play an active role in the contractual arrangement in order to prevent conflicts/ breach of contractual agreement between the two

Table 4. Ranking of various constraints faced by farmers in potato seed contract farming (n = 30).

Constraint	Frequency					WTS	WMS	Ranks
	VS	QS	S	NS	LS			
Pest and disease	8	7	7	4	4	101	3.37	I
Non-availability of labour during peak period	6	7	7	7	3	96	3.20	II
Difficulty in meeting quality requirements	7	7	4	7	5	94	3.13	III
Price fixed is lower than the prevailing market price	3	8	8	8	3	90	3.00	IV
Absence of government's active role	3	9	7	7	4	90	3.00	IV
Faulty grading by an agency	6	7	3	8	6	89	2.97	V
Delay in procurement of produce	4	6	4	10	6	82	2.73	VI
Lack of visits by field officers	2	6	7	11	4	81	2.70	VII
Terms and conditions made in favour of firm	3	7	6	5	9	80	2.67	VIII
Lack of irrigation water	2	7	4	12	5	79	2.63	IX
Lack of credit and crop insurance facilities	3	6	5	9	7	79	2.63	IX
Delay in delivery of inputs	0	6	5	12	7	70	2.33	X
Delay of payment	0	7	3	13	7	70	2.33	X

Rating scale: Very severe (VS) = 5, Quite severe (QS) = 4, Severe (S) = 3, Not so severe (NS) = 2, Least severe (LS) = 1; WTS = Weighted total score, WMS = Weighted mean score

contracting parties. The government should also develop a mechanism for the inclusion of marginal and small farmers in the contract farming system.

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Short communication

Response of China aster (*Callistephus chinensis* (L.) Nees) cv. Kamini to different combinations of NPK and biofertilizers

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ABSTRACT

An investigation was carried out to find a suitable combination of NPK and biofertilizers for maximizing flower yield in China aster cv. Kamini. The experiment was laid out in randomized block design (RBD) with 12 treatment combinations replicated thrice. The observations on various growth and flowering parameters were recorded. Results revealed that maximum plant height (56.67 cm), number of leaves per plant (103.93), flowering branches per plant (8.23), plant spread (32.32 cm), number of flowers per plant (28.58), number of flowers per plot (428.67), duration of flowering (27.20 days), flower yield per plant (73.21 g), flower yield per plot (1098.20 g), shelf-life of flowers (6.43 days) at ambient conditions were recorded in plants receiving 75% NPK (22.5:11.25:7.5 g NPK/ m^2) + *Azotobacter* + PSB. Plants supplied with 100% NPK (30:15:10 g NPK/ m^2) + *Azotobacter* + PSB were noticed with maximum leaf area (15.76 cm^2), whereas, largest flower diameter (5.41 cm) and fresh weight of individual flower head (2.70 g) were found in plants receiving 50% NPK (15:7.5:5 g NPK/ m^2) + *Azotobacter* + PSB.

Key words: China aster, NPK, *Azotobacter*, phosphorus solubilizing bacteria.

China aster (*Callistephus chinensis* (L.) Nees) belongs to family 'Asteraceae' and is native to China. It is an important loose flower in India and is being grown in Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra and West Bengal. Productivity and quality of the flowers of China aster can be improved by using high yielding cultivars through improved nutrition. Though, the chemical fertilizers are important sources of nutrients, these are not only costly but growing awareness of environment pollution and limitations of non-renewable resources may introduce additional constraints. The use of chemical fertilizers also poses a major threat to sustain soil health and crop productivity. Incorporation of biofertilizers, which are eco-friendly, economical and easily available, in combination with chemical fertilizers can, to some extent, prevent the detrimental effects of current practices. Keeping in view the above facts, this investigation was undertaken.

The studies were carried out during June 2015 to November 2015 at the Research Farm of the Department of Floriculture and Landscape Architecture, Dr YSPUH&F, Nauni, Solan, Himachal Pradesh. The experimental site is located at a latitude of 30^o 52' 02" N and longitude of 77^o 11' 30" E with an elevation of 1,276 m above mean sea level. The climate of the area, in general, is sub-temperate to sub-tropical and is characterized by mild summers and cool winters. Mean maximum temperature (24.15°C) was recorded in July 2015 with minimum (15.45°C) in November 2015 during study period. The experiment was laid out in randomized block design (RBD) with twelve fertilizer treatments {T_1: 100% NPK (RDF, *i.e*. 30:15:10 g NPK/ m^2), T_2: 100% NPK + *Azotobacter*, T_3: 100% NPK + PSB, T_4: 100% NPK + *Azotobacter* + PSB, T_5: 75% NPK (22.5:11.25:7.5 g NPK/ m^2), T_6: 75% NPK + *Azotobacter*, T_7: 75% NPK + PSB, T_8: 75% NPK + *Azotobacter* + PSB, T_9: 50% NPK (15:7.5:5 g NPK/m^2), T_{10}: 50% NPK + *Azotobacter*, T_{11}: 50% NPK + PSB, T_{12}: 50% NPK + *Azotobacter* + PSB} replicated thrice. The soil of the experimental field was medium in nitrogen and high in phosphorus and potassium availability. The pH and electrical conductivity of soil was normal with high organic carbon. Nursery of China aster cv. Kamini was raised in elevated bed. There were 36 plots of 1 m x 1 m each having 15 plants with a spacing of 30 cm × 20 cm. Half dose of nitrogen and whole of the phosphorus and potassium were incorporated in soil before planting according to the treatment. The remaining half dose of nitrogen was given after 35 days of planting. *Azotobacter* and phosphate solubilizing bacteria (PSB) were applied immediately before planting by dipping the roots of the seedlings for 30 min. in the slurry prepared by dissolving 200 g of single biofertilizer or 100 g of both biofertilizers in one litre water. Pinching was done by removing the plant part above 6 inches from the ground level

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Table 1. Response of China aster cv. Kamini to different combinations of NPK and biofertilizers.

Treatment	Plant height (cm)	No. of leaves per plant	Leaf area (cm^2)	No. of flowering branches per plant	Plant spread (cm)	No. of days taken for first flower bud formation	No. of days taken for first flower opening	No. of flowers per plant	No. of flowers per plot	Flower dia. (cm)	Duration of flowering (days)	Fresh wt. of individual flower head (g)	Flower yield per plant (g)	Flower yield per plot (g)	Shelf-life of flowers in ambient conditions (days)
T_1	51.12	92.07	13.05	6.73	27.91	71.43	81.67	23.09	346.33	5.12	23.13	2.32	53.96	809.35	4.63
T_2	52.92	96.40	14.44	7.00	29.16	71.10	81.93	24.11	361.67	5.21	25.43	2.44	58.15	872.30	5.13
T_3	52.54	95.07	14.77	7.13	30.76	69.43	80.97	25.91	388.67	5.23	25.67	2.51	62.32	934.75	5.23
T_4	55.78	101.33	15.76	8.03	31.87	68.13	79.30	27.73	416.00	5.35	26.77	2.60	71.50	1072.50	5.57
T_5	49.54	89.87	12.75	6.60	26.06	66.53	78.97	22.98	344.67	5.02	22.90	2.40	53.69	805.35	4.30
T_6	53.44	98.93	13.37	7.70	30.07	68.37	80.10	25.71	385.67	5.16	24.80	2.55	61.17	917.50	6.00
T_7	54.02	99.13	14.02	7.87	31.32	66.93	78.53	27.07	406.00	5.17	24.93	2.57	66.06	990.95	6.13
T_8	56.67	103.93	15.14	8.23	32.32	65.90	77.87	28.58	428.67	5.26	27.20	2.64	73.21	1098.20	6.43
T_9	48.20	88.80	12.39	6.30	25.51	72.30	82.73	22.51	337.67	4.97	22.67	2.27	50.66	759.90	4.07
T_{10}	50.19	94.20	13.49	6.80	27.02	72.93	83.30	23.56	353.33	5.19	23.57	2.43	56.02	840.35	4.77
T_{11}	50.08	90.80	13.61	7.07	26.51	68.73	80.20	24.80	372.00	5.21	24.00	2.48	56.24	843.65	4.97
T_{12}	54.14	97.60	14.97	7.53	28.60	66.17	78.30	26.76	401.33	5.41	26.07	2.70	68.18	1022.65	5.93
$CD_{0.05}$	1.87	4.30	1.10	0.85	2.86	2.33	2.12	1.74	26.11	0.20	2.06	0.15	5.82	87.27	0.77

T_1 = 100% NPK (RDF *i.e.* 30:15:10 g; T_2 = 100% NPK + *Azotobacter* NPK/ m^2), T_3 = 100% NPK + PSB, T_4 : 100% NPK + *Azotobacter* + PSB, T_5 = 75% NPK (22.5:11.25:7.5 g NPK/ m^2), T_6 75% NPK + *Azotobacter*, T_7: 75% NPK + PSB, T_8: 75% NPK + *Azotobacter* + PSB, T_9 = 50% NPK (15:7.5:5 g NPK/m^2), T_{10} = 50% NPK + *Azotobacter*, T_{11} = 50% NPK + PSB, and T_{12} = 50% NPK + *Azotobacter* + PSB

after 30 days of transplanting to break the apical dominance and encourage the emergence of lateral branches. Observations on plant growth and flowering characters were recorded and analyzed statistically.

