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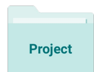

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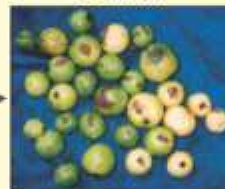

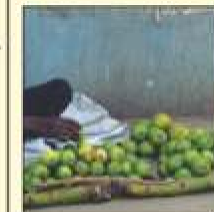

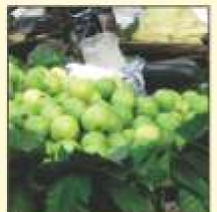

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Cover picture : Post-harvest loss in guava (cv. *Allahabad safeda*) in Karnataka
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Biochemical changes during plantlet regeneration in two accessions of *Mucuna pruriens*

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ABSTRACT

The genus *Mucuna* is an important medicinal herb and is extensively used in traditional Indian systems of medicine for various ailments. *In vitro* culture technique provides an alternative to plant propagation and germplasm conservation. Our aim was to study the biochemical changes occurring during regeneration of shoots (plantlets) from explants of two accessions of *Mucuna pruriens,* by monitoring the efficiency of nitrogen utilization and changes in levels of some hydrolytic enzymes. A rapid micropropagation system was developed using Murashige and Skoog's (MS) medium supplemented with BAP and IAA combined. In both the accessions, 3.0mg l^{-1} 6-BAP, in combination with 0.2mg l^{-1} IAA, induced shoot buds and shoot elongation; however for multiple-shoot induction, a slightly higher concentration of cytokinin, i.e., 3.5mg l^{-1} 6-BAP, in combination with 0.2mg l^{-1} IAA, was required. Results of the present study confirm an active growth of explants revealed by nitrate assimilation enzymes and hydrolytic enzymes. It is concluded that medium composition, growth regulator combination and culture incubation conditions are all vital in both the accessions of *Mucuna pruriens* for induction of *in vitro* plant regeneration.

Key words: *Mucuna*, *in vitro*, biochemical changes, regeneration, enzymes

INTRODUCTION

The genus *Mucuna* belongs to the family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes with pan-tropical distribution. *Mucuna pruriens* L. is a well-known medicinal plant, yet, study on its pharmacological properties and corresponding compounds still continues. Importance of the genus *Mucuna* as a medicinal plant is mainly due to presence of L-Dopa. L-Dopa (3,4 dihydroxy–L–phenylalanine) is a neurotransmitter precursor used for symptomatic relief of Parkinson's disease. Further, it acts as a precursor for several neurologically important catecholamines such as the neurotransmitter dopamine and the important hormones, adrenaline and noradrenalin (Riley, 1997).

Reproducible regeneration of shoots from various explants is desirable in plant tissue culture for crop improvement (Christopher *et al,* 1991). Differentiation of structures in tissue culture is controlled by growth regulators, along with other components of the culture medium (Narender *et al*, 2011). Analysis of activities of various enzymes provides a reasonable and promising approach to understanding the biochemical basis of developmental pathways (Singh *et al*, 2009). Therefore, there is a need to study structural and biochemical aspects underlying initiation of organized development *in vitro* (Sujatha *et al,* 2000). The present study was aimed at investigating the biochemical changes that occur during regeneration of shoots (plantlets) in explants of two accessions of *Mucuna pruriens*, viz., Accession 1 (*M. pruriens* bearing a black seed-coat) and Accession 2 (*M. pruriens* bearing a white seed-coat). This was done by monitoring the efficiency of enzymes involved in nitrogen utilization, and changes in the level of some hydrolytic enzymes.

MATERIAL AND METHODS

Plant material and preparation of explants

Seeds of both the accessions of *Mucuna pruriens* were procured from University of Agricultural Sciences, Bengaluru. The seeds were surface-sterilized with 1% mercuric chloride for 5 min, followed by washing in sterile distilled water 5-6 times to remove traces of the surface-

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sterilant. These were then germinated *in vitro* on basal MS (Murashige and Skoog, 1962) medium. Plants grown thus were used as the explant source. Explants were trimmed aseptically (1.5 to 2.0cm) and inoculated onto MS medium.

Media and culture conditions

MS medium composed of MS salts and vitamins supplemented with sucrose (30g l^{-1}), solidified with 0.8% (w/v) agar and pH adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were maintained at 24 ± 2°C under 16h light/8h dark photoperiod using light provided by cool, white, fluorescent lamps (25µmol m^{-2} s^{-1}) in a growth chamber.

Shoot induction, multiplication and rooting

MS basal medium supplemented with various concentrations (0.5, 1.0 to 5.0mg l^{-1}) of different cytokinins, viz., 6-BAP (benzyl amino prurine), kinetin and 2-ip [6-(-γ, γ-dimethylallylamino purine)] either singly, or in combination, with 0.2mg l^{-1} indole-3-acetic acid (IAA) or α-naphthalene acetic acid (NAA) or no plant growth regulator to evaluate morphogenic potential of the nodal explants. All the cultures were subcultured to fresh medium of the same composition every 28 days (4 weeks). Percentage response of explants producing shoots, number of shoots produced per explants and shoot-length were recorded at weekly intervals.

Rooting of shoots was done on half-strength MS medium supplemented with different concentrations of IAA and NAA (each singly at 0.5mg l^{-1} to 4mg l^{-1}) or in combination with 0.1% activated charcoal.

Enzyme extraction and assay

Enzyme extraction and assays were performed as described below, with slight modifications when necessary, for the present investigation. For Nitrate/ Ammonia assimilating enzymes, extraction for nitrate reductase was carried out as per Altaf Ahmad and Abdin (1999), and enzyme activity was assayed as per Campbell and Smarrelli (1978). Extraction and assay for glutamine synthetase were done as per Philippe Lenee and Yves Chupeau (1989). The same extraction procedure was adopted for glutamate dehydrogenase. Optimum conditions for enzyme activity were maintained, namely, pH, temperature, substrate and cofactor concentrations. Acid and alkaline phosphatase enzyme extraction and assay were carried as per Angosto *et al* (1988). For invertase, the method of Yolanada Cuadrado *et al* (2001) was used for extraction, and the activity was determined using the method of Miller and Ranwala (1994). Extraction and assay of α-amylase was carried out as per Sadasivam and Manickam (2008). Peroxidase extraction was done as per Lorenza M. Bellani *et al* (2002) and its activity was assayed as per Oskar Sanchez *et al* (1989).

Statistical analysis

All the experiments were conducted in three replicates. Data were subjected to statistical analysis using Microsoft Excel (MS Office, 2003) and are presented as Mean ± SE.

RESULTS AND DISCUSSION

Shoot induction and rooting in nodal explants

Organogenesis was observed in nodal segments cultured on MS medium supplemented with each of the concentrations of BAP/ kinetin/ 2-ip (alone, or in combination) with 0.2mg l^{-1} IAA/ NAA in both the accessions of *Mucuna*. Morphogenic response observed was better with the aminopurine class of cytokinins (BAP and 2-ip) than with the furfuryl amine class of cytokinins (kinetin), with BAP showing a better response among the former. Therefore, for further studies, only BAP was used as the cytokinin of choice. Optimum growth of shoot occurred on medium containing 3mg l^{-1} BAP in combination with 0.2mg l^{-1} IAA (Fig. 1a and. 1b) in both the accessions

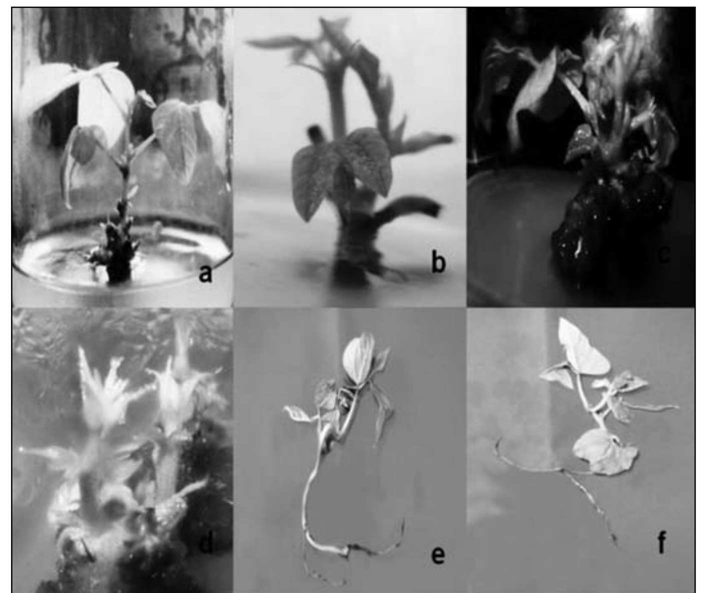

Fig. 1. Micropropagation in two accessions of *Mucuna pruriens* using nodal explants a. Shoot elongation in Accession 1 on MS + BAP (3.0mgl^{-1}) and IAA (0.2mgl^{-1}) b. Shoot elongation in Accession 2 on MS + BAP (3.0mgl^{-1}) and IAA (0.2mgl^{-1}) c. Multiple-shoot induction in Accession 1 on MS + BAP (3.5mgl^{-1}) and IAA (0.2mgl^{-1}) d. Multiple-shoot induction in Accession 2 on MS + BAP (3.5mgl^{-1}) and IAA (0.2mgl^{-1}) e. Rooting in Accession 1 f. Rooting in Accession 2.

(Table 1). A concentration of 3.5mg l^{-1} BAP, in combination with 0.2mg l^{-1} IAA, induced multiple shoots (Fig. 1c and Fig. 1d) in both the accessions. Average number of shoots per culture in Accession 1 was 14.25±2.46, with a survival of 97%. In Accession 2, the average number of shoots per culture was 11.50±2.61, and the survival percentage was 89 (Table 1).

After three subcultures (28-day subculture cycle), individual shoots attained nearly 5-6cm length. Successful root establishment (Fig. 1e and 1f) was achieved in individual shoots on MS medium (half-strength) supplemented with NAA (0.5mg l^{-1}) and IAA (0.5mg l^{-1}), individually, in the presence of 0.1% activated charcoal at 30 days of incubation. Of the two auxins studied, NAA was more effective in induction of rooting than IAA in the case of both the accessions of *Mucuna* (Table 2). Roots induced on NAA were thicker and survival percentage of the plants regenerated was also better (98% for Accession 1, and 92% for Accession 2) compared to IAA (69% for Accession 1, and 64% for Accession 2).

Shoot regeneration was achieved in nodal explants of Accessions 1 and 2 when cultured on MS medium supplemented with 3mg l^{-1} 6-BAP in combination with 0.2mg l^{-1} IAA. Similarly, organogenesis (in the form of root emergence) was also observed in MS medium fortified with NAA/ IAA at 0.5mg l^{-1}. Morphological changes occurring in explants during the course of their proliferation on a suitable medium were monitored by determining some biochemical changes, viz., nitrate/ ammonia utilizing enzymes during shoot regeneration from nodal/ leaf explants, and changes in hydrolytic enzymes during organogenesis.

Changes in nitrate reductase (NR) activity:

Nitrate reductase (NR) is one of the key enzymes involved in the first step of nitrate assimilation in plants (Altaf Ahmed & Abdin, 1999). Table 3 shows the pattern of changes in nitrate reductase activity in both the accessions monitored from the day of inoculation up to the 30th day, at 5-day intervals.

In regenerating nodal explants of Accession 1, the activity peaked on Day 20. Thereafter, it remained the same until Day 30. Whereas, in Accession 2, two peaks of activity were observed on the 10th and 25th day (Table 3).

Changes in GS and GDH activity:

Glutamine synthetase (GS) and Glutamine dehydrogenase (GDH) are the other key enzymes involved in nitrate and ammonia assimilation in plants. In Accession 1, GS activity was found to be higher between the 10th and

Table 1. Effect of cytokinin–auxin combination in MS basal medium on shoot regeneration from nodal explants in two accessions of *Mucuna pruriens*

BAP (mg l^{-1})	NAA (mg l^{-1})	IAA (mg l^{-1})	Average no. of shoots per culture		Average shoot-length (cms)		Per cent survival	
			Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2
0.0	0.2	0.0	0	0	0	0		
0.5	0.2	0.0	1.83 ± 0.30	1.10 ± 0.35	0.82 ± 0.23	0.80 ± 0.20	14.42 ± 0.87	10.92 ± 0.51
1.0	0.2	0.0	1.50 ± 0.34	1.33 ± 0.31	1.33 ± 0.19	1.23 ± 0.13	23.50 ± 1.92	17.67 ± 1.24
1.5	0.2	0.0	1.50 ± 0.34	1.83 ± 0.37	1.83 ± 0.21	1.73 ± 0.21	40.75 ± 2.33	30.42 ± 1.61
2.0	0.2	0.0	1.75 ± 1.33	1.50 ± 0.38	2.25 ± 0.28	2.15 ± 0.18	52.00 ± 1.65	39.42 ± 1.76
2.5	0.2	0.0	1.75 ± 1.33	1.83 ± 0.34	3.08 ± 0.34	3.18 ± 0.34	58.17 ± 1.65	49.08 ± 3.55
3.0	0.2	0.0	2.83 ± 40.23	2.67 ± 1.37	6.42 ± 0.62	5.42 ± 0.42	94.00 ± 1.11	87.33 ± 1.44
3.5	0.2	0.0	12.75 ± 1.66	10.17 ± 2.90	4.83 ± 0.53	4.33 ± 0.23	86.33 ± 1.81	84.42 ± 2.88
4.0	0.2	0.0	1.17 ± 0.23	1.17 ± 0.47	3.58 ± 0.45	2.58 ± 0.25	64.58 ± 0.82	64.25 ± 2.04
4.5	0.2	0.0	1.50 ± 5.05	1.75 ± 0.51	2.00 ± 0.35	2.01 ± 0.35	46.17 ± 1.11	62.92 ± 1.78
5.0	0.2	0.0	1.80 ± 0.30	1.73 ± 0.35	1.83 ± 0.21	0.83 ± 0.12	40.15 ± 1.53	39.32 ± 1.56
0.0	0.0	0.2	0	0	0	0		
0.5	0.0	0.2	1.00 ± 0.33	1.67 ± 0.31	0.82 ± 0.23	0.82 ± 0.20	15.47 ± 0.67	11.12 ± 0.53
1.0	0.0	0.2	1.83 ± 0.41	1.92 ± 0.23	1.33 ± 0.19	1.24 ± 0.13	25.53 ± 1.97	18.68 ± 1.26
1.5	0.0	0.2	1.92 ± 0.29	1.50 ± 0.29	1.83 ± 0.21	1.83 ± 0.23	46.85 ± 2.53	31.41 ± 1.62
2.0	0.0	0.2	1.27 ± 0.51	1.08 ± 0.90	2.25 ± 0.28	2.25 ± 0.28	57.00 ± 1.53	39.72 ± 1.46
2.5	0.0	0.2	1.17 ± 0.97	1.17 ± 0.38	3.28 ± 0.36	3.38 ± 0.24	62.17 ± 1.56	51.13 ± 1.25
3.0	0.0	0.2	3.42 ± 1.69	2.42 ± 2.64	7.42 ± 0.61	5.74 ± 0.42	97.00 ± 1.09	89.13 ± 1.14
3.5	0.0	0.2	14.25 ± 2.46	11.50 ± 2.61	5.83 ± 0.53	4.23 ± 0.23	89.09 ± 1.80	84.02 ± 1.18
4.0	0.0	0.2	1.33 ± 0.66	1.42 ± 0.07	3.58 ± 0.45	2.78 ± 0.52	64.18 ± 0.32	65.24 ± 2.14
4.5	0.0	0.2	1.42 ± 0.01	1.92 ± 0.05	2.00 ± 0.35	2.03 ± 0.30	52.17 ± 1.17	61.82 ± 1.29

(Data represent Mean + S.E.)