The plant growth parameters showed significant results due to different fertilizer treatments as depicted in Table 1. The maximum plant height (56.67 cm), number of leaves per plant (103.93), number of flowering branches per plant (8.23) and plant spread (32.32 cm) was recorded in T_8 comprising of 75% NPK + *Azotobacter* + PSB, however, higher leaf area (15.76 cm^2) was found in T_4, *i.e.* 100% NPK + *Azotobacter* + PSB. Minimum values for these characters were observed in T_9, *i.e.* 50% NPK alone. The combined application of biofertilizers with NPK resulted in better nutrition which lead to increased photosynthesis activity, enhanced cell division and enlargement as nitrogen is important constituent of nucleic acid and it might have increased the synthesis of carbohydrate, amino acids *etc*. from which the phytohormones like auxins, gibberellins, cytokines have been synthesized and phosphorus being an essential component of protoplasm and chlorophyll, caused conversion of photosynthates into phospholipids resulting in adequate vegetative growth. Biofertilizers produce several growth promoting hormones (auxins, cytokinins and gibberellins *etc*.) in addition to increasing the availability of nitrogen and phosphorus to the plants resulting in better plant growth. The reduction in plant growth characters with T_9 may be due to the lack of nutrition to the plants receiving half dose, so, they could not assimilate required food materials to support the vegetative growth and hence lesser plant growth. Similar results of increased plant growth due to combined application of biofertilizers with NPK have been reported by Chaitra and Patil (2), Patil and Agasimani (8) and Kirar *et al*. (4) on China aster; Kumar *et al*. (5) and Gupta *et al*. (3) on marigold; Airadevi (1) and Panchal *et al*. (7) on annual chrysanthemum.

The perusal of data presented in Table 1 discovered the significant results for flowering characters with application of different fertilizer treatments. The application of 75% NPK + *Azotobacter* + PSB resulted in minimum number of days taken for first flower bud formation (65.90) and first flower opening (77.87), which can be attributed to early completion of vegetative growth and changing of vegetative primordia to reproductive primordia, probably due to the secretion of growth promoting substances like auxins, gibberellins, vitamins and organic acids by the biofertilizers, which resulted in early flower bud formation and ultimately early flower opening. Further, phosphorus is an important element and essential for the initiation of flowering and PSB is known to increase the availability of phosphorus resulting in early flowering. Flower bud formation and opening were delayed with application of 50% NPK + *Azotobacter*, which can be due to the fact that the nutritional requirements of the plants were not met, so they took more time to complete their vegetative phase and delayed the flower bud formation and opening. These results got support from the findings of Chaitra and Patil (2) and Kirar *et al*. (4) on China aster; Kumari *et al.* (6) in chrysanthemum and Thane *et al.* (9) on gerbera.

The number of flowers per plant (28.58), number of flowers per plot (428.67), duration of flowering (27.20 days), flower yield per plant (73.21 g), flower yield per plot (1098.20 g) and shelf-life of flowers in ambient conditions (6.43 days) was maximum in plants applied with 75% NPK + *Azotobacter* + PSB (T_8), whereas, the application of 50% NPK, *i.e.* T_9 registered minimum values for these parameters. This might be due to proper nitrogen, phosphorus and potassium assimilation from the combined use of NPK in association with more nitrogen fixing and phosphorus solubilizing proficiency and secretion of hormones by the biofertilizers. The more number of flowering branches might have increased the number of flowers ultimately leading to increased flower yield. Better assimilation of photosynthates resulted in more food reserve, thus, more flowering duration and shelf-life. The maximum flower diameter (5.41 cm) and fresh weight of individual flower head (2.70 g) was seen in T_{12} comprised of 50% NPK + *Azotobacter* + PSB. These results were in agreement with the findings of Kirar *et al*. (4) who reported maximum length and width of flower head and fresh weight of individual flower with 50% NPK + vermicompost + *Azotobacter* + PSB in China aster. From the present studies, it can be concluded that an application of 75% NPK (22.5:11.25:7.5 g NPK/m^2) along with inoculation of *Azotobacter* and phosphate solubilizing bacteria (PSB) was found superior regarding flower production of China aster cv. Kamini.

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Short communication

Postharvest application of $CaCl_2$ and wrapping materials on shelf-life of banana cv. Robusta

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ABSTRACT

Present experiment was conducted to evaluate the effect of $CaCl_2$ and wrapping materials on improving shelf-life of banana. Twelve treatment combinations comprising of $CaCl_2$ (2 or 4%), polyethylene bag, banana dried leaves, news paper, $CaCl_2$ (2%) + polyethylene bag, $CaCl_2$ (4%) + polyethylene bag, $CaCl_2$ (2%) + banana dried leaves, $CaCl_2$ (4%) + banana dried leaves, $CaCl_2$ (2%) + news paper, $CaCl_2$ (4%) + newspaper with control were taken. $CaCl_2$ (4%) + polyethylene bag treatment resulted in minimum spoilage (19.65%), highest marketability (80.35%), fair organoleptic score, maximum total sugars (20.10%), ascorbic acid (2.96 mg/ 100 g of fresh weight) with increased shelf-life (16 days) of banana cv. Robusta fruits.

Key words: Banana, $CaCl_2$, post-harvest storage, shelf-life, wrapping material.

Bihar is an important banana growing state which produces 4.8% of total banana production in the country (Anon, 1). Robusta is the prime choicest cultivar of this state, which predominantly grown in Bhagalpur, Koshi in Purnea and Gandak in Vaishali districts with its high yield prospective and excellent fruit quality. As far as postharvest losses of banana fruits are concerned it is approximately 30-40 per cent. It happens due to the perishable nature of this fruit, wretched handling practices as well as inadequate storage facilities. Wrapping revealed immense role in lengthening the shelf-life as well as reducing the wastage by inhibiting undesirable physiological events, bruising and pathological deterioration during storage, transportation and marketing (Sahay *et al*., 7). The appropriate wrapping materials afford congenial surroundings which decreases the ethylene synthesis, unwanted bio-chemical changes, ripening, slows down the rate of respiration, desiccation and pathological deterioration of fruits (Singh *et al.*, 8).

Polyethylene bags are used broadly to prolong shipment and storage life of banana and other fruits. Singh *et al.* (8) found that the shelf-life of strawberry was maintained up to six days when they were packed in high-density polyethylene pouches. A number of chemicals were also reported to have role in postharvest management of banana fruits by delaying the ripening process. The use of calcium salts itself as well as combined action of chemical dip with 1% (w/v) calcium chloride, 0.75% (w/v) ascorbic acid and 0.75% (w/v) cysteine help to maintain firmness of fresh cut banana (Vilas-Boas and Kader, 9) and improve quality of many fruits during storage by minimizing the respiration rate, disease incidence and weight loss.

The present experiment was carried out at the Department of Horticulture (Fruit & Fruit Technology), BAU, Sabour, Bhagalpur. The bunches of banana cv. Robusta were dehanded, washed and treated with $CaCl_2$ (2 or 4%) solution. The surface moisture was dried under shade. The treated or untreated (control) bunches were packed in newspaper, dried banana leaves and LDPE polythene bags of 150 gauge thickness of pouch size (120 × 60 cm). The total surface area of each polythene bag was 7,200 cm^2 with perforation (8 holes /bag) and each whole having 0.50 cm^2 surface areas (Sahay *et al.*, 7).

The fruits were handpicked to avoid any injury and carried to the experimental laboratory in bamboo basket. Only firm healthy fruits of uniform size and maturity, free from pest, disease, injuries, bruises and blemishes were selected for the experiment. Banana hands were selected from mature uniform bunches. One hand with 12 fingers was considered as one experimental unit. Aqueous solution of $CaCl_2$ (2 or 4%) solutions were used in which the fruits were dipped (10 min.). The treated fruits were air-dried. There were 12 treatment combinations replicated thrice under completely randomized design (CRD). Each treatment had two sets. First set was used for estimation of spoilage and marketability, while the second set was used for bio-chemical analysis as well as organoleptic evaluation. Marketability of the fruits was characterized on the account of their firmness, colour and appearance at alternate day interval on the strength of initial fruit weight. For storage studies, the maximum temperature ranged from 27.4° to

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32.4°C and minimum temperature varied from 9.5° to 16.8°C, while, the relative humidity varied from 25.0 to 88.0% during the storage period. The total sugars and ascorbic acid contents were estimated following standard methods. Sensory evaluations were conducted to assess the consumer acceptability of the stored fruits by the score and corel system with the panel of five judges (Larmond, 4).