Table 2. Effect of different auxins (in half-strength MS medium supplemented with 0.1% activated charcoal) on root induction in two accessions of *Mucuna pruriens*

Auxin (mg l-1)	Rooted shoots (%)		Mean no. of roots/shoot		Mean root-length (cm)		Plant survival (%)	
	Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2	Accession 1	Accession 2
No auxin	07.2 + 0.95	07.2+ 0.95	1.4 + 0.07	1.4 + 0.07	0.8 + 0.02	0.8 + 0.02	16.1 + 0.78	16.1+ 0.78
IAA								
0.2	34.2 + 1.28	32.4 +1.28	5.6 + 0.12	3.5 + 0.12	1.4 + 0.01	1.2 + 0.01	35.4 + 1.35	32.4+ 1.31
0.3	48.4 + 1.14	42.4+ 1.12	7.6 + 0.07	6.7 + 0.06	2.7 + 0.02	2.3 + 0.02	41.6 + 1.19	38.6+ 1.13
0.4	76.5 + 2.12	72.5+ 2.02	9.6 + 0.10	8.6 + 0.01	3.3 + 0.28	3.0 + 0.21	53.7 + 1.82	49.7+ 1.72
0.5	94.8 + 2.96	92.8+ 2.86	11.9 + 0.1	10.9 + 0.1	4.1 + 0.03	3.6 + 0.02	69.2 + 2.73	64.2+ 1.72
1.0	72.2 + 2.08	68.2+ 1.67	5.2 + 0.06	4.8 + 0.05	2.8 + 0.28	2.2 + 0.18	22.1 + 1.89	18.1+ 1.74
NAA								
0.2	63.5 + 4.72	61.5+ 3.62	6.9 + 0.08	5.6 + 0.04	2.7 + 0.28	2.3 + 0.25	34.2 + 0.48	32.4+ 0.34
0.3	78.6 + 3.26	72.4+ 2.36	7.7 + 0.08	7.4 + 0.07	3.1 + 0.22	2.9 + 0.12	60.2 + 0.94	58.2+ 0.84
0.4	89.2 + 2.33	85.2+ 2.13	9.9 + 0.09	9.4 + 0.08	4.0 + 0.32	3.8 + 0.26	76.2 + 0.96	72.4+ 0.86
0.5	96.2 + 6.63	94.2+ 5.52	12.4 + 0.24	12.2+ 0.23	4.4 + 0.38	4.1 + 0.28	98.6 + 0.96	92.6+ 0.76
1.0	46.3 + 2.88	43.4+ 2.68	5.6 + 0.06	5.2 + 0.05	2.9 + 0.22	2.7+ 0.21	30.2 + 0.33	28.2+ 0.23

(Data represent Mean + S.E.)

20th day, and decreased thereafter. Accession 2 had higher GS activity between the 5th and 20th day, and decreased thereafter (Table 3).

In Accession 1, it was observed that activity of both the isoforms of GDH (NAD^+ and NADH isoforms) from Day 0 and Day 5 remained the same; but, there was an increase in activity on Day 10, and it peaked on Day 15, decreasing thereafter. But, the activity was greater in the NADH isofarm on Day 10 compared to the NAD isoform (Table 3). In Accession 2, there was a gradual increase in the activity of both the isoforms of glutamate dehydrogenase (NAD^+ and NADH isoforms) up to Day 10 and Day 15 in NADH and NAD isoforms, respectively, and decreased thereafter (Table 3).

Acid and alkaline phosphatases

Activity of acid phosphatase in regenerating shoots in Accession 2 was found to increase gradually from Day 0 to Day 30, whereas, in Accession 1, the activity of this enzyme increased from Day 0 to Day 5, and showed a dip on day '10' and, thereafter, a gradual increase until Day 30 (Table 3).

Activity of alkaline phosphatase in regenerating shoots in Accession 1 increased progressively from Day 0 to Day 30, whereas, in Accession 2, there was a reduction until Day 10, and thereon, the activity increased and peaked on Day 25 (Table 3).

Invertases

Invertases exist in at least two isoforms, such as the soluble (extracellular) and wall-bound form; and, acid and alkali isoforms. In the present study, both acid (pH 5) and alkali (pH 7.5) isoforms were studied in organ-forming and non-organ-forming regenerating shoot cultures.

Wall-bound invertase

Activity of the wall-bound invertases in Accession 1 is presented in Table 3. Acid invertase peaked on Day 15. In the case of alkaline invertase, there was no increase in activity at all; rather, there was a gradual decrease in its activity from Day 0 to Day 30.

The activity of wall-bound invertase of Accession 2 peaked on Day 5, and, gradually decreased from Day 10 to Day 30 (Table 3); whereas alkaline invertase showed a little increase in activity on Day 5, and decreased thereafter.

Extracellular invertase

Activity of acid isoforms in Accession 1 peaked on Day 10, and gradually decreased thereon. Alkaline isoforms also showed maximum activity on Day 10 (Table 3). The activity in Accession 2 showed a gradual increase from Day 0 to Day 10 and remained constant up to Day 15, decreasing thereafter; whereas, the activity of alkaline isoforms peaked on Day 10.

α-amylase

Activity of á-amylase remained the same on Day 0 and Day 5 in both the accessions, and gradually increased from Day 10 to Day 30 (Table 3).

Peroxidase

Peroxidase activity in Accession 1 peaked on Day 25, and decreased thereafter; whereas, in Accession 2, peak activity was observed on Day 20.

Table 3. Changes in nitrate/ammonia utilizing enzymes and some hydrolytic enzymes during plantlet regeneration in two accessions of *Mucuna pruriens*

Enzyme	Accession	Days of incubation						
		0	5	10	15	20	25	30
Nitrate reductase(µmole NO_2 g^{-1} min^{-1})	Accession 1	25.24	25.24	25.24	49.4	73.95	73.95	73.95
	Accession 2	49.4	49.4	73.95	49.4	49.4	73.95	73.95
Glutamine synthetase (GS)(n moles of γ glutamate formed mim^{-1} g^{-1} protein)	Accession 1	230	250	250	250	250	150	140
	Accession 2	200	230	230	230	230	140	130
Glutamate dehydrogenase (NADH-GDH)(µmole NADH g^{-1} F.W.)	Accession 1	100	100	240	260	120	150	100
	Accession 2	100	140	200	180	140	100	100
Glutamate dehydrogenase (NAD^+-GDH)(µmole NAD^+ g^{-1} F.W.)	Accession 1	100	100	160	260	100	80	100
	Accession 2	100	100	120	180	60	100	100
Acid phosphatase(n moles of PNP released min^{-1} g^{-1} F.W.)	Accession 1	508	508	434	579	650	675	711
	Accession 2	394	394	394	431	468	662	712
Alkaline phosphatase(n moles of PNP released min^{-1} g^{-1} F.W.)	Accession 1	468	468	394	434	468	662	712
	Accession 2	418	418	431	418	468	529	712
Wall-bound invertase(µg of reducing sugar released min^{-1} g^{-1} F.W.)	pH 5	3.05	3.05	8.76	12.8	0.876	0.292	0.292
	pH 7	2.3	2.3	2.00	1.75	0.876	0.292	0.292
Wall-bound invertase(µg of reducing sugar released min^{-1} g^{-1} F.W.)	pH 5	3.000	3.504	4.080	3.504	0.379	0.292	0.292
	pH 7.5	2.000	2.300	1.750	0.876	0.876	0.292	0.292
Extracellular invertase(µg of reducing sugar released min^{-1} g^{-1} F.W.)	pH 5	1.460	1.460	2.920	1.168	0.584	0.000	0.000
	pH 7.5	0.876	0.876	5.548	3.500	2.920	0.584	0.000
Extracellular invertase(µg of reducing sugar released min^{-1} g^{-1} F.W.)	pH 5	2.336	2.336	3.050	0.876	0.292	0.000	0.000
	pH 7.5	2.62	2.62	3.504	3.050	0.292	0.000	0.000
α-Amylase(µg maltose released min^{-1} g^{-1} F.W.)	Accession1	30	30	32	36	38	42	46
	Accession2	28	28	30	34	36	38	40
Peroxidase(Units ml^{-1} min^{-1})	Accession1	400	480	720	360	600	840	780
	Accession2	300	360	400	400	600	450	660

Mean±SE of 3 observations is not indicated due to the large amount of data

Nitrate uptake system in a plant must be versatile and robust, because, plants need to transport adequate amount of nitrate to satisfy the demand in the face of external nitrate concentration that can vary by five orders of magnitude (Crawford, 1995). Nitrate supplement in the medium must be converted into NH_4^+ in plants before the nitrogen can enter amino acids and other nitrogen compounds. Nitrate reductase has been studied intensively because its activity often controls protein synthesis rate in plants absorbing NO_3^- as a major nitrogen-source (Srivastava, 1980; Naik *et al,* 1982). Genes for this have been cloned from several plants and mutants, and transgenic lines are available too (Lam *et al,* 1996; Lochab *et al,* 2007). Results of the present investigation clearly suggest that there is a synergy that operates among enzymes for nitrate and ammonia assimilation when nitrate concentrations in the medium are high (~30mM in MS medium). This induces production of nitrate reductase, and subsequently GS, as nitrate is converted into ammonia.

Activity of nitrate reductase persists continuously even after cultures enter the stationary phase; whereas, production of GS is directly or indirectly dependent on NR activity. From the results above, it can be concluded that decrease in GS activity when cultures enter the stationary phase may be attributed to decrease in the activity of nitrite reductase; also, this could be due to exhaustion of sucrose in the medium. Results of the present investigation are supported (in other plant species) by Philippe Lenee & Yves Chupeau (1989) and Suzuki *et al* (1987).

In the present study, lower levels of phosphatases during the initial culture-period may be because of the high

inorganic-phosphate levels in the medium. Pronounced increases in the activity of phosphatases before manifestation of a visible morphogenic event (observed to happen prominently on Day 20), i.e., on Day 15 in both the accessions, suggests that these enzymes may have a role in biochemical degradation of plasmodesmata. Such degradation may facilitate penetration of roots/ elongation of shoots (Naidu & Kavi Kishor, 1995; Kumar, 1998). Together with this, peroxidase activity was found to peak on Day 20 in Accession 2, and Day 25 in Accession 1, with gradual and progressive increase witnessed from Day 0. Peroxidases are a large group of enzymes involved in a number of biological processes such as lignification (Lagrimini *et al,* 1997a), cross-linking of cell wall proteins (Bradley *et al,* 1992) and auxin catabolism (Lagrimini *et al,* 1997b). Perhaps, an increase in enzyme-level indicates a role of these enzymes in tissue proliferation and differentiation. On examining involvement of acid phosphatases in initiation and formation of adventitious roots in *Impatiens* sps., Malik and Kumari (1977) attributed these roles to the enzymes. It was speculated that glycodisases may cleave wall-linkages and facilitate growth.

Increased activity of hydrolytic enzymes seen in the present investigation indicates that different compounds degrade in tissues, and this is concurrent with a high synthetic activity occuring during organogenesis. Results of the present investigation are supported by previous findings of Brown & Thorpe (1980), Kavi Kishor & Mehta (1988), Naidu & Kavi Kishor (1995), and Kumar (1998), in other plant species.

CONCLUSION

With a view to confirm whether nutrients or, growth regulators added to the medium and conditions like of source light and temperature at which cultures were incubated, affected growth of plants *in vitro*, biochemical studies were undertaken. In higher plants, nitrate-assimilating and hydrolytic enzymes are regulated by light, hormones, sugars, and, carbon and nitrogen metabolites. Results of the present study confirm active growth of explants revealed by the activity of nitrate assimilating enzymes and hydrolytic enzymes (excepting GDH, the activity of which decreased, as, it is a stress induced enzyme). Thus, it may be concluded that medium composition, growth regulator combination and culture incubation conditions are cited for optimal growth *in vitro* in both the accessions of *Mucuna pruriens* for plant regeneration.

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Variation in relation between yield and yield attributes in 'Thompson Seedless' grape and its clones

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ABSTRACT

To study variation in the relationship between yield and yield attributes in 'Thompson seedless', 'Tas-A-Ganesh' and '2A clone' vines grafted onto Dogridge rootstock and trained on the extended Y training system, data collected from 120 vines in each variety were subjected to correlation and regression analysis. Numbers of clusters per vine was the main contributing factor for yield in all these varieties. It determined the yield by 87.9, 42.0 and 51.5%, respectively, in 'Thompson Seedless', 'Tas-A-Ganesh' and '2A clone', with the optimum number of clusters at 27.3, 43.1 and 46.5, respectively. Contrary to that in vars. Thompson Seedless and Tas-A-Ganesh, increase in number of canes was associated with higher cluster/cane ratio. Yield depended upon cluster weight in 'Thompson Seedless', mediated through number of clusters, but was not a contributory factor as evidenced by a negative correlation between cluster-weight and yield. Increase in cluster weight was associated with increase in number of berries in all the varieties. Increase in berry weight was related to cluster weight in only Thompson Seedless and Tas-A-Ganesh. While berry number and berry weight together determined cluster weight by 96.3 and 92.4%, respectively, in vars. Thompson Seedless and Tas-A-ganesh, this value was just 39.0% in '2A clone'. These studies provide a clue that for realizing higher yield, cluster size needs to be greater while limiting the number of canes/vine in vars. Thompson Seedless and Tas-A-Ganesh. Increase in the number of canes would benefit '2A clone' by adopting suitable cultural practices.

Key words: Correlation, variation, yield, yield attributes, Thompson Seedless, Tas-A-Ganesh, 2A clone

INTRODUCTION

Yield in grapevine is determined by number of clusters and mean weight of the cluster. Number of clusters in a vine, in turn, is determined by the number of canes, and cane-productivity as measured by cluster/cane ratio. Earlier studies have revealed that increase in number of canes does not result in proportionate increase in cluster number on a vine. Cane density of 5 to 6/m^2 was found as optimum in bower-trained 'Thompson Seedless' vines with reference to cane-productivity and number of clusters/vine. Higher number of canes/unit area gave a reduced cluster/cane ratio, eventually reduced number of clusters/vine (Shikhamany, 1983). Cluster/cane ratio is an outcome of the number of fruitful buds on a cane. Mutual shading of shoots in a dense vine canopy hampers incident light required for fruit-bud formation in 'Thompson Seedless' which requires over 3600 ft. candles of light (Buttrose, 1970). Since varietal variation was observed in requirement of light for fruit-bud formation (Buttrose, 1969), optimum cane density may be different for Tas-A-Ganesh and 2A Clone, for Thompson seedless even, when vines are trained on extended Y trellis to afford open canopies.

Mean cluster weight, the other yield attribute, is determined by number of berries in a cluster and mean berry-weight.

Variation in relation of the above stated yield attributes to yield, in the different varieties studied, can provide guidelines for formulating specific sets of cultural practices for each variety to obtain higher yields, since, all these attributes are amenable to regulation by cultural operations.

MATERIAL AND METHODS

The present investigation was carried out on 'Thompson Seedless', 'Tas-A-Ganesh' and '2A clone' in 2013-2014 cropping season in growers' vineyards around

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Nashik, Maharashtra. Details of the vineyards selected are given below:

Thompson Seedless vineyards of:

1. Shri Suresh Kalamkar, Mohadi
2. Shri Arun More, Pimpalgaon

Tas-A-Ganesh vineyards of:

1. Shri Ashokrao Gaikwad, Palkhed
2. Shri Jagannathrao Khapre, Kothure

2A Clone vineyards of:

1. Shri Kailashrao Bhosale, Sarole Khurd
2. Shri Manikrao Patil, Khedgaon

Tas-A-Ganesh and 2A Clone are mutants of Thompson Seedless. The former was identified by the Late Vasantrao Arve, a progressive grower in 1976, in his vineyard at Borgaon, Sangli district, Maharashtra, the latter was identified at Kearney Experimental Station, UC Davis, California, USA. Tas-A-Ganesh is cultivated widely in Maharashtra, whereas, 2A Clone was introduced only in 1999, and is gaining popularity.

To work out variation in the relation of yield attributes to yield in these varieties, 120 vines (60 from each vineyard, under each variety) were selected at random. All the vines selected were in the age group of 6-7 years, grafted onto Dogridge rootstock, spaced uniformly at 2.7 x 1.8m, trained on extended Y training system, grown under similar agro-climatic conditions and subjected to similar cultural practices, including sub-cane development; application of Ethrel for pre-pruning defoliation, hydrogen cyanamide for bud-break, GA_3 sprays for cluster elongation, girdling and dipping in CPPU solution for berry sizing. Data were collected on the following yield-attributes and yield, separately for each vine:

No. of canes/vine: Number of canes left on the vine after Forward Pruning.

Cluster/cane ratio: This is an index of vine productivity, derived by dividing the number of clusters borne on a vine by the number of canes retained on it.

No. of clusters/vine: Number of clusters borne on each vine, counted at harvest.

Cluster weight: Mean weight of the cluster was derived by dividing mean yield/vine by mean number of clusters/vine.

Yield/vine: Recorded in kg for each vine at harvest

No. of berries/cluster: Average number of berries in five bunches selected at random in each vine

Mean berry weight: Average weight of 25 berries selected at random in five selected clusters, at the rate of five berries from each cluster

Berry diameter: Average diameter of 25 berries, measured at middle length of the berry, using callipers

Statistical analysis: Correlation was worked out to assess the relation of yield and cluster-weight to their respective attributes. Multiple regression equations for these parameters, with all their respective attributes as independent variables, were also worked out. Optimized models and optimum values for the critical attribute for yield, cluster-weight and cluster-compactness were derived.

RESULTS AND DISCUSSION

Yield/vine

Yield correlated positively with number of canes/vine in 2A Clone, cluster/cane ratio in 'Thompson Seedless', and number of clusters/vine in all the varieties, but, was negatively correlated with cluster-weight in 'Thompson Seedless' (Table 1). Among the yield attributes studied, number of clusters/vine correlated significantly with yield in all the varieties. While cane is a unit of vine productivity, cluster/

Table 1. Correlation between yield and yield attributes

Correlation	Correlation coefficient		
	Thompson Seedless	Tas-A-Ganesh	2A clone
1. Yield/vine vs. no. of canes/vine	-0.048	0.160	0.226**
2. Yield/vine vs. Cluster/cane ratio	0.211*	-0.042	0.152
3. Yield/vine vs. no. of clusters/vine	0.940**	0.643**	0.696**
4. Yield/vine vs. cluster weight	-0.255**	-0.001	0.125
5. Yield/vine vs. betty TSS	-0.713**	-0.479**	-0.535**
6. Clusters/vine vs. no. of canes/vine	-0.104	0.185	0.330**
7. No. of clusters/vine vs. cluster/cane ratio	0.235*	-0.065	0.286**
8. Cluster weight vs. no. of canes/vine	0.204*	-0.019	0.081
9. Cluster weight vs. cluster/cane ratio	-0.221*	-0.152	0.052
10. Cluster weight vs. no. of clusters/vine	-0.314**	0.073	0.169
11. Cluster/cane ratio vs. no. of canes/vine	-0.234*	-0.222*	0.427**

Significance of 'r' value at 5% = 0.195, and at 1% = 0.254
(0.361 and 0.463, respectively, at 5% and 1% for yield/vine vs. berry TSS)

Table 2. Variation in yield attributes

Attribute	Thompson Seedless			Tas-A-Ganesh			2A Clone		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Yield/vine(kg)	1.1	18.8	9.14	5.1	16.5	10.7	4.4	28.0	17.37
No. of canes/vine	17	53	32.7	10	52	25.9	18	62	44.1
Cluster/cane ratio	0.8	2.0	1.41	0.4	2.4	1.68	1.2	2.8	1.81
No. of clusters/vine	4	66	26.3	11	62	32.7	11	73	47.4
Cluster weight (g)	137.3	791.9	380.3	162.6	534.5	335.5	184.7	580.1	367.4

cane ratio is a measure of cane-productivity. An inverse relationship of cane number with cluster/cane ratio was reported, with optimum cane-density of 5/m^2, in bower-trained vines of 'Thompson Seedless' (Shikhamany, 1983). This was attributed to inadequate intensity of light received by the vines for fruit-bud formation.

However, the relation of number of canes to cluster/cane ratio was not significant in 'Thompson Seedless' or 'Tas-A-Ganesh'. This could be due to exposure of the canes to more sunlight in an open canopy in vines trained on extended Y training system in the present study, where, increase in cane-density did not impair fruit-bud differentiation and, consequently, cluster/cane ratio. Data in Table 2 corroborating with vine spacing of 4.9m^2 reveals that cane density was 6.7 in 'Thompson Seedless', 5.3 in 'Tas-A-Ganesh' and 9.0 in '2A Clone'. It is pertinent to note that in spite of a higher number of canes/vine and a higher cane-density (9/m^2), cluster/cane ratio and number of clusters/vine were highest in '2A Clone' compared to the other two varieties. Moreover, i) the relationship of number of clusters/vine with number of canes/vine was not significant in 'Thompson Seedless' or 'Tas-A-Ganesh', but was significant in '2A Clone', and ii) Cluster/cane ratio correlated negatively with number of canes/vine in the two former varieties, but correlated positively and highly significantly in '2A Clone' (Table 1). Number of clusters/cane ratio, a measure of cane-productivity, depends upon the inherent ability of a variety to develop fruitful buds on the canes under a given set of agro-climatic conditions. Despite having higher number of canes/vine, cane-productivity was higher in '2A Clone'. These results imply that higher cane density of up to 9/m^2 is not detrimental to cane-productivity and yield/vine in '2A Clone', unlike in 'Thompson Seedless' and Tas-A-Ganesh.

Increase in yield/vine was associated with reduced total soluble solids (TSS) content in the berry in all the varieties studied (Table 1). Depressing effect of yield on TSS content in berry is a well-established fact in several varieties of grape (Chadha *et al*, 1974; Lider *et al*, 1974; Purohit *et al*, 1979; Chittiraichelvan *et al,* 1985).

Table 3. Regression of yield attributes on yield in various varieties

Regression equation	Variety		
	Thompson Seedless	Tas-A-Ganesh	2A clone
Intercept	2.66	0.59	-5.8
Slope of x_1(no. of canes/vine)	0.03	0.009	0.045
Slope of x_2 (cluster/cane ratio)	0.96	-0.091	-0.58
Slope of x_3 (no. of clusters/vine)	0.29	0.142	0.27
Slope of x_4 (cluster weight)	0.04	-0.009	-0.003
Slope of x_5 (berry weight)	0.095	0.08	0.81
Slope of x_6 (no. of berries/cluster)	-0.014	0.04	0.0001
Slope of x_7 (berry diameter)	-0.16	0.313	0.44
Determination Co-efficient (R^2)	0.885	0.44	0.53

Number of clusters/vine

Number of clusters/vine is dependent on number of canes/vine and the cluster/cane ratio, and was correlated positively with number of canes/vine in '2A Clone' but not in the other two varieties (Table 1). Probable reason for this variation in relationship is the inherent character of a variety in converting growth into productivity, as explained earlier. Number of clusters/vine had a positive relationship with cluster/cane ratio in 'Thompson Seedless' and '2A Clone', but not in 'Tas-A-Ganesh' (Table 1). From the data presented in Table 2, estimated number of clusters/vine (product of number of canes and cluster/cane ratio) is 46.1, 43.5 and 79.82 in 'Thompson Seedless', 'Tas-A-Ganesh' and '2A Clone', respectively; whereas, number of clusters observed is 26.3, 32.7 and 47.4, respectively. Thus the percentage of observed number of clusters to estimated number of clusters works out at 57.0, 75.2 and 59.4, respectively. This implies that the proportion of productive canes was higher in 'Tas-A-Ganesh' compared to that in the other two varieties. Hence, the deviation in relationship.

In multiple regression analysis involving seven yield-attributes, it was observed that all these yield attributes could together determine yield by 88.5% in 'Thompson Seedless', but only by 44% in 'Tas-A-Ganesh' and 53% in '2A Clone' (Table 3). Number of clusters/vine was the major contributing factor in determining yield in all the varieties. Optimized regression model revealed that 87.9% of the yield was determined by number of clusters/vine in 'Thompson

Seedless', while, the corresponding values were 42.0 and 51.5%, respectively, for 'Tas-A-Ganesh' and '2A Clone. Values of 27.3, 43.1 and 46.5 clusters/vine were optimum, respectively, for 'Thompson Seedless', 'Tas-A-Ganesh' and '2A Clone' (Table 4).

Cluster weight

Cluster weight correlated positively with number of canes/vine, but negatively with number of clusters/vine, cluster/cane ratio and yield/wine in 'Thompson Seedless', but not in 'Tas-A-Ganesh' or '2A Clone' (Table 1).

The positive relationship observed between number of canes/vine and cluster weight can be explained by a negative relationship of canes/vine with cluster/cane ratio, coupled with the negative relationship of cluster weight with cluster weight with cluster/cane ration (Table 1). When cluster/cane ratio simultaneously correlated negatively with number of canes number of cluster/vine and cluster weight, the latter two parameters would correlate positively.

While the number of cluster/cane ratio denotes the physiological sink, carbohydrate reserves and the current metabolites in a cane denote the source. Similarly number of clusters/vine denote the sink and its corresponding source is the total carbohydrate reserves in a vine. At a given level of source, increasing number of sinks result in a reduced size (weight) of an individual sink (cluster). This is the reason for a negative correlation of cluster weight with number of clusters/vine and number of clusters cane ratio.

Number of clusters/vine and cluster/cane ratio are attributes of yield and these correlated positively with yield/vine (Table 1). When these correlated negatively with the cluster weight, yield/vine would also correlate negatively.

Lack of negative correlation of cluster-weight to yield in 'Tas-A-Ganesh' and '2A Clone' indicates that contribution of cluster-weight in determining yield is much less in these varieties compared to that in 'Thompson Seedless' evidenced by the meagre values of slope of cluster-weight in the multiple regression function of yield in these varieties (Table 3). Physiologically, this can be explained by variation in source-sink relation among varieties. Cluster-size being larger in 'Thompson Seedless', any additional cluster on the cane can reduce cluster-weight more drastically than in the other two varieties (where clusters were relatively smaller). Positive correlation of cluster weight with number of canes/vine can be explained in the light of the inverse relationship of yield with cluster-weight, and, with the number of canes/vine. When the number of canes increases, yield decreases and there is a simultaneous increase in cluster-weight, resulting in a positive correlation between number of canes/vine and cluster-weight.