The spoilage of fruits increased with successive increment in the storage period irrespective of treatments (Table 1). The highest spoilage of banana fruits during storage was recorded under control to the tune of 79.35% (T_{12}), while significantly lower spoilage (19.65%) was found in $CaCl_2$ (4%) + fruits packaged in polyethylene bag (T_7) followed by $CaCl_2$ (4%) + fruits wrapped in newspaper (21.04%) on the 16th day of experiment. This might be due to existing pathogens on the fruit surface, which easily invaded and increased decaying of banana fruits with storage period (Emerald and Sreenarayanan, 2). The unpacked fruits were exposed openly with the direct contact of air and temperature of contiguous situation, so that control might have respired faster and transpired rapidly that causes loss of water and enhanced the microbial infection. At this time, ethylene evolution, degradative metabolism and pectin hydrolysis were also at higher rate resulting in more decay. The polyethylene bag acted as an effective barrier to surrounding atmosphere and reduced exposure of fruits to micro-floras. Ventilated polyethylene bags slowed down the rate of respiration, ethylene evolution, oxidative metabolism and pectin hydrolysis resulting in retention of firmness. It might have imparted some resistance against the growth of the pathogens on fruits.

The marketability of banana fruits was evaluated on the basis of shrinkage, softness, appearance and taste of fruits. In all treatments marketability declined gradually and successively with the prolongation of storage period (Table 2). The highest marketability were noticed with $CaCl_2$ (4%) + polyethylene bag (90.41%) followed by $CaCl_2$ (2%) + polyethylene bag (90.17%) at the end of storage period as compared to control (69.22%). Improvement in marketability percentage of fruits treated with $CaCl_2$ along with different wrapping materials is due to good texture, better edible quality, less physiological and spoilage losses.

Total sugars increased gradually with the advancement of storage period and after attaining its peak, the value reduced slightly (Fig. 1). The fruits packed with $CaCl_2$ (2%) + polyethylene bag or $CaCl_2$ (4%) + polyethylene bag (20%) exhibited continuous enhancement in total sugars content till the end of the experiment, whereas in remaining treatments, it declined after 14th day of storage. At the end of experiment, the highest total sugars were obtained in fruits packaged with $CaCl_2$ (4%) + polyethylene bag (20%), which showed statistical parity with $CaCl_2$ (2%) + polyethylene bag and minimum value was recorded under control (16%). The increase in total sugars content may be due to loss of moisture and hydrolysis of polysaccharides and transformation of organic acids into soluble sugars. The declined

Table 1. Effect of $CaCl_2$ and wrapping material on spoilage of banana fruits during storage.

Treatment	Period of storage (days)									Mean
	00	02	04	06	08	10	12	14	16	
T_1- $CaCl_2$ 2%	0.00	0.00	2.12	3.02	4.35	6.85	11.59	19.95	28.60	10.93
T_2- $CaCl_2$ 4%	0.00	0.00	2.02	2.89	4.15	6.73	11.45	19.71	28.02	10.71
T_3- Polyethylene bag	0.00	0.00	3.40	4.16	10.12	13.62	32.57	61.12	68.35	27.62
T_4- Banana dried leaves	0.00	0.00	4.36	5.28	10.86	18.65	35.95	64.24	73.46	30.40
T_5- Newspaper	0.00	0.00	5.93	6.58	12.16	19.25	40.35	67.35	75.20	32.40
T_6- $CaCl_2$ 2% + polyethylene bag	0.00	0.00	1.80	2.50	5.35	8.20	11.34	15.20	20.05	9.21
T_7- $CaCl_2$ 4% + polyethylene bag	0.00	0.00	1.60	2.42	5.10	8.01	11.04	15.04	19.65	8.98
T_8- $CaCl_2$ 2% + Banana dried leaves	0.00	0.00	1.80	2.95	6.02	8.82	11.59	20.02	23.45	10.66
T_9- $CaCl_2$ 4% + Banana dried leaves	0.00	0.00	1.90	2.89	5.68	8.69	11.04	17.79	23.04	10.15
T_{10}- $CaCl_2$ 2% + Newspaper	0.00	0.00	2.60	3.49	7.35	10.21	13.34	17.24	21.20	10.78
T_{11}- $CaCl_2$ 4% + News paper	0.00	0.00	2.50	3.30	9.20	12.17	15.15	17.04	21.04	11.49
T_{12}- Control	0.00	0.00	6.59	7.63	12.95	20.65	42.35	69.35	79.35	34.12
Mean	0.00	0.00	3.05	3.93	7.77	11.82	20.65	33.67	40.12	
CD at 5%	T = 0.55; D = 0.46; T × D = 1.65									

Table 2. Effect of $CaCl_2$ and wrapping material on marketability of banana fruits.

Treatment	Period of storage (days)									Mean
	00	02	04	06	08	10	12	14	16	
T_1- $CaCl_2$ 2%	100.00	100.00	97.88	96.98	95.65	93.15	88.41	76.13	71.40	88.51
T_2- $CaCl_2$ 4%	100.00	100.00	97.98	97.11	95.85	93.27	88.55	76.35	71.96	88.72
T_3 -Polyethylene bag	100.00	100.00	96.60	95.84	89.88	86.38	67.43	36.56	31.65	72.05
T_4- Banana dried leaves	100.00	100.00	95.60	85.72	89.14	83.62	67.13	42.60	26.75	70.09
T_5- Newspaper	100.00	100.00	96.07	95.14	88.95	81.80	82.31	35.90	24.80	72.14
T_6- $CaCl_2$ 2% + polyethylene bag	100.00	100.00	98.02	97.50	94.65	91.80	88.60	80.64	79.95	90.17
T_7- $CaCl_2$ 4% + polyethylene bag	100.00	100.00	98.40	97.50	94.90	91.99	88.96	80.80	80.35	90.41
T_8- $CaCl_2$ 2% + Banana dried leaves	100.00	100.00	97.40	95.07	92.65	89.79	86.66	78.80	78.76	88.43
T_9- $CaCl_2$ 4% + Banana dried leaves	100.00	100.00	97.50	95.29	93.50	89.04	87.40	78.89	78.96	88.65
T_{10}- $CaCl_2$ 2% + Newspaper	100.00	100.00	98.20	95.34	93.88	91.18	88.41	76.06	75.10	88.31
T_{11}- $CaCl_2$ 4% + Newspaper	100.00	100.00	98.10	97.11	94.32	91.32	88.96	76.28	75.95	88.86
T_{12}- Control	100.00	100.00	95.01	94.18	89.37	87.68	87.21	49.74	23.35	69.22
Mean	100.00	100.00	97.23	95.23	92.73	89.25	84.17	65.72	59.92	
CD at 5%	T= 2.46; D = 2.13; T x D = 7.37									

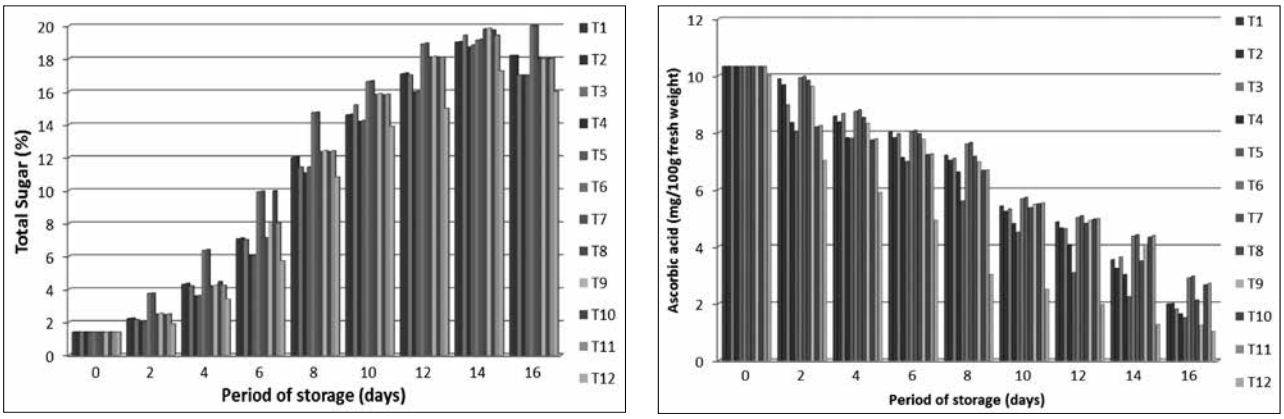

Fig. 1. (a) Total sugars (%) and (b) ascorbic acid contests in banana fruits during storage under various packaging treatments.

rate of physiological changes and slow conversion of starch and polysaccharides into simple sugars and less utilization in respiration and other catabolic process might be the reasons of estimation of the highest content of total sugars in fruits dipped in solutions of calcium salts. Similar findings have also been reported by Prasad *et al.* (6) and Jagadeesha *et al.* (3) in banana.