Cluster-weight is an important yield attribute in grape, alterable as desired by cultural operations, primarily with use of growth regulators. The main components of cluster weight are: number of berries in a cluster, and, mean berry-weight. Increase in the number of berries in a cluster was associated with increase in weight of the cluster. Correlation here was highly significant in all the varieties studied. Cluster-weight also varied significantly with mean berry weight in 'Thompson seedless' and 'Tas-A-Ganesh' but not in '2A Clone'. Number of berries in a cluster and mean berry-weight correlated negatively in all the varieties (Table 5). Although increase in the number of berries reduced mean berry-weight in '2A Clone', the reduction seemed to be inadequate in masking the positive effect of number of

Table 4. Determination of yield in various varieties

Variety	Per cent yield determination by no. of clusters/vine	Optimum number of clusters/vine
Thompson Seedless	87.9	27.3
Tas-A-Ganesh	42.0	43.1
2A clone	51.5	46.5

Table 5. Correlation between cluster weight and other attributes in various varieties

Attribute	Correlation coefficient		
	Thompson Seedless	Tas-A-Ganesh	2A clone
Cluster weight vs. no. of berries/cluster	0.661**	0.754**	0.449**
Cluster weight vs. mean berry weight	0.477**	0.296**	0.164
Mean berry weight vs. no. of berries/cluster	- 0.316**	- 0.349**	- 0.484**

Significance of 'r' value at 5% = 0.195, and at 1% = 0.254

Table 6. Variation in cluster attributes

Cluster attribute	Thompson Seedless			Tas-A-Ganesh			2A Clone		
	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
Cluster weight (g)	137.3	791.9	380.3	162.6	534.5	335.5	184.7	580.1	367.4
No. of berries/cluster	30	132	74.5	35	108	68.9	44	128	76.6
Berry weight (g)	2.41	8.63	5.21	3.23	7.52	4.97	3.51	6.84	4.86

Table 7. Regression of cluster weight attributes on cluster weight in different varieties

Regression equation	Thompson Seedless	Tas-A-Ganesh	2A clone
a) Intercept	-301.78	-213.07	-49.19
b) Slope of x_1 (berry weight)	68.03	57.82	47.61
c) Slope of x_2 (No. of berries/cluster)	5.14	4.56	3.04
Determination Coefficient (R^2)	0.963	0.924	0.39

Table 8. Determination of cluster weight in different varieties

Variety	Per cent determination of cluster weight	Optimum values	
		Number of berries	Mean berry weight
Thompson Seedless	96.3	85.7	7.32
Tas-A-Ganesh	92.4	84.5	4.96
2A clone	39.0	104.3	4.32

berries on cluster-weight. This assumption gains support from less variation seen in the number of berries in a cluster, berry-weight and cluster-weight in '2A Clone' (Table 6). Multiple regression function involving berry number and berry weight determined cluster weight by 96.3% in 'Thompson Seedless' and 92.4% in 'Tas-A-Ganesh', but only 39.0% in '2A Clone' (Table 7). A model optimized for cluster-weight indicated that 85.7 berries/cluster was optimum in 'Thompson Seedless', 84.5 in 'Tas-A-Ganesh' and 104.3 in '2A Clone'. Optimum weight of the berry was 7.32, 4.96 and 4.32 grams, respectively, for the three varieties in that order (Table 8).

Based on variation observed in the relation of yield-attributes to yield in various varieties in the present study, it can be inferred that for obtaining higher yields, cluster-size needs to be increased while limiting number of canes/vine in 'Thompson Seedless' and 'Tas-A-Ganesh'; but, an increase in number of canes in '2A clone' by appropriate cultural practices would be useful.

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Induction of off-season flowering in custard apple (*Annona squamosa* L.) cv. Balanagar

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ABSTRACT

Pruning and defoliation are essential operations for inducing off-season flowering and fruiting to yield better quality and quantity of fruits in custard apple. Trees were subjected to two levels of pruning (25% and 50%) combined with use of chemical defoliants (urea 5%, Ethrel 2000ppm, potassium iodide 1%, or ortho-phosphoric acid 1%) besides the Control, with each treatment replicated thrice. Early initiation of flowering and better vegetative growth was seen in pruned (25%) and defoliated trees than in the Control or other treatments. Maximum off-season yield (10.33kg/ plant) was obtained in T_4 (25% pruning, combined with 5% urea spray as defoliant) and T_6 (25% pruning, combined with 1% potassium iodide-spray as defoliant). Findings of this investigation helped standardize pruning and defoliation practices on a scientific basis for off-season production of custard apple fruits.

Key words: Pruning, defoliation, off-season, custard apple, urea

INTRODUCTION

Custard apple belongs to the family Annonaceae which has 46 genera and around 500 to 600 species, most of them found in the Tropics. Of the several species of *Annona*, at least five are available in India and yield edible fruit. These are: custard apple (*Annona squamosa* L.), cherimoya (*Annona cherimola* Mill.), soursop (*Annona muricata* L.), 'Ramphal' (*Annona reticulata* L.) and atemoya (*Annona atemola* Hort.). Custard apple has been performing well under dryland conditions where other crops do not. The tree is small, more or less shrub-like, shedding leaves in winter. The flowers are borne on current season's growth (newly emerging young shoots). Flowers are bisexual and distinctly protogynous (Sampath and Jalikop, 2000). Pruning and defoliation are essential components for inducing off-season flowering while aiming at quality and quantity of fruits. In custard apple, fruiting occurs on the current season's growth. With this in view, the present investigation was conducted to test the effect of different pruning intensities in combination with chemical defoliation on induction of off-season cropping in custard apple cv. Balanagar.

MATERIAL AND METHODS

The experiment was laid out in the experimental orchard of custard apple at ICAR - Indian Institute of Horticultural Research, Bengaluru, during 2013-2014. Eleven-year-old trees of cv. Balanagar showing uniform vigour were selected for the study. Randomized Complete Block Design (RCBD) was followed, with two levels (25% and 50%) of pruning intensity combined with defoliating chemicals (urea 5%, Ethrel 2000ppm, potassium iodide 1%, ortho-phosphoric acid 1%) with Control. Each treatment was replicated thrice. Number of shoots that emerged (secondary and tertiary shoots were counted), length of the emerged shoots (from the point of emergence to the tip, in cm), number of flowering shoots, number of flowers per shoot, and number of flowers per plant were noted at monthly interval; days taken to first flower, duration of flowering, days taken to fruit-set from onset of pruning, average number of fruits per tree, and fruit yield (kg/tree) were recorded.

RESULTS AND DISCUSSION

Growth attributes

Pruning, when performed appropriately, provides the tree with a proper shape and size. It also enables essential operations for custard apple for enhancing production of quality fruits. Significant differences among treatments at different dates of observations for number of shoots that emerged were observed (Table 1). Maximum number of shoots emerged at 30, 60 and 90 days in treatment T_4 (66.0,

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86.00 and 125.67, respectively), which was on par with T_5, T_6, T_8, T_7, T_2 and T_{10}, and, no branches were seen in T_1 (Control- no pruning and no chemical spray). This could be due to greater availability of leaf area on shoots from 25% pruning compared to 50% pruning. Further, as T_4 is a combination (25% pruning + spray of 5% urea), more numerous shoots may have been induced, resulting in greater leaf area, consequently increased photosynthetic activity. The present findings are in line with Pandey *et al* (1998) who reported maximum number of shoots under 25% pruning in ber. At 120 days from treatment, T_1 (198.0) was found to be highly significant relative to other treatments; minimum shoot number was seen in treatments T_{11}, T_9, T_{10} and T_8. This could be attributed to the fact that Control trees were neither pruned nor sprayed with chemicals, and, sprouting occurred as a natural consequence of leaf shedding and production of new growth from all the buds, 120 days after treatment-imposition in the other trees. Bajwa *et al* (1986) found significantly high total number of shoots in unpruned ber trees. Lal and Prasad (1971) also found unpruned ber trees as producing more shoots compared to the pruned trees.

Data on length of shoot (Table 1) indicate significant differences among treatments, at monthly intervals. At 30 days of observation, maximum shoot length was seen in T_3 (6.3) and T_2 (5.5), at 60 days in T_{11} (13.57), T_3 (12.93) and T_8 (12.67), at 90 days in T_3 (25.23), and, at 120 days in T_5 (30.74) which was on par with T_{10}, T_3, T_{11} and T_9. Minimum shoot length was observed in T_1 (5.60). No shoots emerged in Control T_1 at 30, 60 or 90 days. Longest shoots were observed in the lightly pruned (25%) trees during initial stages of growth, in severely pruned (50%) trees at the end of growth period, and shortest shoots were seen in unpruned trees, throughout the growth period. Similar results were obtained by Dhaliwal *et al* (2014) in kinnow, and by Trevor and Steven (2009) in custard apple.

Yield attributes

Significant differences were noticed for number of flowering shoots per tree at monthly intervals (Table 2). At 30 days, maximum flower number was found in treatment T_4 (46.0), at 60 days in T_{11} (74.67), at 90 days in T2 (85.0), and was on par with T_5, T_6, T_8, T_{10}, T_2, T_{11}, T_7, T_3 and T_9. No shoots or flowers emerged in Control T_1 at 30, 60 or 90 days. These findings are in agreement with Guimond *et al* (1998) who found pruning in cherry trees to influence number of flowering shoots. Similar results were reported by Braswell and Spiers (2005) and Lord *et al* (1979). At 120 days, T_1 (148.33) was more significant than the other treatments and the least number of flowers were seen in T_3 (120.33), which was on par with T_7, T_8, T_9 and T_2. Control trees showed maximum number of flowering shoots at this stage, because, it was the main season for flowering in custard apple under Bengaluru conditions. Flowering period in treated trees ended at 120 days, as, the trees had started flowering early in the season, and, all the differentiated buds had bloomed. Similar results were reported by Mohamed and Fawzi (2010) in custard apple, and by Bruno and Evelyn (2001) in cherimoya.

At 30 days, higher number of flowers per shoot appeared in T_3, T_7 and T_8 (6.67); at 60 days, most number of flowers were found in T_{10} (6.67), and at 90 days in T_4 (6.33), which was on par with the other treatments, excepting T_1 and T_9 (4.67). At 120 days, more flowers per shoot were found in T_1 (12.67), followed by T_5 (7.00); the least number was seen in T_2 (5.00) and other treatments (Table 2). More

Table 1. Effect of various pruning levels and defoliants on shoot emergence and shoot length

Treatment	Number of shoots emerging per tree (days after pruning)				Length of emerged shoots (cm) (days after pruning)			
	30	60	90	120	30	60	90	120
T_1 Control (no pruning, no chemicals)	0.00	0.00	0.00	198.00	0.00	0.00	0.00	5.60
T_2 25% pruning (no chemicals)	61.00	81.00	121.00	176.00	5.50	9.53	18.03	27.30
T_3 50% pruning (no chemicals)	59.00	79.00	118.67	165.33	6.30	12.93	25.23	29.57
T_4 25% pruning + Urea 5%	66.00	86.00	125.67	175.00	4.10	9.23	23.67	29.20
T_5 25% pruning + Ethrel 2000ppm	64.67	83.33	123.67	171.33	5.23	10.13	21.00	30.74
T_6 25% pruning + Potassium iodide 1%	64.00	82.67	123.33	168.67	4.53	12.03	24.30	29.20
T_7 25% pruning + Ortho-phosphoric acid 1%	61.33	83.33	123.00	166.33	4.57	12.10	23.83	27.23
T_8 50% pruning + Urea 5%	63.00	83.00	121.67	162.33	5.33	12.67	23.83	26.30
T_9 50% pruning + Ethrel 2000ppm	60.67	80.00	119.33	160.67	3.53	13.20	23.27	29.27
T_{10} 50% pruning + Potassium iodide 1%	61.33	81.67	122.00	162.00	4.03	12.20	24.53	30.50
T_{11} 50% pruning + Ortho-phosphoric acid 1%	60.33	78.67	117.00	157.00	4.57	13.57	23.73	29.43
SEm±	1.637	1.436	1.558	1.842	0.311	0.330	0.683	0.519
CD (*P=0.05)	4.830*	4.237*	4.957*	5.433*	0.918*	0.974*	2.015*	1.532*

Table 2. Effect of various pruning levels and defoliants on number of flowering shoots and number of flowers

Treatment	Number of flowering shoots per tree (days after pruning)				Number of flowers per shoot (days after pruning)			
	30	60	90	120	30	60	90	120
T_1 Control (no pruning, no chemicals)	0.00	0.00	0.00	148.33	0.00	0.00	0.00	12.67
T_2 25% pruning (no chemicals)	41.00	61.67	85.00	123.33	5.67	5.00	5.00	5.00
T_3 50% pruning (no chemicals)	32.33	59.67	82.33	120.33	6.67	4.67	5.33	6.33
T_4 25% pruning + Urea 5%	46.00	64.67	83.00	124.33	5.00	5.67	6.33	5.00
T_5 25% pruning + Ethrel 2000ppm	44.67	62.00	83.67	123.33	5.33	5.00	5.67	7.00
T_6 25% pruning + Potassium iodide 1%	43.67	65.00	82.33	124.00	6.00	5.33	6.00	6.00
T_7 25% pruning + Ortho-phosphoric acid 1%	40.33	61.00	81.67	121.00	6.67	5.00	5.00	6.00
T_8 50% pruning + Urea 5%	42.00	62.00	82.67	122.33	6.67	4.67	5.00	6.00
T_9 50% pruning + Ethrel 2000ppm	40.00	61.00	79.67	122.67	6.33	5.67	4.67	6.00
T_{10} 50% pruning + Potassium iodide 1%	41.33	63.00	81.00	129.00	5.33	6.67	5.00	6.00
T_{11} 50% pruning + Ortho-phosphoric acid 1%	40.67	74.67	81.67	128.00	6.00	5.33	5.33	6.33
SEm±	2.140	9.798	1.409	1.830	0.526	0.469	0.536	0.521
CD *(P=0.05)*	6.313*	28.90*	4.157*	5.398*	1.551*	1.384*	1.582*	1.537*

Table 3. Effect of various pruning levels and defoliants on number of flowers

Treatment	Number of flowers per tree (days after pruning)			
	30 days	60 days	90 days	120 days
T_1 Control (no pruning, no chemicals)	0.00	0.00	0.00	989.00
T_2 25% pruning (no chemicals)	232.33	309.00	422.67	617.67
T_3 50% pruning (no chemicals)	216.67	277.33	438.33	762.67
T_4 25% pruning + Urea 5%	230.67	368.00	526.00	623.33
T_5 25% pruning + Ethrel 2000ppm	241.67	311.33	474.00	863.33
T_6 25% pruning + Potassium iodide 1%	261.00	347.33	494.00	744.33
T_7 25% pruning + Ortho-phosphoric acid 1%	269.00	305.00	410.67	724.67
T_8 50% pruning + Urea 5%	280.67	289.33	411.00	732.00
T_9 50% pruning + Ethrel 2000ppm	254.67	344.67	372.67	737.67
T_{10} 50% pruning + Potassium iodide 1%	219.33	422.00	403.00	773.33
T_{11} 50% pruning + Ortho-phosphoric acid 1%	241.67	504.33	436.00	810.67
SEm±	27.339	61.357	42.946	63.950
CD *(P=0.05)*	80.642*	180.98*	126.67*	188.62*

flowers per shoot were observed in pruned trees than in unpruned trees, as light-pruning removes apical dominance, resulting in bud-break from the lower portion of the shoot. These findings are in agreement with George and Nissen (1987) in custard apple. However, at 120 days, number of flowers per shoot in T_1 was more because of presence of more number of shoots at the time, naturally resulting in the highest number of flowers in unpruned trees. Similar results were obtained by Trevor and Steven (2009) in custard apple, and by Dhaliwal *et al* (2014) in kinnow. Kahn *et al* (2001) found an increase in the number of flowers per shoot after pruning in cherimoya.