The ascorbic acid content in fruits reduced gradually and progressively with the prolongation of storage period, irrespective of treatments (Fig. 1a, b). The minimum depletion on termination of experiment was observed in fruits treated with $CaCl_2$ (4%) + polyethylene bag (2.96 mg/100 g of fresh weight) and $CaCl_2$ (2%) + polyethylene bag (2.90 mg /100 g of fresh weight). The depletion in ascorbic acid content was due to oxidation of L-ascorbic acid into dehydro-ascorbic acid by enzymes ascorbinase (Mapson, 5). Higher retention of ascorbic acid in banana during storage with calcium compound has also been reported by Prasad *et al.* (6) and Jagadeesha *et al.* (3). There was a gradual enhancement in score of organoleptic rating under all the wrapping materials and afterwards it declined with prolongation of storage period. The initial increase in organoleptic score was due to softness increase in TSS, flavour, development of colour and decrease in acidity. Fruits stored under $CaCl_2$ (4%) + polyethylene bag had high organoleptic score upto 16th day of storage of banana fruits at ambient temperature (Table 3). It was shown that shelf-

Table 3. Sensory evaluation of banana fruit during storage.

Treatment	Period of storage (days)						Mean
	06	08	10	12	14	16	
T_1-$CaCl_2$ 2%	58	71	82	88	74	62	72.50
T_2- $CaCl_2$ 4%	60	73	86	92	78	56	74.16
T_3- Polyethylene bag	51	71	86	82	74	54	69.67
T_4- Banana dried leaves	50	70	84	80	70	58	68.67
T_5- Newspaper	52	76	79	78	70	42	66.16
T_6- $CaCl_2$ 2% + polyethylene bag	60	75	89	94	81	72	78.50
T_7- $CaCl_2$ 4% + polyethylene bag	62	77	90	96	85	74	80.67
T_8- $CaCl_2$ 2% + banana dried leaves	58	72	88	91	82	70	77.00
T_9- $CaCl_2$ 4% + banana dried leaves	60	74	89	92	83	71	78.17
T_{10}- $CaCl_2$ 2% + newspaper	55	66	76	90	83	59	71.50
T_{11}- $CaCl_2$ 4% + newspaper	56	78	82	92	84	60	75.33
T_{12}- Control	50	70	76	80	60	45	63.50
Mean	56.00	72.75	83.92	87.92	77.00	60.25	
Rating (Score)	Excellent = 90-100; Good = 80-90; Fair = 70-79						

life of banana fruits was enhanced with application of $CaCl_2$ (4%) + polyethylene bag or $CaCl_2$ (2%) + polyethylene bag, which also reduced the spoilage, prolonged marketability with fair organoleptic score.

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Short communication

Influence of chitosan coating and storage temperatures on postharvest quality of guava

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ABSTRACT

The effect of chitosan coating on postharvest quality of guava cv. Allahabad Safeda fruits stored at room temperature, 12° and 8°C was investigated. The fruits were either treated with chitosan (1 and 2%), acetic acid 1% or untreated and various quality attributes were studied at the end of storage period. Among the different treatments and temperatures, the chitosan 1% treated fruits stored at 12°C had shown higher firmness, TSS, titratable acidity and maintained greenness with a slow increase in yellow colour by the end of storage life. Also, the total antioxidant capacity, total phenols and total flavonoids of these fruits were well maintained by the time they are full ripe. Chitosan 1% and storage temperature of 12°C can be used for extending the storage life of guava upto 21 days with least deterioration in postharvest quality.

Key words: Antioxidants, acetic acid, edible coating, post-harvest.

Chitosan is one among various per-treatments extensively used nowadays for postharvest treatment of fruits. Chitosan has a chemical structure close to that of cellulose, has long been known to protect perishable produce from deterioration by reducing transpiration, respiration and maintaining the textural quality. Chitosan (poly β-(1-4)N-acetyl-d-glucosamine), a deacetylated form of chitin, is a natural compound obtained from crustacean shells (crabs, shrimp and cray fishes) either by chemical or microbiological processes and can be produced by some fungi too. India has a vast cost line (7,517 km) owing to its high capability of harvesting crustaceans from the sea, producing a large quantity of crustacean shell waste. Chitosan has been successfully tried and recommended for enhancing the shelf life of several fruits such as litchi, mango and guava.

Although, there has been some research into the use of chitosan as a preservative coating in some fruits, but very few published information on the use of chitosan coatings and different storage temperatures on postharvest quality of guava is available. In the present investigation, an attempt has been made to know the interaction effect of chitosan coating and storage temperatures on physio-chemical characteristics of guava after harvest.

Physiologically mature green fruits of guava cv. Allahabad Safeda were harvested manually from nearby orchards of IIHR, Bengaluru, during early hours (8.00-9.00 am). The fruits were transported to the laboratory in plastic crates, where they were sorted out to remove immature, misshaped, bruised, diseased and insect-infested fruits if any. These fruits were graded as floaters (≤1) and sinkers (>1) based on their specific gravity among which floaters (mature) were taken for the experiment. The fruits were then washed, air-dried and treated with chitosan. Acetic acid (1%) was used to dissolve and prepare 1% (C_2) and 2% (C_3) chitosan solutions. The solution was stirred for sufficient time using mechanical stirrer for complete dissolution of chitosan. Fruits were dipped in these chitosan solutions for 2 min., drained and surface dried. Acetic acid (1%) (C_1) was also taken as one of the treatment since the same was used in dissolving and preparing the chitosan solutions and un-treated as control (C_0). These fruits were then packed in non-ventilated CFB boxes, each with 20 fruits and stored at room temperature (T_1) (28-32°C and 32-41% RH), 12°C (T_2) and 8°C (T_3).

Fruit firmness, as the force required to puncture the fruit, was measured using an Instron-Universal testing machine (Model 4201, USA) and expressed as kg/cm^2. Quality components like total soluble solids (TSS) and titratable acidity were estimated according to standard AOAC methods (Ranganna, 8). Total antioxidants were estimated using FRAP (Ferric Reducing Antioxidant Potential) method as described by Benzie and Strain (3). Total phenols were estimated according to the procedure given by Singleton and Rossi (9). Total flavonoids in the methanol extract were determined as per Chun *et al.*

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(4). The surface colour of the fruit was measured with colour difference meter (Model: Color Reader, CR-10, Konica Minolta, Japan) in terms of (*L*, *a*, *b*) values. The experiment consisted of 12 treatments, *i.e.*, three storage conditions (including room temperature) and four pre-treatments (including control) under each storage condition. These treatments were replicated thrice. The observations recorded under each parameter at the end of storage life (7th at RT, 21st at 12°C and 35th at 8°C) were statistically analysed using factorial completely randomised design.

It is quite apparent from the table 1 that at the final stage of ripening, the higher firmness was retained at room temperature (RT) followed by 12°C irrespective of the pre-treatment given. Among the pre-treatments, chitosan 2% (5.39 kg/cm^2) treated fruits retained significantly higher firmness followed by chitosan (1%) treated fruits than acetic acid (1%) treated fruits and control. The interaction effect revealed that T_1C_3 has the highest firmness followed by T_2C_2 and the least firmness was observed in T_3C_1. Softening of guava fruit was remarkably delayed with chitosan (1 and 2%) treatment during storage at all the temperatures (Table 1). The increase in pectin solubilisation and disruption of the xyloglucan–cellulose micro fibril networks of guava fruit moderated by an increase in the activities of exo-polygalacturonase (PG), pectin methylesterase, β(1→4)-glucanase and β-galactosidase has been proposed to be associated with the rapid softening of fruit (Ali *et al*., 2). The maintenance of firmness in the fruits treated with 1 and 2% chitosan coatings could be due to the covering of the cuticle and lenticels and their higher antifungal activity thereby reducing infection, respiration and other ripening processes during storage.The retardation of fruit softening in response to chitosan treatment has been reported in many fruits such as papaya (Al Eryani *et al*., 1).

It was evident from the table 1 that, irrespective of the pre-treatments, at the fully ripe stage the titratable acidity was significantly retained at 12°C followed by 8°C. Among the pre-treatments, chitosan (2%) treated fruits retained significantly higher titratable acidity (0.62%), which is on par with chitosan (1%) treated fruits than acetic acid (1%) treated fruits and control. The interaction effect revealed that T_2C_2 (0.79%) shown higher titratable acidity which is on par with T_2C_3 and lowest was observed in remaining all other interaction effects which are on par with each other. The decrease in acidity during storage may be attributed to an increase in malic enzyme and pyruvate decarboxylation reaction during the climacteric period in apples. The fruits treated with Chitosan maintained higher acidity during storage probably due to delay in the ripening process (Table 1). Al Eryani *et al*. (1) observed lower acidity loss during storage in papaya.