As for number of flowers per tree (Table 3), at 30 days of treatment, more flowers per tree were observed in treatment T_8 (280.67), at 60 days in T_{11} (504.33), at 90 days in T_4 (526.00), and at 120 days in T_1 (989.0). Due to a higher number of flowering shoots, and more flowers per shoot in these treatments, the number of flowers per tree was high too. Similar results reported by Kahn *et al* (2001) revealed that pruning increased the number of flowers per tree in cherimoya and so did Trevor and Steven (2012) in atemoya.

All the pruning treatments together with defoliation gave better results as for early initiation of flowering. Significant difference was seen between pruning-with-defoliation, and unpruned trees (Table 4). Minimum number of days taken for emergence of the first flower were seen in T_8 (22.6), while longest time taken for the appearance of first flower was seen in Control T_1 (95.3) (Table 4). By pruning, apical dominance could be arrested thus, directing the movement of photosynthates to the lateral buds, thereby aiding flower initiation. Similar results were reported by Trevor and Steven (2012) in atemoya, and by Laura and Julian (2008 and 2009) in cherimoya.

Table 4 shows longer duration of flowering as observed in pruned defoliated tress than in Control trees; T_8 (130.0) showed the longest duration, followed by the other

Table 4. Effect of various pruning levels and defoliants on reproductive growth and yield

Treatment	Days taken to first flower	Duration of flowering (days)	Time taken for fruit-set (days)	Average no. of fruits per tree	Fruit yield/tree (estimated) (kg)
T_1 Control (no pruning, no chemicals)	95.3	73.00	156.00	32.00	8.00
T_2 25% pruning (no chemicals)	26.6	121.67	121.00	37.33	9.33
T_3 50% pruning (no chemicals)	24.6	123.00	117.67	30.00	7.50
T_4 25% pruning + Urea 5%	25.3	127.33	116.67	41.33	10.33
T_5 25% pruning + Ethrel 2000ppm	26.3	128.00	114.00	39.33	9.83
T_6 25% pruning + Potassium iodide 1%	26.6	129.00	111.67	41.33	10.33
T_7 25% pruning + Ortho-phosphoric acid 1%	25.6	129.00	112.33	40.33	10.08
T_8 50% pruning + Urea 5%	22.6	130.00	110.00	37.67	9.42
T_9 50% pruning + Ethrel 2000ppm	24.3	129.33	111.67	37.00	9.25
T_{10} 50% pruning + Potassium iodide 1%	24	126.33	110.67	38.33	9.58
T_{11} 50% pruning + Ortho-phosphoric acid 1%	23.6	127.67	109.00	36.67	9.17
SEm±	0.686	1.210	1.367	1.214	0.303
CD *(P=0.05)*	2.023*	3.56*	4.302*	3.583*	0.895*

treatments. This could be attributed to the fact that treated trees flowered earlier and continued to flower into the normal season. Minimum duration of flowering was seen in Control T_1 (73.00) where trees flowered in the normal season only. Similar results were reported by Trevor and Steven (2012) in atemoya, and by George and Nissen (1987) in custard apple.

Minimum number of days taken to fruit-set seen in treatment T_{11} (109.0) was on par with the other treated trees. Control T_1 (156.0) took longer to set fruit (Table 4), as photosynthate pruning increases photosynthate translocation to flower buds causing them to fruit earlier than in the Control. These findings are in accordance with that of Naira and Moieza (2014) and Lal *et al* (2000) in guava.

Average number of fruits per tree differed significantly among treatments. Pruning regimes, including defoliation, increased the mean number of fruits per tree over Control (Table 4). More fruits were seen in T_4 (41.33) and T_6 (41.33), whereas, fewest fruits were seen in T_1 (30.0). Pruning along with defoliation appears to have resulted in increase in new growth, culminating in higher translocation of photosynthates from the leaves to the shoots. Our results are in agreement with findings of Farre *et al* (2000) and Kahn *et al* (2001) in cherimoya.

Data presented in Table 4 reveal significant difference between treatments. Pruning at 25% produced higher yield than 50% pruning or that in unpruned trees. Pruning operation removed apical dominance, released lateral buds from correlative inhibition, and, changed the tree form and construction. This, in turn, enhanced flower-bud initiation in lateral buds, leading to increased yield. Maximum yield was obtained in T_4 (10.33) and T_6 (10.33), as more number of fruits were borne on the tree. Similar results were reported by Mohamed *et al* (2011) in plum, and by Demirtas *et al* (2010) in apricot. Minimum fruit yield was recorded in T_3 (7.50) and T_1 (8.0 which may be attributed to 50% pruning in T_3, resulting in reduced tree-size and available photosynthates. Mohamed and Fawzi (2010) reported a similar phenomenon in *Annona*.

The regular in season crop of *Annona* under Bengaluru conditions coincides with the South-West monsoon that is August; therefore, quality of the fruit is affected due to rains. The present investigation on induction of off-season flowering through pruning and chemical defoliants resulted in achieving off-season flowering and fruiting (in June) in cv. Balanagar. As *Annona* fruits are not available in the market during this period, growers will be able to get better market price and profits. In our findings, maximum off-season yield was obtained in T_4 (25% pruning, combined with 5% urea as spray) and T_6 (25% pruning, combined with 1% potassium iodide as spray). Our findings have helped standardize the cultural practices required on a scientific basis for off-season production of *Annona* fruits.

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Fertilizer-prescription equations for targeted yield in radish under integrated nutrient management system

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ABSTRACT

Soil test crop response correlation studies under integrated plant nutrient system were carried out in Inceptisol of West Bengal during 2008 – 2010, with radish as a test crop following Ramamoorthy's 'inductive-cum-targeted yield model'. Four fertilizer (NPK) and three farmyard manure (FYM) levels were randomized in three well-established fertility gradients, each comprising 21 plots. Soil and plant analysis data were interpreted to formulate fertilizer adjustment equations, with or without FYM, at varying yield targets. It was computed that 1.40, 0.17 and 2.8 kg N, P and K, respectively, were required for producing 100 kg of radish. Contribution of fertilizer-source to the total NPK uptake in radish was far higher than that obtained from FYM and soil available sources. A ready-reckoner developed from soil-test based fertilizer adjustment equations showed that FYM application at 10 t ha^{-1}, along with NPK fertilizer, resulted in a net saving of 15, 1.8 and 5.0kg ha^{-1} of N, P and K, respectively, for cultivating radish.

Key words: Fertility gradient, fertilizer-prescription equation, Inceptisol, radish, ready-reckoner, yield target

INTRODUCTION

Increase in the rate of population growth in India (1.3% in 2012, Economic Survey, 2012-13). has necessitates production of additional food from a shrinking land area, without deterioration of soil health. This needs extensive research to help develop a scientific model for enhancing and sustaining food production and soil productivity, entailing minimal environment degradation. To attain this, it is essential that nutrients removed from the soil are replaced through judicious use of fertilizers and manures. Intensive cropping and imbalanced fertilizer application are major causes for depletion of macronutrients like N, P and K from soil. Indian agriculture is running at a 'net negative nutrient balance' of a staggering 8-10 million tonnes per year (Tandon, 2004), a figure set to reach around 15 million tonnes by 2025. Application of fertilizers by farmers without information on soil-fertility status and crop nutrient requirement affects both the soil and the crop adversely (Ray *et al*, 2000). Soil testing is an ideal scientific means for a quick and reliable estimation of soil-fertility status. Soil test crop response studies in field provide soil-test calibration between level of soil nutrients determined in the laboratory, and crop response to fertilizers observed in the field, for predicting fertilizer requirement of a crop. It is well-documented through various experiments across the country that the actual on-farm yield is lower than potential yield of a crop (Aggarwal *et al*, 2000; Ladha *et al*, 2003). These yield gaps provide ample scope for improving yield levels using techniques that prescribe fertilizer nutrients based on soil-test values and yield-targets desired. Fertilizer recommendations based on soil test crop response correlation (STCRC) are more quantitative, precise and meaningful, because, a combined use of soil and plant analysis is involved. This presents a balance between nutrients applied and available nutrients (nutrients already present) in the soil. Besides, it takes into account the farmer's ability to invest in a crop. STCR treatments are also known to record positive responses in terms of biomass yield and net returns more so when integrated sources of nutrients are used (Santhi *et al*, 2002). So far, STCRC-IPNS studies have not been made in vegetable crops in West Bengal. As radish (*Raphanus sativus* L.) is an important vegetable crop grown extensively in India, the present study was made with an objective of developing fertilizer prescription equations for targeted yield in this crop using organic manure (FYM) and chemical fertilizers combined.

MATERIAL AND METHODS

Experimental site

To develop a scientific basis for prescribing fertilizer recommendations in radish, field experiments were carried out during 2008 - 2010 at Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur, Nadia, India (22°58' N latitude and 88°29' E longitude), with fodder maize (cv. Prakash) as the gradient, and radish (cv. Red culpin) as the test crop. Soil at the experimental site was Inceptisol, sandy loam in texture, at pH 6.9 and with organic carbon content of 0.6%. Initial, available N, P and K level in the soil was 308, 24 and 155 kg ha^{-1}, respectively.

Fertility gradient experiment

Fertility-gradient experiment was conducted prior to the test-crop experiment as per inductive methodology proposed by Ramamoorthy *et al* (1967), during summer 2008-09, by dividing the experimental field into three rectangular strips along the breadth. Fertility gradients were created by applying graded doses of fertilizer N, P and K on the strips as shown in Table 1. Fodder maize was grown exhaustively to help the fertilizers undergo transformation in soil by the plant and microbes.

Test-crop experiment

After harvesting the exhaustive crop, each strip was divided into three sub-strips to impose three levels of FYM (0, 5 and 10 t ha^{-1}). Each sub-strip was further divided into seven sub-sub-strips, or plots, of 5m x 5m size. Thus, 21 plots constituted each strip. The test-crop experiment was conducted during *rabi* season (2008-09 and 2009-10) with radish (var. Red culpin) by superimposing 21 treatment combinations consisting of four levels of N (0, 40, 60 and 80 kg ha^{-1}), four levels of P (0, 9, 13 and 18 kg ha^{-1}) and four levels of K (0, 25, 33 and 50 kg ha^{-1}). In the 21 plots, all the 18 selected treatment-combinations, along with three Controls, were superimposed in each gradient strip, as per Fractional Factorial Randomized Block Design (or incomplete split-plot randomized block design) following the technical programme of All India Coordinated Research Project on Soil Test Crop Response Correlation (AICRP on STCRC) Studies. Half the N, along with full amount of P and K, was applied as a basal dose at the final land-preparation stage, and the rest half of N was top-dressed at 30 days after sowing.

Table 1. Graded dose of fertilizer applied to a gradient crop, maize

Strip	Level of fertilizer			Fertilizer dose (kg ha^{-1})		
				N	P	K
I	N_0	P_0	K_0	0	0	0
II	N_1*	P_1*	K_1*	100	22	83
III	N_2	P_2	K_2	200	44	166

* Recommended dose for fodder maize

Biometric observation

Total biomass yield in radish, comprising root and leaf, was recorded plot-wise after crop harvest under each treatment, under all the three fertility strips.

Soil analysis

Soil samples (0-0.2m depth) were collected before crop-sowing, prior to FYM and fertilizer application, and, after the harvest of both the gradient (maize) and the test (radish) crops. Soil samples were analyzed for available N, P and K as per standard methods (Jackson, 1973).

Plant analysis

At harvest, representative plant samples were collected from the test crop, washed thoroughly in tap water, followed by a wash in double distilled water. The plant samples were then dried at 60°C to a constant weight, ground and ashed at 550°C for 2 h in a muffle furnace. The ash was dissolved in 2N HCl for determining P and K content as per Chapman and Pratt (1961). Nitrogen content in the dried samples was estimated separately by digesting plant samples with sulphuric acid in the presence of digestion mixture ($CuSO_4$+K_2SO_4+ Se powder) (Micro-Kjeldahl digestion method), and, subsequently distilled and titrated (Jackson, 1973). Nutrient uptake was computed by multiplying the total dry-matter yield with nutrient concentration.

Data computation

From data on soil-test values, crop dry-matter yield and nutrient uptake, basic parameters like nutrient requirement (NR), soil efficiency (CS), fertilizer efficiency (CF) and organic efficiency (CO) were calculated, using the following formulae (developed as per Ramamoorthy *et al*, 1967):

$$\text{Nutrient requirement (NR) (kg of nutrient per 100kg of produce)} = \frac{\text{Total uptake of nutrient (kg ha}^{-1}\text{)}}{\text{Total biomass yield (100kg ha}^{-1}\text{)}}$$

$$\text{Soil efficiency or \% contribution from soil (CS)} = \frac{\text{Total uptake in Control plot (kg ha}^{-1}\text{)}}{\text{Soil-test value (STV) of nutrient in Control plot (kg ha}^{-1}\text{)}} \times 100$$

$$\text{Fertilizer efficiency or \% contribution from fertilizer (CF)} = \frac{\text{Total uptake in fertilized plot (kg ha}^{-1}\text{)} - \text{(STV of nutrient in fertilizer treated plot X CS)}}{\text{Fertilizer dose (kg ha}^{-1}\text{)}} \times 100$$

$$\text{Organic efficiency or \% contribution from organic component (CO)} = \frac{\text{Total uptake in organic plot (kg ha}^{-1}) - \text{(STV of nutrient in organic-treated plot X CS)}}{\text{Organic fertilizer dose (kg ha}^{-1})} \times 100$$

From these basic parameters, fertilizer prescription equations were developed for radish using targeted yield calculator (TYC) software of AICRP on STCR, developed by Indian Institute of Soil Science, Bhopal. Based on the equations, fertilizer recommendation was prescribed as a ready-reckoner for arriving at desired yield-target in radish.

RESULTS AND DISCUSSION

Creation of fertility gradient at the experimental site

In the present investigation, all the variation needed in soil fertility level was created deliberately in the same field. The gradient crop developed variability in soil fertility in the three experimental strips in a differential manner. Available-nitrogen after harvest increased to 90.4 and 99.1 kg ha^{-1} in medium (Strip-II) and high (Strip-III) fertility-gradient strip, respectively, from that in the low fertility Strip-I (Table 2). On the other hand, available phosphorus content increased to 37.9 and 44.5 kg ha^{-1}, while, available potassium content increased to 35.2 and 81.4kg ha^{-1} in medium- and high-fertility gradient strips from the low fertility ones, respectively. Increased availability of N, P and K in soil was due to fertilizer application in graded doses, thus creating a fertility gradient in the same field. The gradient developed with regard to K was stiffer and uniform (Fig. 1), while, the same for N and P was rather non-uniform. Occurrence of non-uniform gradients for N and P is not uncommon (AICRP Bi-annual Report, STCR, 2007-08). This was due to loss of N through different mechanisms and by locking of P by soil components. Maize has been found to develop fertility gradients for the three major nutrients in the experimental strips, because, maize is an exhaustive crop causing overmining of plant nutrients, thus leaving relatively stable nutrient sinks in the soil that result in creating the fertility gradient.