A perusal of data in Table 1, showed that with respect to storage temperatures guava fruits stored at 8°C had shown highest TSS (12.85°Brix) followed by the fruits stored at 12°C which was on par with RT. Among the pre-treatments, C_2 treated fruits had the highest amount of TSS followed by C_3 treated fruits. The interaction of the two factors showed T_3C_3 and T_3C_2 had a higher content of TSS, followed by T_3C_0, T_3C_1 and T_3C_1 while lowest TSS content was found in T_1C_0 (10.13°Brix) and T_1C_1. The fruit treated with chitosan registered maximum TSS content, while the lowest average TSS was recorded by control and acetic acid 1% treated fruits. The increase in TSS/ sugars during storage/ripening may be possibly due to hydrolysis of starch into sugars and on complete hydrolysis of starch, no further increase occurs and subsequently a decline in these parameters is predictable as they along with other organic acids are primary substrate for respiration. Chitosan delayed

Table 1. Effect of Chitosan coatings on firmness, titratable acidity and TSS in guava cv. Allahabad Safeda fruits at the end of storage.

Treatment	Firmness At harvest (34.94 kg/ cm^2)				Titratable acidity At harvest (1.25%)				TSS At harvest (11.10°B)			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
C_0	3.56	2.41	2.41	2.79	0.50	0.62	0.48	0.53	10.13	11.12	12.37	11.21
C_1	3.81	3.19	2.26	3.09	0.48	0.55	0.49	0.51	10.63	11.47	12.23	11.44
C_2	5.97	6.71	2.67	5.12	0.50	0.79	0.55	0.61	12.27	11.47	13.33	12.36
C_3	8.50	5.02	2.65	5.39	0.50	0.73	0.62	0.62	10.97	11.27	13.47	11.90
Mean	5.46	4.33	2.50		0.50	0.67	0.54		11.00	11.33	12.85	
	T	C	T×C		T	C	T×C		T	C	T×C	
CD at 1%	0.03	0.03	0.06		0.03	0.04	0.07		0.46	0.53	0.92	

C_0 = Control, C_1 = Acetic acid (1%), C_2 = Chitosan (1%), C_3 = Chitosan (2%), T_1 = Room temperature, T_2 = 12°C, T_3 = 8°C.

metabolic activity of fruits during storage due to reduced respiration rate with consequent delay in ripening as shown in Table 1. Keqian *et al*. (6) reported the delayed metabolic activity and respiration rate in the chitosan treated guava fruits.

The interaction effect showed that T_2C_3, which was on par with T_2C_2 is the best treatment combination to achieve higher total anti-oxidant capacity and the least total anti-oxidant capacity (177.41 mg ascorbic acid eqv. /100 g) was found under the treatment combination T_3C_2. The changes in the total antioxidant capacity of the guava fruits are shown in Table 2. The total antioxidant capacity of the guava fruits increased at ripe stage compared to harvest. However, the results obtained were contradictory to Neeraj *et al.* (7) who reported the decline of antioxidants in fruits during their ripening. Among the storage temperatures, guava fruits stored at 12°C had shown higher amounts of total antioxidant capacity followed by those stored at RT and lowest at 8°C. The reduced antioxidant activity at 8°C might be due to more utilization of the antioxidants to neutralize the free radicals produced by the low-temperature stress (chilling injury). Among the pre-treatments highest anti-oxidant capacity was noticed in chitosan (2%) treated fruits at the full ripe stage, while the acetic acid (1%) treated fruits had the lowest antioxidant capacity. The chitosan treated guava fruits stored at 12°C had shown significantly higher total antioxidant capacity than other treatments, which shows the loss in antioxidants at RT was mainly due to increased respiration rate in this temperature compared to 12°C.

The interaction studies reveal that T_2C_3 (650.93 mg gallic acid eqv./100 g) had significantly higher total phenols followed by T_1C_3, while lowest total phenols were recorded in T_3C_2, which is on par with T_3C_3. Total phenols were high in fruits stored at RT, followed by 12°C, whereas the fruits stored at 8°C have shown reduced total phenol content (Table 2). Similar observations were recorded in guava fruits (Hussain *et al.*, 5) stored at 10 or 20°C for 3 weeks and found that total phenols decreased significantly as storage period and temperature increased. Among pre-treatments chitosan treated fruits had highest total phenols content followed by control fruits whereas acetic acid (1%) treated fruits had the lowest phenol content. This might be due to, the reduction of ripening rate and respiration, which lead to maintained phenols in the post-storage ripening period.

Flavonoids are one of the major compounds contributing to the total antioxidant capacity of the fruits and vegetables. In nature, very large quantities of flavonoids are present in the form of catechins. The interaction of storage temperatures with pre-treatments showed T_1C_0 (395.09 mg catechin eqv. /100 g) as the best treatment followed by T_2C_2, which is on par with T_1C_2, while the poor performing treatment was T_3C_3. In this experiment, there were high total flavonoids at full ripe stage compared to the day of harvest and the total flavonoids were highest at 12°C followed by RT and lowest at 8°C (Table 2). Among the pre-treatments the highest flavanoid content in control might be due to a lesser number of days taken by them to reach a full ripe stage, which is followed by chitosan (1%) and chitosan (2%). The low flavonoids were found in acetic acid treated fruits.

At full ripe stage, irrespective of the storage period and pre-treatment given (Table 3), the L-values were significantly high at 12°C (68.86) followed by RT and were significantly low at 8°C (61.22). Among the three pre-treatments, control treated fruits had significantly higher L-values than acetic acid (1%)

Table 2. Effect of Chitosan coating on total antioxidant capacity, total phenols and total flavonoids in guava cv. Allahabad Safeda fruits at the end of storage.

Treatment	Total antioxidant capacity (mg ascorbic acid eqv./ 100 g) At harvest (122.90)				Total phenols (mg gallic acid eqv. / 100 g) At harvest (690.89)				Total flavonoids (mg catechin eqv. / 100 g) At harvest (98.54)			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
C_0	235.51	233.16	197.12	221.93	625.48	607.55	452.14	561.72	395.09	311.17	234.88	313.71
C_1	186.75	241.95	192.18	206.96	604.01	594.41	455.09	551.17	197.13	301.74	142.48	213.78
C_2	232.56	274.91	177.41	228.30	618.00	625.36	443.50	562.29	322.14	330.86	208.76	287.25
C_3	245.25	279.34	234.78	253.12	634.87	650.93	445.62	577.14	258.86	295.54	183.18	245.86
Mean	225.02	257.34	200.37		620.59	619.56	449.09		293.31	309.83	192.33	
	T	C	T×C		T	C	T×C		T	C	T×C	
CD at 1%	5.11	5.98	10.25		4.39	5.07	8.79		5.11	5.90	10.22	

C_0 = Control, C_1 = Acetic acid (1%), C_2 = Chitosan (1%), C_3 = Chitosan (2%), T_1 = Room temperature, T_2 = 12°C, T_3 – 8°C.

Table 3. Effect of Chitosan coating on surface colour (*L*, *a* and *b* values) in guava cv. Allahabad Safeda fruits at the end of storage.

Treatment	*L* At harvest (51.22)				*a* At harvest (-14.48)				*b* At harvest (35.58)			
	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean	T_1	T_2	T_3	Mean
C_0	66.73	72.12	65.14	67.99	5.16	5.23	3.80	4.73	44.84	44.13	41.87	43.61
C_1	65.89	66.37	66.26	66.17	6.65	8.96	1.43	5.68	42.93	42.34	41.88	42.38
C_2	64.54	68.21	58.85	63.87	0.45	3.55	6.62	3.54	42.90	46.94	39.70	43.18
C_3	59.43	68.73	54.63	60.93	-5.06	4.26	2.52	0.57	39.63	46.32	35.95	40.63
Mean	64.15	68.86	61.22		1.80	5.50	3.59		42.58	44.93	39.85	
	T	C	T×C		T	C	T×C		T	C	T×C	
CD at 1%	0.09	0.10	0.18		0.08	0.10	0.17		0.06	0.07	0.12	

C_0 = Control, C_1 = Acetic acid (1%), C_2 = Chitosan (1%), C_3 = Chitosan (2%), T_1 = Room temperature, T_2 = 12°C, T_3 = 8°C.

treated fruits followed by chitosan (1%) and chitosan (2%). The interaction effect between the temperature and the pre-treatments indicate that T_2C_0 and T_2C_3 were the best possible combinations followed by T_2C_2 while T_3C_3 was the poorest performing combination.