Table 2. Soil chemical properties at completion of the gradient crop

Strip	pH_w	Organic C (%)	Available nutrient		
			N	P	K
			kg ha^{-1}		
I	7.21	0.50	267.4	20.2	130.7
II	7.04	0.65	357.8	58.1	165.9
III	7.01	0.62	366.5	64.7	212.1

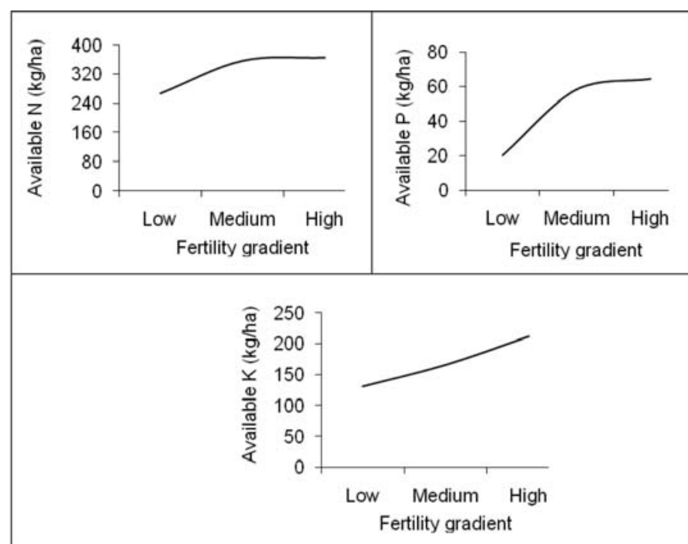

Fig. 1. Fertility gradient of the experimental field with reference to available N, P and K in soil

Yield response in radish

Results showed that yield in radish was significantly influenced by soil fertility gradient and level of FYM and NPK. On an average, irrespective of the dose of organic or NPK fertilizers, highest yield (48.8 t ha^{-1}) was recorded in medium-fertility strip, followed by high (41.6 t ha^{-1}) and low (38.7 t ha^{-1}) fertility strips (Table 3). Profuse growth of leaves in the high-fertility strip may have failed to translocate photosynthates to the roots, resulting in relatively low yield. Application of FYM at 5 t ha^{-1} produced the highest yield (45.1 t ha^{-1}), followed by 'no FYM' and FYM at 10 t ha^{-1}, irrespective of the gradient and dose of mineral NPK applied.

Across fertility gradients and FYM levels, yield in radish was significantly influenced by level of N imposed. Maximum yield (45.6 t ha^{-1}) was obtained with application of the highest level of N (80kg ha^{-1}). However, this yield was statistically at par with that obtained with 40 or 60kg N ha^{-1}. This indicates that the optimum dose of N for radish could be 40kg ha^{-1}. With increasing application of N, only vegetative growth increased. Yield increased significantly with increasing levels of P and K, and maximum yields were obtained with 13 and 33 kg ha^{-1} of P and K, respectively; thereafter, it dropped significantly with further increase in levels of both these nutrients. Thus, optimum levels of P and K for growing radish would be 13 and 33 kg ha^{-1}, respectively.

Nutrient uptake

Irrespective of the levels of FYM or NPK, no significant difference was seen in N uptake by the crop due to different fertility gradients, but the uptake of P and K was significantly influenced (Table 3). However, in all other cases too, the same trend was seen as in yield. Again,

Table 3. Effect of fertility gradient, FYM and NPK level on yield and NPK uptake in radish (mean of two years' data)

Gradient	Yield ($t\ ha^{-1}$)	Nutrient uptake ($kg\ ha^{-1}$)		
		N	P	K
Low	38.7[b]	45.1	11.6[b]	102.6[b]
Medium	48.8[a]	49.9	14.7[a]	126.8[a]
High	41.6[b]	48.3	13.7[a]	111.1[b]
		NS		
FYM level ($t\ ha^{-1}$)				
0	41.8	47.2	11.0[c]	111.1
5	45.1	49.9	13.8[b]	116.1
10	42.2	46.3	15.2[a]	113.3
	ns	ns		ns
N level ($kg\ ha^{-1}$)				
0	28.5[b]	22.2[c]	6.5[c]	52.6[b]
40	45.6[a]	56.1[a]	15.9[a]	125.5[a]
60	45.2[a]	48.9[b]	13.0[b]	119.0[a]
80	45.6[a]	58.6[a]	14.0[b]	132.9[a]
P level ($kg\ ha^{-1}$)				
0	28.5[c]	22.2[b]	6.5[c]	52.6[c]
9	44.2[ab]	47.0[a]	12.6[b]	110.5[b]
13	48.4[a]	54.1[a]	15.3[a]	132.8[a]
18	42.1[b]	53.9[a]	15.1[a]	122.2[ab]
K dose ($kg\ ha^{-1}$)				
0	28.5[c]	22.2[c]	6.5[b]	52.6[b]
25	46.1[a]	49.8[ab]	14.4[a]	123.1[a]
33	47.3[a]	56.1[a]	14.7[a]	127.6[a]
50	41.0[b]	48.9[b]	14.2[a]	117.7[a]

Values of mean followed by a different letter were significantly different at $p \leq 0.05$ using Duncan's Multiple Range Test (DMRT); NS indicates non-significant

irrespective of the gradient and NPK levels, FYM application had no significant effect on uptake of either N or K by radish. However, significant variation was observed in P uptake by the crop. Maximum uptake (15.2 $kg\ ha^{-1}$) resulted from application of 10 t FYM ha^{-1}, while, the minimum was with zero level of FYM.

Across fertility-gradients and FYM levels, application of different levels of N caused significant changes in uptake of N, P and K by radish. Maximum uptake of N and K was associated with the highest level of N application (80kg ha^{-1}), while, minimum values were obtained with no N input (Table 3). However, magnitude of NPK uptake due to application of different levels of P and K followed the same trend as that for yield. Uptake increased with increasing levels of P and K and, maximum NPK uptake was seen with application of optimum levels of P and K, as mentioned earlier, i.e., 13 and 33 kg ha^{-1}, respectively.

Developing targeted-yield equations in radish:

Basic parameters

Pre-sowing soil-test values, and, data on dry matter yield and nutrient uptake by radish, were used for calculating

Table 4. Basic parameters of targeted yield equation for radish

Parameter	Basic data		
	N	P	K
Nutrient requirement (kg nutrient per 100kg dry matter yield)	1.40	0.17	2.8
Contribution from soil (soil efficiency, %)	6.8	7.1	26.4
Contribution from fertilizer (fertilizer efficiency, %)	47.3	27.4	186.7
Contribution from organics (organic efficiency, %)	17.6	4.9	29.8

basic parameters, viz., nutrient requirement (NR) in kg for producing one quintal of radish, per cent contribution from soil (CS), fertilizer (CF) and organic source (CO). Average nutrient requirement for producing 100kg dry matter yield in radish was 1.40, 0.17 and 2.8 kg of N, P and K, respectively (Table 4). This is in close conformity with results of Bera *et al* (2006) and Thilagam and Natesan (2009). Contribution of N, P and K as estimated from soil, FYM and fertilizer sources was 6.8, 7.1 and 26.4; 17.6, 4.9 and 29.8, and 47.3, 27.4 and 186.7%, respectively. These results indicate that nutrient contribution from fertilizer sources is greater than that from soil or organic sources. These findings are in agreement with Ray *et al* (2000), Meena *et al* (2001), Shrinivas *et al* (2001) and Bera *et al* (2006). Interestingly, it was observed that contribution of K from fertilizer was more than 100% (186.7%). This high value of K could be due to an interaction effect of higher doses of N and P, and the primary effect of starter K dose, in treated plots leading to release of soil K, and consequent higher uptake from native soil sources by the crop (Ray *et al*, 2000). Similarly, high efficiency of potassic fertilizer was reported for rice by Ahmed *et al* (2002) and Bera *et al* (2006) in alluvial soil, and for maize (Reddy *et al*, 2000) and jute (Ray *et al,* 2000) in Inceptisol.

Fertilizer-prescription equations, developed using basic parameters estimated by the whole-field method, are presented below:

Prescription equations for fertilizer NPK alone

$$\begin{cases} F_N = 2.95\,T - 0.14\,S_N \\ F_P = 0.62\,T - 0.26\,S_P \\ F_K = 1.47\,T - 0.14\,S_K \end{cases}$$

Prescription equations for fertilizer NPK plus organics (FYM)

$$\begin{cases} F_N = 2.95\,T - 0.14\,S_N - 0.37\,O_N \\ F_P = 0.62\,T - 0.26\,S_P - 0.18\,O_P \\ F_K = 1.47\,T - 0.14\,S_K - 0.16\,O_K \end{cases}$$

where,

F_N, F_P and F_K=fertilizer N, P and K required ($kg\ ha^{-1}$); T=yield target [(100 kg) ha^{-1}]; S_N, S_P and S_K=soil available N, P and K ($kg\ ha^{-1}$), and O_N, O_P and O_K=quantity of N, P

and K added as FYM (FYM contains 0.46% N, 0.09% P and 0.37% K).

A ready-reckoner for fertilizer recommendation in radish

Based on generated equations, a ready-reckoner was prepared for different soil-test values for yield target of 35 and 45 t ha^{-1}, under NPK alone and under NPK+FYM. Results showed that for producing 35 t ha^{-1} of radish at average soil nutrient status of 300, 30 and 200 kg N, P and K ha^{-1}, respectively, fertilizer nutrient required was 39, 4.4 and 10.8 kg ha^{-1} N, P and K, respectively (Table 5); But, the requirement was reduced to 24, 2.6 and 5.8 kg ha^{-1} N, P and K, respectively, when the fertilizer was applied together with 10 t ha^{-1} FYM. This resulted in a saving of 15, 1.8 and 5.0 kg ha^{-1} N, P and K, respectively. Again, for producing 45 t ha^{-1} of radish at the same soil-available-nutrient levels, fertilizer nutrient requirement was 63, 6.6 and 20.8 kg ha^{-1} N, P and K, respectively (Table 6); But, the requirement was reduced to 48, 4.8 and 15.8 kg ha^{-1} N, P and K, respectively, when used with FYM. This resulted in similar magnitude of nutrient savings, i.e., 15, 1.8 and 5.0 kg ha^{-1} N, P and K, respectively. Thilagam and Natesan (2009) also reported that application of FYM at 15 t ha^{-1} together with chemical fertilizer resulted in a saving of 35, 10.9 and 23.3 kg ha^{-1} N, P and K, respectively, in cauliflower.

Table 5. A ready reckoner for fertilizer dose at varying soil-test values for a yield target of 35 t ha^{-1}

Soil-test value (kg ha^{-1})			Fertilizer nutrient required (kg ha^{-1}) for yield target of 35 t ha^{-1}					
			Inorganic			Inorganic+ FYM (10 t ha^{-1})		
N	P	K	N	P	K	N	P	K
250	5	100	47	7.0	22.5	32	5.7	17.5
275	10	125	43	6.6	20.0	28	4.8	14.2
300	15	150	39	6.1	16.7	24	4.4	11.7
325	20	175	36	5.2	13.3	21	3.9	8.3
350	25	200	32	4.8	10.8	17	3.1	5.8
375	30	225	29	4.4	7.5	15	2.6	2.5
400	35	250	25	3.5	5.0	10	2.2	0.0

Table 6. A ready reckoner for fertilizer dose at varying soil-test values for a yield target of 45tha^{-1}

Soil-test value (kg ha^{-1})			Fertilizer nutrient required (kg ha^{-1}) for yield target of 45 t ha^{-1}					
			Inorganic			Inorganic+ FYM (10 t ha^{-1})		
N	P	K	N	P	K	N	P	K
250	5	100	70	9.2	32.5	55	7.4	26.7
275	10	125	67	8.7	29.2	52	7.0	24.2
300	15	150	63	8.3	26.7	48	6.6	20.8
325	20	175	60	7.4	23.3	45	6.1	18.3
350	25	200	56	7.0	20.8	41	5.2	15.8
375	30	225	52	6.6	17.5	37	4.8	12.5
400	35	250	49	5.7	15.0	34	4.4	9.2

CONCLUSION

Irrespective of fertility gradient or FYM level, optimum dose of N, P and K for cultivating radish was 40, 13 and 33 kg ha^{-1}, respectively. This also corresponded to a higher removal of N, P and K by biomass of the harvested crop. Contribution of N, P and K from the soil-available pool was 6.8, 7.1 and 26.4% to total N, P and K uptake by the crop, respectively, while, such contribution from applied fertilizer was 47.3, 27.4 and 186.7%, and, that from applied FYM was 17.6, 4.9 and 29.8%, respectively. A ready-reckoner developed using soil-test based fertilizer adjustment equations in radish showed that application of FYM at10 t ha^{-1} along with chemical fertilizer resulted in a net saving of 15, 1.8 and 5.0 kg ha^{-1} of N, P and K, respectively, for cultivating radish at average soil-nutrient status of 300, 30 and 200 kg ha^{-1} N, P and K, respectively. This indicates the usefulness of STCRC-IPNS technology for achieving higher-crop production and a more rational use of fertilizer nutrients.

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Evaluation of cut-foliage plants for Eastern Ghats

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ABSTRACT

A maiden attempt was made at Horticultural Research Station (HRS), a constituent research unit of Tamil Nadu Agricultural University, located at Yercaud, Salem District, Tamil Nadu, India during 2012-2013 to assess the suitability of various ornamental foliage plant species under Shevroys /Eastern Ghats conditions. Considerable variation was found in quantitative and qualitative parameters among the foliage species. The study recommends *Nephrolepis cordifolia* and *Asparagus sprengeri* as suitable liners, while, large-leaved species like *Cordyline fruticosa* and *Philodendron xanadu* as background materials in larger arrangements, and the smaller-leaved *Dracaena reflexa* var. *angustifolia* for smaller arrangements.

Key words: Foliage plants, arrangements, liner, background material

INTRODUCTION

Cut-foliage industry has made a major breakthrough in floriculture business. Most foliage plants are indigenous to tropical and subtropical regions. In general, foliage plants are grown as understory plants in the canopy of giant trees. As a result, foliage plants are native to this type of environment, are tolerant to low light, sensitive to chilling temperature and are day-neutral to photoperiod. In subtropical climes, temperature as well as humidity may vary with season. Among various parameters, leaf characters assume significance for their use as cut-foliage.

Of the total turnover and supply of floricultural products during 2010 (€4130 million), indoor foliage plants alone contributed €1445 million (Rs. 99.23 billion) in global floricultural trade (Anon., 2011a).

Some of the important indoor foliage plants (genera) occupying world-rank lists in 2010 *Anthurium, Kalanchoe, Dracaena, Ficus, Spathiphyllum, Hedera, Begonia, Chrysalidocarpus (lutescens)* and *Zamioculcas*. Recent data showed that floricultural products (live trees and other plants, bulbs, roots and the like; cut-flowers and ornamental foliage) exported from India stood at Rs. 28,645 lakh during the 2010-11 fiscal years. In the same period, imports were valued at Rs. 4,548 lakh (Anon., 2011b). The trend shows that India has been slowly accelerating its pace in the international trade. As for the foliage plant industry, during 2008-2009 more than 39% of the total export from India was contributed by foliage products, fresh or dry. However, in view of the importance of foliage ornamentals, an experiment was formulated to evaluate 29 foliage species collected from various sources to identify suitable cut-foliage species for the Shevroys region.

MATERIAL AND METHODS

An experiment was conducted using 29 foliage species (Table 1) at Horticultural Research Station, Tamil Nadu Agricultural University, Yercaud, during the year 2012-2013 to evaluate their suitability for foliage. The experimental site is geographically situated between 11° 04" and 11° 05" North Latitude and 78° 05" to 78° 23" East Longitude, at an altitude of 1500m above Mean Sea Level. Average maximum and minimum temperatures during the experimental period were 31.0°C and 12.4°C, respectively. The mean annual rainfall received by Yercaud was 1572mm in 47 rainy days. Average relative humidity was 75%. Irrigation was provided at intervals of 5-6 days throughout the period of experiment, depending upon soil moisture status and weather conditions. All the foliage species were planted at a spacing of 1m × 0.8m. The study was patterned on Randomized Block Design, with three replications. Five plants from each replication were observed for biometrics on plant height (cm), plant spread (cm), leaf length (cm), leaf breadth (cm), number of shoots, leaf area, longevity, petiole length and girth (cm), and internodal length (cm) besides qualitative characters like leaf shape, margin, texture, venation, leaf

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apex and foliage colour. Data was compiled, analyzed and is presented in Tables 2 & 3.

Post-harvest treatments like pulsing and holding solution were also studied. In pulsing treatment, mature leaf from each species was harvested and treatments imposed for six hours. Details of the pulsing treatments are as follows: P_o – Filtered water, P_1- Acidified water (pH 3.5), P_2- Sucrose 5%, P_3- Sucrose 5% + $AgNO_3$ 50ppm, and, P_4- Sucrose 5% + $AgNO_3$ 100ppm. After pulsing, the foliage was transferred to water for comparing the effects of treatments. Foliage from different species was subjected to the following holding treatments: H_o – Filtered water, H_1- Acidified water (pH 3.5), H_2- Sucrose 5%, H_3- Sucrose 5% + $AgNO_3$ 25ppm and, H_4- Sucrose 5% + $AgNO_3$ 50ppm. Vase-life was calculated by noting the time taken to develop symptoms like leaf-drop, yellowing and wilting (factors that rendered the foliage unfit for arrangement). Observations on vase-life in combination treatments were noted for a period of ten weeks.

Table 1. List of foliage species evaluated in Shevroys of Eastern Ghats in Tamil Nadu

Botanical name	Family	Common name
Aglaonema crispum	Araceae	Chinese evergreen
Anthurium andreanum	Araceae	Lady Jane
Asparagus sprengeri	Liliaceae	*Sprengeri* fern
Asparagus densiflorus	Liliaceae	Asparagus fern
Asparagus setaceous	Liliaceae	Fern asparagus
Cordyline Chocolate queen	Agavaceae	Ti plant
Cordyline Chocolate swirl	Agavaceae	Ti plant
Cordyline compacta	Araceae	Ti plant
Cordyline fruticosa	Agavaceae	Ti plant
Cordyline negra	Agavaceae	Ti plant
Cordyline tango	Agavaceae	Ti plant
Cordyline terminalis	Agavaceae	Ti plant
Dracaena 'Purple compacta'	Agavaceae	Ti plant
Dracaena compacta	Agavaceae	Dracaena
Dracaena fragrans 'Lemon lime'	Agavaceae	Dracaena
Dracaena fragrans 'Massangeana'	Agavaceae	Corn plant
Dracaena marginata	Agavaceae	Red-edged Dracaena
Dracaena reflexa var. angustifolia	Agavaceae	Song of India
Dracaena reflexa var. Tropical	Agavaceae	Dracaena
Dracaena reflexa 'Song of Jamaica'	Agavaceae	Song of Jamaica
Dracaena sanderiana	Agavaceae	Corn plant
Heliconia rostrata	Heliconiaceae	Lobster claw
Nephrolepis cordifolia	Polypodiaceae	Erect sword Fern
Nephrolepis falcata	Polypodiaceae	Fishtail sword Fern
Philodendron 'Ceylone gold'	Araceae	Philodendron
Philodendron Green emerald	Araceae	Philodendron
Philodendron imbe 'Variegata'	Araceae	Philodendron
Philodendron Red emerald	Araceae	Philodendron
Philodendron xanadu	Araceae	Philodendron

RESULTS AND DISCUSSION

On evaluation, it was found that all the foliage plants had significant differences in the characters studied. Quantitative characters of different foliage species is presented in Table 2. Plant height recorded ranged from 37.70cm to 31.40cm. *Cordyline fruticosa* recorded the highest plant height (131.4cm), followed by *Dracaena* Purple compacta (102cm) and *Asparagus sprengeri* (92.7cm). Lowest plant height was recorded in *Dracaena fragrans* 'Lemon lime', with 37.7cm. A similar trend was also reported by Russ and Pertuit (2001) in various foliage plants like *Dracaena*, *Philodendron*, *Schefflera,* and some indoor ferns.

Plant-spread is an important character when considering the foliage for its growing environment. It gives an idea about the number of plants that can be accommodated in a given area (plant density). However, in climbers, plant-spread had lesser relevance compared to that in the others that had vertical growth. The highest plant-spread of 117.59cm East-West, and 118.18cm North-South, was noticed in *Asparagus sprengeri*. Lowest plant-spread was noticed in *Philodendron* Green Emerald (30.63cm E-W, 33.37cm N-S). Similar variations were observed by Eapen (2003).

Number of leaves ranged from 13.51 to 196.67. Maximum number of leaves was recorded in *Dracaena reflexa* 'Song of Jamaica' (196.67), followed by *Dracaena reflexa* var. *angustifolia* (185.00), *Asparagus setaceous* (137.27) and *Dracaena marginata* (78.95). Lowest number of leaves was observed in *Philodendron* 'Ceylon gold' (13.5). Basically, species with larger leaves tended to produce less number of leaves, whereas, species with smaller leaves had greater number of leaves. This variation was due to several factors like genetic make-up, partition of the photosynthates, production of more number of branches and tillers, etc. Our results confirmed the findings of Bulle and Dejongh (2001) and Benedetto *et al* (2006).

Number of shoots too is an important characters contributing to yield. In the present study, shoot number differed significantly between species. *Dracaena reflexa* var. *angustifolia* registered higher number of shoots (7.7), followed by *Dracaena reflexa* (Song of Jamaica) (6.5)

Table 2. Quantitative characters of various foliage species

Species	Plant height (cm)	Plant spread E-W (cm)	Plant spread N-S (cm)	Leaf no.	Shoot number	Leaf area (cm²)	Petiole length (cm)	Petiole girth (cm)	Inter-nodal length (cm)	Leaf longevity (d)
Aglaonema crispum	59.80	55.93	50.74	19.50	2.10	130.6	7.75	3.19	-	24.50
Anthurium andreanum	69.90	86.63	88.53	17.50	-	93.06	17.30	2.29	-	22.70
Asparagus sprengeri	92.70	117.59	118.18	55.37	-	3.19	5.24	2.23	-	24.00
Asparagus densiflorus	57.80	34.22	41.50	14.57	-	14.89	5.79	1.50	-	22.40
Asparagus setaceous	53.20	44.73	44.35	133.27	-	33.02	6.25	1.44	-	22.50
Cordyline Chocolate queen	54.80	34.68	36.27	30.10	3.30	86.53	4.72	3.66	-	19.00
Cordyline Chocolate swirl	61.90	40.07	44.13	30.18	3.40	89.60	4.82	3.47	-	21.70
Cordyline compacta	37.80	39.38	42.80	15.58	1.00	67.62	3.85	2.49	-	19.10
Cordyline fruticosa	131.40	68.03	67.53	30.89	3.30	214.1	6.07	3.28	-	19.90
Cordyline negra	45.40	39.71	41.10	16.52	1.80	171.1	4.19	2.69	-	17.90
Cordyline tango	42.00	42.25	44.20	30.82	4.20	67.98	2.81	2.50	-	18.80
Cordyline terminalis	81.20	59.88	64.44	28.41	1.00	80.16	7.77	2.67	-	21.80
Dracaena 'Purple compacta'	78.30	32.69	37.11	74.97	2.50	42.79	2.63	2.59	-	23.00
Dracaena compacta	54.00	33.68	39.00	71.28	4.30	41.81	-	-	-	22.40
Dracaena fragrans 'Lemon lime'	37.70	47.64	58.43	24.42	-	39.91	-	-	-	23.50
Dracaena fragrans 'Massangeana'	79.10	77.86	83.94	39.52	1.90	289.7	-	-	-	26.70
Dracaena marginata	102.0	67.16	70.68	78.95	1.80	32.33	-	-	-	23.20
Dracaena reflexa var. angustifolia	48.20	62.88	71.93	185.0	7.70	17.28	-	-	2.09	19.70
Dracaena reflexa var. Tropical	53.60	39.83	45.01	36.62	3.80	20.49	-	-	1.36	23.50
Dracaena reflexa 'Song of Jamaica'	72.10	65.98	70.12	196.67	6.50	32.42	-	-	-	23.20
Dracaena sanderiana	56.90	63.09	66.60	25.08	1.00	70.44	-	-	-	23.10
Heliconia rostrata	65.80	63.00	71.31	22.40	1.00	47.19	5.61	2.73	-	23.00
Nephrolepis cordifolia	58.20	49.81	50.93	32.08	-	72.13	7.44	0.45	-	22.70
Nephrolepis falcata	64.30	57.34	51.73	19.07	-	43.67	4.53	0.27	-	23.60
Philodendron 'Ceylon gold'	36.80	54.40	56.13	13.51	1.00	38.47	5.79	2.64	2.96	19.50
Philodendron Green emerald	50.90	30.63	33.37	21.11	1.00	56.76	14.29	3.28	1.72	18.00
Philodendron imbe 'Variegata'	53.80	34.93	56.93	14.57	1.00	321.6	16.81	3.65	-	24.60
Philodendron Red emerald	70.00	86.80	97.63	19.27	1.00	240.7	5.85	3.82	3.62	22.80
Philodendron xanadu	47.60	70.24	73.83	42.40	-	61.15	22.23	2.67	-	18.50
S.Ed.	3.66	3.13	3.96	10.72	0.57	20.11	0.31	0.13	0.19	0.64
CD *(P=0.05)*	7.33	6.28	7.94	21.48	1.15	40.3	0.63	0.27	0.38	1.28

and the lowest number of shoots (1.0) was observed in the species of *Heliconia rostrata*, *Philodendron* Red emerald, *Cordyline compacta*, *Cordyline terminalis*, *Philodendron* Green emerald, *Philodendron* 'Ceylon gold' and *Philodendron imbe* 'Variegata'.

Highest leaf area was observed in *Philodendron imbe* 'Variegated' (321.67cm²) followed by *Dracaena fragans* 'Massangeana' (Corn plant) with 289.79cm², and *Philodendron* Red emerald (240.75cm²⁾. Lowest leaf area was observed in *Asparaus sprengeri* (3.19cm²).

Length and girth of petiole are important characters for cut-foliage giving physical support to the leaf. Also, length of the leaf contributes to the spread of a plant. More the petiole length, greater the plant spread. If the petiole is short, high compactness is noticed in leaf arrangement. Petiole length ranged from 2.63cm to 22.23cm.

Table 3. Qualitative characters of various foliage species

Treatment	Leaf type	Venation	Leaf shape	Leaf margin	Leaf tip	Leaf orientation	Texture of the leaf	Foliage colour
Aglaonema crispum	Simple	Pinnate	Oblong	Entire	Acute	Cuneate	Smooth	Pale green
Anthurium andreanum	Simple	Pinnate	Acuminate	Entire	Acute	Cuneate	Smooth	Deep green
Asparagus sprengeri	Simple	none	Linear	Entire	Acute	Cuneate	Fine	Deep green
Asparagus densiflorus	Simple	none	Linear	Entire	Acute	Cuneate	Fine	Deep green
Asparagus setaceous	Simple	none	Linear	Entire	Acute	Cuneate	Fine	Deep green
Dracaena 'Purple compacta'	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Deep purple
Dracaena compacta	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Deep green
Dracaena fragrans 'Lemon lime'	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Fine	Yellow
Dracaena fragrans 'Massangeana'	Simple	Parallel	Lanceolate	Undulate	Acute	Attenate	Coarse	Deep green
Dracaena marginata	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Purple
Dracaena reflexa var. *angustifolia*	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Pale yellow
Dracaena reflexa 'Song of Jamaica'	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Deep green
Dracaena reflexa 'Green'	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Deep purple
Dracaena sanderiana	Simple	Parallel	Lanceolate	Undulate	Acute	Attenate	Coarse	Pale green
Cordyline Chocolate queen	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Deep green
Cordyline Chocolate swirl	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Pale sandal
Cordyline compacta	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Deep purple
Cordyline fruticosa	Simple	Parallel	Lanceolate	Entire	Acute	Attenate	Smooth	Deep green
Cordyline negra	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Deep pink
Cordyline tango	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Deep purple
Cordyline terminalis	Simple	Parallel	Lanceolate	Entire	Acute	Decurrent	Smooth	Deep green
Heliconia rostrata	Simple	Pinnate	Ovate	Entire	Acute	Cuneate	Smooth	Deep green
Nephrolepis cordifolia	Simple	none	Lanceolate	Entire	Acute	Cuneate	Fine	Deep green
Nephrolepis falcata	Simple	none	Lanceolate	Entire	Acute	Cuneate	Fine	Deep green
Philodendron 'Ceylon gold'	Simple	Pinnate	Lanceolate	Entire	Acute	Cuneate	Smooth	Golden Yellow
Philodendron Green emerald	Simple	Pinnate	Lanceolate	Entire	Acute	Cuneate	Smooth	Deep green
Philodendron imbe 'Variegata'	Simple	Pinnate	Lanceolate	Entire	Acute	Cuneate	Smooth	Deep green
Philodendron Red emerald	Simple	Pinnate	Saggitate	Revolute	Acute	Cuneate	Coarse	Deep purple
Philodendron xanadu	Simple	Pinnate	Entire	Entire	Acute	Decurrent	Coarse	Deep green

Philodendron xanadu recorded the longest petiole (22.23cm), the shortest petiole was observed in *Dracaena* 'Purple compacta' (2.63cm). Maximum petiole girth (3.82cm) was recorded in *Philodendron* Red emerald. Minimum petiole girth was observed in *Nephrolepsis falcata* (Fishtail sword fern), with 0.27cm. These results are in accordance with those of Wang and Chen (2003) and Mollick *et al* (2011).