It is evident from the data in table 3 that, with respect to storage temperatures alone, RT (1.80) had significantly lower a-value compared to 12°C and 8°C at thefull ripe stage. Among the pre-treatments given chitosan (2%) treated fruits had the lowest a-value followed by chitosan (1%), control and acetic acid (1%) treated fruits. The interaction studies reveal that T_1C_3 had significantly lower a-value than all other treatment combinations followed by T_1C_2. The data presented in the table 3 shows that, at full ripe stage irrespective of the pre-treatment, highest b-value was observed for fruits stored at 12°C followed by RT, while control, chitosan (1%) treated fruits had attained higher b-value compared to acetic acid 1% and chitosan (2%) treated fruits. The effect of interaction between storage temperature and pre-treatments showed that T_2C_2 was the best treatment combination followed by T_2C_3 and T_3C_3 was the poorly performed combination.

In our present study, chitosan (1 and 2%) treatments significantly delayed the green colour loss in guava fruits (Table 3). The occurrence of yellow colour on fruits was further delayed with a reduction in storage temperature of chitosan treated fruits. However, a slow but continuous increase in yellowness value of fruit was observed in chitosan (1 and 2%) treated fruits during later days of storage at 12° and 8°C. Similar results were reported by Yueming *et al*. (10) in chitosan treated longan fruits. But fruits treated with chitosan (2%) at RT did not turn yellow at all which may be due to the high CO_2 accumulation in tissue of the fruit, which completely retarded the yellow colour development. There were green mosaic patches on the fruits, attributed to the CO_2 injury. Among all the treatments and temperatures, chitosan (1%) and 12°C was found more appropriate in retention of fruit quality at the end of storage period, *i.e.*, upto 21 days. Even though 8°C extended the storage life for more than 30 days, it showed chilling injury after the fruits were shifted to room temperature condition. chitosan (2%) is also not recommended as the higher concentration lead to uneven ripening of fruits at room temperature.

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Short communication

A process for preparation of ketchups from mango and guava fruits and their storage study

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ABSTRACT

A process for preparation of ketchups from ripe mango and guava fruits was developed, comprised extraction of pulp by conventional methods, homogenization and addition of sugar, salt, spices, acetic acid *etc.* The contents were heated to reduce the volume to one third, added with class II preservatives and bottled. Changes in TSS, acidity, non enzymatic browning, ascorbic acid, antioxidants along with microbial and sensory qualities were recorded at one month interval for a period of nine months under ambient storage conditions. The ascorbic acid content decreased from 98.3 to 81.8 and 60.0 to 49.9 mg/100 g in guava and mango ketchups, respectively, while the NEB increased from 0.089 to 0.174 and 0.373 to 0.577 after nine month storage. Slight increase in TSS and acidity were observed. The anti-oxidant value decreased from 32.8 to 18.8 mM/ ml in guava ketchup and 26.9 to 16.2 mM/ ml in mango ketchup after 9 month storage. No microbial population could be detected during the period of study. The overall acceptability score ranged from 7.0 to 7.8 and 7.2 to 8.5 in guava and mango, respectively.

Keywords: Fruits, guava, ketchup, mango, storage.

India is blessed with a variety of fruits having peculiar aroma and taste. Among these, mango and guava are the widely grown and commercially important fruit crops of India. The country produced 3.7 million tonnes of guava and 18.4 million tonnes of mango during year 2013-14 (NHB, 1). These are valuable sources of carbohydrates, minerals and vitamins, particularly vitamins A in mango and vitamin C in guava. Pickle, chutney, dried slices, amchoor, puree, squash, RTS drink, jam, canned slices, bar, etc. are the major traditional products prepared from mango, while guava products include squash, RTS drink, jam, jelly, cheese and toffee. However, in view of huge production of mango and guava in the country, there is a need to increase the level of processing as well as development of newer products from these crops.

Ketchup is often used as a supplement with various food preparations served either hot or fried. It is an integral part of fast food dishes like pizzas, burgers, noodles, etc. all over the world. Ketchup enhances taste and flavour of food stuffs and also serves as an appetizer. With changing food habits of modern generation and rapid growth of fast food industry in India, consumption of ketchup has also grown up fast. Mango and guava are also highly nutritional fruits. Mango is rich in beta-carotene (vitamin-A), a potential antioxidant compound like lycopene in tomato. Lupeol, a triterpene present in mango is known to exhibit a number of pharmacological properties including antioxidant, antilithiatic, and antidiabetic effects (Prasad *et al.*, 2). Guava fruit is considered as highly nutritious because it contains a high level of ascorbic acid and has several carotenoids such as phytofluene, β-carotene, β-cryptoxanthin, γ-carotene, lycopene, rubixanthin, cryptoflavin, lutein, and neochrome. Phenolic compounds such as myricetin, apigenin, ellagic acid, etc. are also at high levels in guava fruits (Verma *et al.*, 3). Apart from these chemical properties, mango and guava fruits bear pleasant aroma, an additional quality not found in tomato. Keeping in view, use of these fruits as alternate to tomato for production of ketchup from guava and mango pulps using spices and other additives was undertaken.

Disease-free, mature ripe fruits of mango and guava were washed thoroughly with tap water. Mangoes were peeled while guavas were cut into pieces using stainless steel knife. Smooth pulps were obtained by subjecting them to electric fruit pulper using 20 per cent water. Both the pulps were kept for heating separately in stainless steel vessels. Ingredients like onion, ginger and garlic along with coarsely ground spices (Table 1) were taken in a small piece of muslin cloth, tied and dipped in heating pulp in order to extract the aroma and taste of spices without adding turbidity to the product. Sugar and salt were added to the pulp and mixture was heated further till consistency of ketchup was achieved. The spice bag was removed after squeezing its extract

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in to pulp. Now, desired quantity of acetic acid (Table 1) was poured into it and pulp was heated for 5 minutes more. Finally, vessel was removed from flame and preservatives, *viz*. potassium metbisulphite and sodium benzoate (Table 1), dissolved in small quantities of water, were added. The prepared ketchup was filled hot in glass bottles and sealed with caps. The bottles were pasteurized in boiling water for 15 min. cooled to room temperature, labeled and stored in cool dry place.

Changes in TSS, acidity, non enzymatic browning, ascorbic acid, antioxidants along with microbial and sensory qualities were recorded at one month interval for a period of nine months under ambient storage conditions. The total soluble solids of ketchup were recorded by using hand refractometer (Erma, Japan). Titratable acidity, ascorbic acid and non-enzymatic browning were determined as per the methods described by Ranganna (4). Ascorbic acid content of beverage was measured by titrating samples against dye (2,6-dichloro phenol indophenol, sodium salt) solution. The amount of reducing sugars was determined by spectrophotometric method as per Folin and Wu (5). The anti-oxidant property of product in terms of FRAP values was determined as per Benzie and Strain (6). The microbial examination of mango and guava ketchups was carried out as per method detailed by Speck (7). The organoleptic evaluation of beverage was carried out by a panel of semi-skilled judges, using a 9-point Hedonic scale as prescribed by Amerine *et al.* (8). Sensory attributes like colour, flavour and taste were scored individually. The overall rating was obtained by calculating the average of the scores.

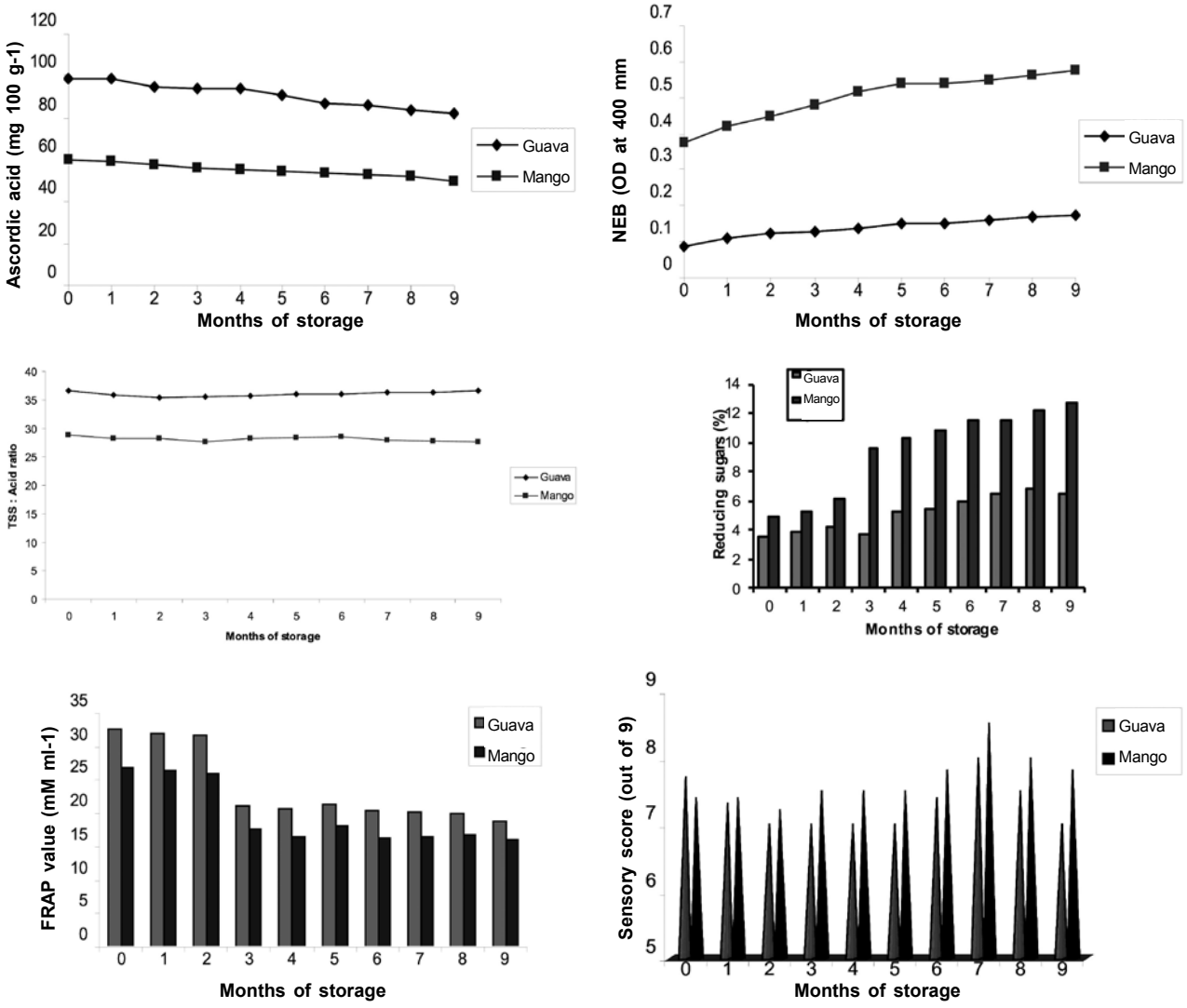

Fig. 1. Changes in bio-chemical and sensory parameters of guava and mango ketchups during storage.

Table 1. Ingredients used for preparation of guava and mango ketchups.

Ingredient	Quantity	
	Guava ketchup	Mango ketchup
Pulp	1000 g	1000 g
Sugar	110 g	67 g
Salt	26 g	15 g
Onion	37 g	50 g
Ginger	9.0 g	10 g
Garlic	5.0 g	5.0 g
Red chili powder	2.0 g	5.0 g
Spice mixture*	2.0 g	2.7 g
Acetic acid	5.0 ml	7.0 ml
Sodium benzoate	0.5 g	0.5 g
Potassium metabisulphite	0.25 g	0.25 g

*Spice mixture = 100 g contained 40 g cumin seeds, 12 g black pepper, 11 g cinnamon, 11 g mace, 4 g green cardamom, 2 g clove, 0.5 g big cardamom, 3.5 g nut meg and 16 g dried ginger

The microbial examination of the mango and guava ketchups revealed no microbial growth during storage. The total soluble solids (TSS) - acid ratio of guava and mango ketchups were 36.6 and 28.8 at zero time. Marginal changes in the TSS - acid were observed during storage of the product (Fig. 1). Initially, reducing sugar content of guava and mango ketchup were 3.50 and 4.93 per cent, respectively. It increased regularly with the storage period and finally reached to 6.49 and 12.69 in guava and mango samples, respectively after 9 months of storage (Fig. 1). The increase in reducing sugars content may be attributed to break-down of sucrose into glucose and fructose units during storage period (Kalra and Tandon, 9). Gradual decline in ascorbic acid content during storage was observed in both ketchups. It decreased from 98.3 to 81.8 and 60.0 to 49.9 mg/100g in guava and mango ketchups, respectively (Fig. 1). Similar trend in ascorbic acid content was observed by Famurewa *et al.* (10) in tomato paste during storage. Loss in ascorbic acid content may be attributed to gradual oxidation of ascorbic acid during storage. The non-enzymatic browning (NEB) (Optical Density of methanol extracted colour at 440 nm wave length) increased from 0.089 to 0.174 and 0.373 to 0.577 in guava and mango ketchups, respectively, after nine months of storage (Fig. 1). Woolfe (11) attributed formation of aldehydes such as furfural and hydroxymethyl furfural for non-enzymatic browning of products during storage. The anti-oxidant value taken as FRAP values decreased from 32.8 to 18.8 mM/ml in guava ketchup and 26.9 to 16.2 mM/ml in mango ketchup after 9 months of storage (Fig. 1). Vallverdú-Queralt (12) also reported decrease in anti-oxidant values of tomato ketchup during storage. The decrease in anti-oxidant value might be due to decrease in ascorbic acid and other anti-oxidant compounds with the storage period. During sensory evaluation of products on the basis of colour, flavour and taste, guava and mango ketchups obtained high organoleptic scores of 7.7 and 7.4 scores (out of 9), respectively (Fig. 1) at zero time. Both products retained good acceptability even after 9 months of storage, scoring 7.0 and 7.8, respectively, for guava and mango ketchups. The study indicated that fruit ketchups from mango and guava had good stability over a long period of time in terms of nutritional and organoleptic qualities. It may suggest guava and mango ketchups as potential alternate/additional products to tomato ketchup due to the presence of fruity flavour.

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Short communication

Enhancement of shelf-life of coriander leaves through storage in a novel high humidity storage box

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ABSTRACT

A high humidity storage box was designed, fabricated and evaluated for freshness retention of coriander leaves. Coriander leaves in bunches were stored in storage box as well as in non-ventilated plastic crates covered with wet gunny cloth (control). Periodical observations on shelf-life, freshness and moisture loss were recorded. At the end of the storage period, the samples were analysed for quality. Coriander leaves stored in novel high humidity storage box had a shelf-life of 72 h under ambient conditions (Temp: 26-28°C, RH: 58%) as compared to 48 h in control. Samples stored in the acrylic boxes showed higher freshness retention compared to those stored in commercial practice. Moreover, coriander leaves stored in high humidity storage box had higher freshness, lesser physiological loss in weight (PLW%) (12%) and better retention of ascorbic acid (58.4%), iron (99%) and calcium (64%) compared to control. Therefore, this study showed that novel, custom designed high humidity storage box hold potential for storage and shelf-life extension of coriander leaves.

Key words: Coriander leaves, high humidity storage box, plastic crate, shelf-life.

Coriander leaves are highly perishable and their shelf-life in terms of loss in freshness, turgidity, weight loss *etc*. is only 24h with a possibility of reaching 48 h depending on the storage conditions. However, in most of the developing tropical countries like India, coriander is supplied by the push cart vendors or by vegetable vendors in retail vegetable markets and only a small part is sent to stores having refrigeration facility. In order to retain freshness and maintain a good visual perception of coriander, the common practice is to cover the leafy vegetables with wet gunny cloth and sprinkle water to maintain high relative humidity (RH%). This process causes spoilage of the produce due to condensation of water on the produce, increase in microbial proliferation with chances of presence of coli form bacteria on account of poor quality. Keeping this in view, to retain freshness with minimal spoilage of the produce, a high humidity vegetable storage box was specially designed, fabricated and tested with coriander leaves.

A high humidity storage box of size 900 mm × 600 mm × 150 mm (L×B×H), made of transparent acrylic sheet of 6 mm thickness with ability to maintain RH of more than 90% was fabricated. The box was divided into three equal chambers using acrylic sheet of 5 mm thickness with partition having perforation of 3 mm diameter for the cross ventilation. In each chamber, coriander leaves (cv. Hessaraghatta Local) in bunches (4 bunches weighing 0.5 kg each) were placed. The coriander leaves in bunches (4 bunches weighing 0.5 kg each) was placed in plastic crates measuring 400 × 300 × 150 mm covered with wet gunny bag was taken as control. Experiments were conducted at ambient temperature (Av. temp: 26-28°C, RH 58%). Samples were analysed for physiological loss in weight (PLW%), ascorbic acid content (Ranganna, 5), mineral content (Ca, Mg, Fe, Mn and Zn) (Bhargava and Raghupathi 1). Sensory analysis was done to assess the quality of fresh coriander leaves at the end of the storage period for colour, freshness, flavour/ aroma and overall acceptability of the samples with a panel of 15 untrained judges in 5-point hedonic scale (Harry and Hildegarde, 4). Microbial analysis of the coriander leaves at end of the storage period was determined by pour plate technique (Downes and Lto, 3). All experiments were statistically analyzed using completely randomised design using WASP 2.0 software (Bhuvaneswari *et al*., 2).

As evident, there was gradual increase in PLW (%) of coriander leaves during storage in both the storage systems (Fig. 1a). However, the weight loss was higher in plastic crate at the end of 48 h (15.13%) compared with those kept in storage box, which had lower weight loss (12.87%) at 72 h of storage. The coriander leaves stored in plastic crate withered and lost its acceptability in 48 h; whereas the samples in storage box were fresh and acceptable upto 72 h.

A drastic reduction in the moisture content of coriander leaves during storage in storage box as compared to control during storage was observed. In the storage box the moisture reduction was 0.07, 1.16 and 1.32% after 24, 48 and 72 h of storage,

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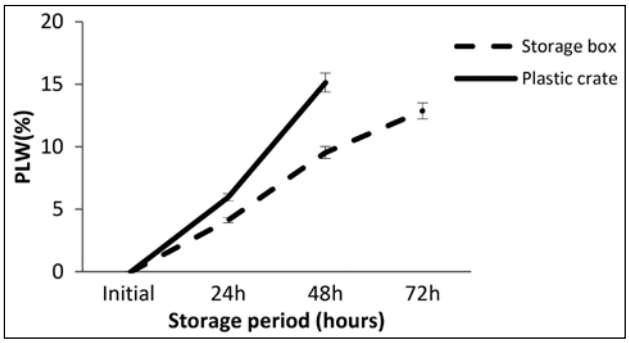

Fig. 1a. Physiological loss in weight (%) of coriander leaves during storage

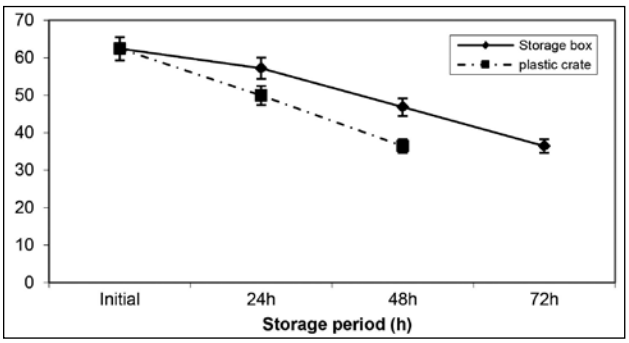

Fig. 1c. Change in ascorbic acid content of coriander leaves during storage.

respectively, where the moisture reduction of the samples placed in plastic crate was 2.11 and 3.2% after 24 and 48 h of storage respectively (Fig. 1b). The samples in the plastic crate lost freshness rapidly as compared to those stored in storage box after 48 h of storage. It was found that moisture retention was higher in samples stored in storage box than in plastic crate. In order to maintain the sensory qualities such as texture and appearance during storage period it is important for fresh green leafy vegetables to remain well hydrated. Latif and EL-Aal (6) noticed the significant reduction in moisture content of fresh coriander leaves in polythene pack after 8 days of storage at 5°C.

Change in ascorbic acid content of the samples kept in storage box as well as control during storage is presented in Fig. 1c. A reduction in ascorbic acid content in both the cases was observed. However, higher reduction was noted in plastic crate covered with moist gunny cloth in 48 h (36.4%) as compared to those kept in storage box which had lower reduction in ascorbic acid content (46.8%) in 48 h of storage. The retention of ascorbic acid content in storage box was 58.4% even after 72 h of storage. It was found from the study that samples kept in storage box retained more ascorbic acid content compared to those in plastic crate. At the end of storage, coriander leaves retained 58.4% of the initial ascorbic acid content in storage box samples. Samples placed in storage box did not have much ascorbic acid degradation during storage. Earlier, Zepplin and Elvehjein (8) found that leafy vegetables held at 6°C lost 10% of their AA content in 6 days; while those held at room temperature lost 20% in only 2 days.

From the Fig. 1d, it was observed that chlorophyll content of stored in both storage box and plastic crate continuously decline during storage. The loss was at lower level (1.3%) for the samples stored in storage box after 72 h as compared to those stored in plastic crates (1.67%) after 48 h. Chlorophyll degradation is accompanied by the loss of colour during storage. This loss of chlorophyll is responsible for the yellowing of leaves. Since the chlorophyll degradation is less in sample stored in storage box, yellowing of leaves was less even after 72 h of storage. Similar findings were noted by Latif and EL-Aal (6) where there was slight decrease in chlorophyll content in fresh coriander leaves after 5-8 days.

Coriander leaves are a rich source of minerals such as, iron, potassium and calcium. At the end of storage period of 72 h, coriander leaves in storage box had calcium 0.785 g/100 g, magnesium 0.403 mg/100 g and iron 6.81 mg/100 g (Table 1).

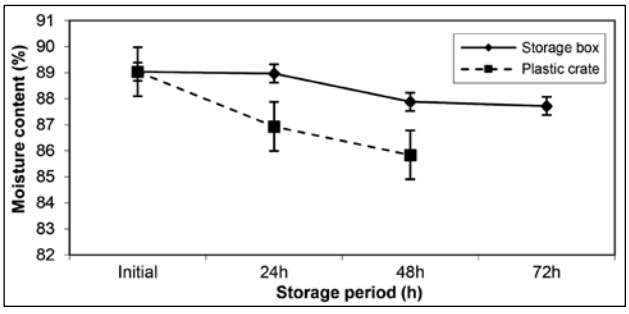

Fig. 1b. Change in moisture content of coriander leaf during storage.

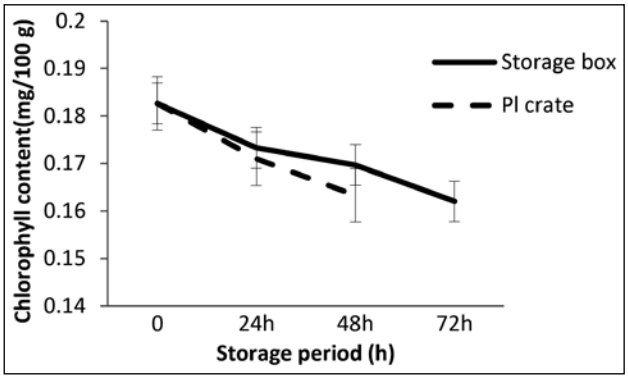

Fig. 1d. Change in chlorophyll content of coriander leaves during storage.

Table 1. Mineral composition of coriander leaves during storage.

Mineral nutrient	Quantity (mg/100 g)
Phosphorous	56
Potassium	22
Calcium	785
Magnesium	403
Iron	6.81
Zinc	3.96
Copper	1.33

The coriander leaves kept in the plastic crate withered and became unmarketable after 48 h of storage at ambient conditions. Sensory evaluation of the samples which had a storage life of 72 h in storage box under ambient conditions was done to determine the freshness and marketability of the coriander leaves. The coriander leaves had its characteristic light green colour with no yellowing of leaves. The average sensory score for colour from a panel of 15 semi trained judges was 4.5/5 (Fig. 1e). which corresponds to the very good rating. Flavour retention in storage box was 4.14 (between good and very good) in 5-point hedonic scale. Overall acceptability is the important factor which determines whether the samples after storage have consumer acceptability. The overall acceptability of the sample had a sensory score of 4.32 out of 5. Similar findings on retention of colour and freshness of coriander leaves upto 72 h in film package in low temperature storage was reported by Luo *et al.* (7). Microbial analysis of the coriander leaves showed that the total plate count is 2×10^4 units and the leaves are microbially safe at the end of the storage period of 3 days. Yeast and molds were in very low numbers in coriander leaves kept in storage box (data not shown). Similar findings were reported for fresh cut coriander leaves where leaves after sequential wash, packaged in polythene bags had reduced microbial count during storage (Luo *et al.*, 7).

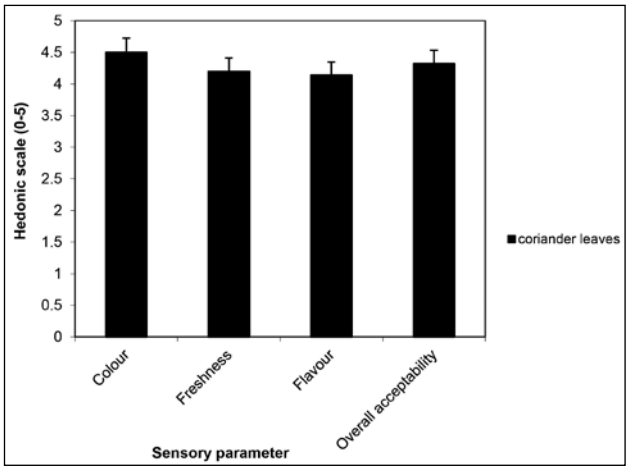

Fig. 1e. Sensory score of coriander leaves during storage.

The coriander leaves placed in high humidity storage box had a shelf-life of 72 h, whereas those placed in plastic crate covered with wet gunny cloth had a shelf-life of 48 h only under similar conditions. It was observed that the storage box retained high humidity more than 90% RH inside the box; which is very much essential for shelf-life extension at under ambient conditions with prevailing temperature 25-28°C and RH 55-62%. for leafy vegetables.

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