As for internode length, most species had short and compact internodes, the very first qualities sought out in decoration. Highest internode length was observed in *Philodendron* Red emerald (3.62cm), followed by *Philodendron* 'Ceylon gold' (2.96cm) while, the minimum was observed in *Dracaena reflexa* var. Tropical (1.36cm). Leaf longevity on the plant is linked to leaf production intervals. If a plant produces leaves at longer intervals, longevity of the leaf is found to be higher. *Longevity of the leaves on a plant depends upon environmental conditions, genetic factors and incidence of pests and diseases. Longer life of leaves on the plant also helps stagger harvest of the leaves. Under normal conditions, foliage of Dracaena fragrans* 'Massangeana' (26.7 days), *Philodendron imbe* 'Variegata' (24.6 days) and *Aglaonema crispum* (24.5 days) was found to have t*he highest longevity among the plants evaluated. However, shrub-like Cordyline negra* (17.9 days) showed lower longevity of leaves than other species (Alex, 2012).

Qualitative traits of different foliage plants are presented in Table 3. Characters like texture, type, shape, margin, tip, base, pigmentation, venation, arrangement of leaves and branching habit, were considered as these relate to aesthetic value of the plants and the arrangement. Plants like *Dracaena reflexa* var. *angustifolia* (Song of India), *Dracaena reflexa* 'Song of Jamaica', *Anthurium*

andreanum (Lady Jane), *Philodendron* 'Ceylon gold' and *Asparagus sprengeri* (*Sprengeri* fern), need adequate staking, as, these tend to bend. *Nephrolepis cordifolia* (Erect sword fern), *Nephrolepis falcata* (Fishtail sword fern) and *Asparagus setaceous* (Asparagus fern) need adequate pruning.

Plants were also rated according to their quality (characters like colour, texture and pigmentation). Among *Dracaena* species, *Dracaena reflexa* var. *angustifolia* rated as good. Similarly, in *Cordyline* species - *Cordyline fruticosa*, *Philodendron* species - *Philodendron xanadu*, *Nephrolepis* species - *Nephrolepis cordifolia,* and *Asparagus* species - *Asparagus sprengeri,* performed well under Eastern Ghats. These can be recommended as the best foliage plants, possessing all the qualities (to be grown in any type of growing conditions); these are also well-suited for testing under open conditions. This type of visual quality-grading was done earlier by Wang *et al* (2005).

Keeping-quality is of prime commercial importance in the trade of cut-foliage, besides aesthetics. Pre-harvest and post-harvest factors, together with the stage and time of harvest, determine keeping-quality of the foliage for vase-life. If harvested at the immature or over-mature stage, the foliage does not keep well, and, the desired effect of foliar variegation is not fully achieved by a foliage arrangement. Generally, foliage is cut when mature, having fully attained its shape, colour and size. Kumar and Bhattacharjee (2003) reported foliage of *Calathea ornata*, *Codiam varigatam*,

Table 4. Effect of pulsing treatment on cut foliage at Shevroys condition (days)

Name of the species	P_0	P_1	P_2	P_3	P_4
Aglaonema crispum	8.40	10.1	12.3	10.3	15.8
Anthurium andreanum	10.0	10.1	11.8	10.8	17.4
Asparagus sprengeri	7.60	8.50	10.5	8.90	17.5
Asparagus densiflorus	7.50	7.90	9.50	8.30	18.6
Asparagus setaceous	8.00	9.20	12.3	9.90	17.9
Cordyline Chocolate queen	8.70	10.4	11.8	10.3	20.3
Cordyline Chocolate swirl	7.80	8.30	10.3	8.80	17.1
Cordyline fruticosa	8.60	8.20	9.10	8.70	19.7
Cordyline negra	8.30	9.50	10.6	9.50	18.5
Cordyline tango	10.4	10.5	11.5	10.8	18.3
Cordyline terminalis	8.30	9.50	10.8	9.50	19.4
Cordylne compacta	8.50	10.4	10.1	9.70	19.5
Dracaena 'Purple compacta'	9.20	10.1	10.6	10.0	20.6
Dracaena compacta	9.60	9.80	9.60	9.70	16.8
Dracaena fragrans 'Lemon lime'	9.30	11.7	12.7	11.2	16.5
Dracaena fragrans 'Massangeana'	10.40	9.00	12.9	10.8	19.0
Dracaena marginata	8.10	9.90	12.0	10.0	20.1
Dracaena reflexa	12.9	9.80	10.2	10.9	17.1
Dracaena reflexa var. Tropical	8.50	8.20	9.50	8.70	18.4
Dracaena reflexa 'Song of Jamaica'	9.10	8.90	9.40	9.10	17.9
Dracaena sanderiana	9.10	9.00	9.70	9.20	19.7
Heliconia rostrata	7.90	8.10	13.3	9.80	19.9
Nephrolepis cordifolia	8.90	8.90	11.2	9.70	19.3
Nephrolepis falcata	8.80	8.30	10.5	9.20	17.1
Philodendron 'Ceylone gold'	7.40	8.30	10.8	8.80	17.0
Philodendron Green emerald	8.20	7.80	10.3	8.80	19.6
Philodendron imbe 'Variegata'	7.70	8.20	11.3	9.10	17.5
Philodendron Red emerald	9.20	8.90	11.3	9.80	17.9
Philodendron xanadu	8.00	8.20	10.1	8.70	20.0
SEd	0.48	0.55	0.61	0.88	0.74
CD *(P=0.05)*	0.97	1.11	1.23	1.77	1.49

P_0 – Filtered water, P_1- Acidified water (pH 3.5), P_2- Sucrose 5%, P_3- Sucrose 5% + $AgNO_3$ 50ppm, P_4- Sucrose 5% + $AgNO_3$ 100ppm

Table 5. Effect of the holding solution on cut foliage plants at Shevroys condition (days)

Name of the species	H_0	H_1	H_2	H_3	H_4
Aglaonema crispum	8.40	6.70	10.7	14.0	13.6
Anthurium andreanum	6.40	6.50	11.3	15.0	15.5
Asparagus sprengeri	6.70	7.20	11.5	15.3	15.3
Asparagus densiflorus	5.70	6.40	10.0	12.7	14.1
Asparagus setaceous	7.30	5.80	11.3	13.6	15.5
Cordyline Chocolate queen	7.40	7.50	12.5	12.9	13.7
Cordyline Chocolate swirl	6.90	5.70	13.4	14.7	15.2
Cordyline fruticosa	7.20	7.50	10.9	13.3	14.5
Cordyline negra	6.40	6.60	11.4	12.3	14.1
Cordyline tango	7.00	7.50	12.2	13.4	13.7
Cordyline terminalis	7.30	6.20	9.90	13.3	14.5
Cordylne compacta	7.00	7.00	11.6	13.5	15.3
Dracaena 'Purple compacta'	7.10	6.10	11.5	12.9	12.9
Dracaena compacta	6.20	6.30	11.7	14.1	15.7
Dracaena fragrans 'Lemon lime'	7.10	6.20	11.2	12.4	16.2
Dracaena fragrans 'Massangeana'	8.40	7.30	11.5	15.0	15.5
Dracaena marginata	5.20	5.40	10.4	14.5	15.2
Dracaena reflexa	7.00	7.50	8.60	15.1	14.7
Dracaena reflexa var. Tropical	6.50	6.60	11.3	13.6	14.5
Dracaena reflexa 'Song of Jamaica'	7.20	5.60	10.3	15.9	16.3
Dracaena sanderiana	6.50	7.20	12.6	14.5	16.1
Heliconia rostrata	7.40	6.00	11.3	13.7	14.3
Nephrolepis cordifolia	7.80	6.50	10.1	12.1	14.4
Nephrolepis falcata	7.60	6.90	12.2	14.3	15.3
Philodendron 'Ceylone gold'	6.50	5.90	11.9	10.0	13.4
Philodendron Green emerald	7.70	6.00	12.1	14.1	15.4
Philodendron imbe 'Variegata'	6.30	6.80	11.4	15.3	15.1
Philodendron Red emerald	7.70	6.70	11.7	13.1	16.2
Philodendron xanadu	7.90	6.40	11.9	12.9	14.4
S.Ed.	0.40	0.36	0.70	1.10	0.74
CD *(P= 0.05)*	0.81	0.72	1.40	2.21	1.49

*H_o - Filtered water, H_1- Acidified water (pH 3.5), H_2 - Sucrose 5%, H_3 - Sucrose 5% + $AgNO_3$ 25ppm, H_4 - Sucrose 5% + $AgNO_3$ 50ppm

Dracaena sp. and *Nephrolepis* sp. as having longer vase-life when the leaves were mature and fully expanded.

Pulsing is a short-term treatment given to cut-foliage immediately following harvest, to improve keeping quality. Data on effect of pulsing solutions on vase-life of different species of cut-foliage are furnished in Table 4. Among the pulsing solutions used, highest vase-life was recorded in *Dracaena* 'Purple compacta' under P_4 (Sucrose 5% + $AgNO_3$100ppm), with 20.6 days. This was significantly superior to the other pulsing solutions and was followed by *Cordyline* 'Chocolate queen' in P_4 (Sucrose 5% + $AgNO_3$100ppm), with 20.3 days. Minimum vase-life of 7.5 days was recorded in P_o (Filtered water) in *Asparagus densiflorus*.

Data on effect of holding solutions on vase-life of different species of cut-foliage are furnished in Table 5. Holding solutions significantly influenced vase-life. Among the holding solutions tested, highest vase-life was recorded in H_4 (Sucrose 5% + $AgNO_3$ 50ppm), with 16.3 days in *Dracaena reflexa* 'Song of Jamaica'. This was significantly superior to other holding treatments, followed by H_3 (Sucrose 5% + $AgNO_3$ 25ppm) with 16.2 days in *Dracaena fragrans* 'Lemon lime' *and Philodendron* Red emerald. A minimum vase-life of 5.4 days was recorded in *Dracaena marginata* in H_1 (Acidified water).

In conclusion, *Nephrolepis cordifolia* and *Asparagus sprengeri* can be recommended as suitable liners, while, large-leaved species like *Cordyline fruticosa* and *Philodendron xanadu* as background materials for larger arrangements, and the smaller-leaved *Dracaena reflexa var. angustifolia* for smaller arrangements.

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Metabolite profiling for six 'B' vitamins using LC-MS in tomato genotypes at different stages of fruit maturity

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ABSTRACT

Vitamins are essential nutrients in food crucial for maintaining good health. Tomato, being a widely consumed vegetable, provides a good quantity of vitamins. Metabolite profiling of vitamins at different stages of fruit maturity in a crop helps identify the right stage for better quality. Based on preliminary screening for quality parameters, tomato lines rich in TSS, antioxidants, lycopene and beta-carotene were selected for the present study. Eight genotypes and a wild species were profiled for 'B' vitamins at three different stages of fruit maturity, viz., green, breaker and ripe stage. A simple and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) method for simultaneous determination of six 'B' vitamins was developed and validated by us. Among the genotypes studied, IIHR-249-1 recorded higher niacin, pantothenic acid and biotin content. Pyridoxine content was higher in the hybrid, Arka Rakshak. The wild species, LA-1777*(Solanum habrochaites*) was found to be rich in pantothenic acid, riboflavin and thiamine. Content of most of the vitamins increased with ripening of the fruit. IIHR-249-1 and LA-1777 were found to be rich in 'B' vitamins, earlier reported to be also rich in antioxidants and lycopene. These genotypes can be used for improving the nutritive value of tomato under crop improvement programmes, through conventional breeding or biotechnological approaches.

Key words: Tomato, B vitamins, LC-MS/MS-MRM, fruit ripening, green stage, breaker stage

INTRODUCTION

Tomato is rich in lycopene, β-carotene, phenols and flavonoids, having moderate amounts of Vitamin C (Stewart *et al,* 2000; Beutner *et al,* 2007). It is also a good source of Vitamin E, thiamine, niacin, pyridoxine, folate, vitamin K and dietary fibre (USDA, 2006). With high levels of health-promoting bioactive compounds and antioxidants, tomato fruit has also been identified as a functional and nutraceutical food (Agarwal and Rao, 1998; Canene-Adams *et al,* 2005).

Vitamins are nutrients essential in our diet. Eight of the water-soluble vitamins are known as Vitamin B-complex group. Thiamine (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxine (B_6), biotin (B_7), folic acid (B_9) and cyanocobalamin (B_{12}) constitute Vitamin B-complex. The B vitamins function as coenzymes helping the body obtain energy from food. These are also important for good vision, a healthy skin, nervous system and red blood cell formation. Various methods like microbiological assays, spectrophotometric assays, capillary electrophoresis, TLC, HPLC and a few LC-MS based methods have been used for estimation of 'B' vitamins (Chen *et al,* 2006). Tomato is a very widely consumed vegetable globally and is moderately rich in vitamins, but it needs to be improved for content of 'B' vitamins - very crucial for maintaining optimal health. Metabolite profiling of the tomato fruit for vitamins can help improve its quality.

The present study was undertaken to study variations in the profile of 'B' vitamins in eight selected genotypes that included hybrids, varieties, an elite germplasm line and a wild species, at three different stages of fruit ripening. We also developed a simple, sensitive and reliable LC-MS/MS method for quantification of six 'B' vitamins, namely, thiamine, riboflavin, niacin, pantothenic acid, pyridoxine and biotin.

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MATERIAL AND METHODS

Plant material

Based on initial screening of a large number of tomato lines for quality parameters, a total of nine genotypes of tomato including released varieties, commercial hybrids, cherry tomato, an elite germplasm line and a wild species were used for profiling ‘B’ vitamins, as follows:

- Commercial hybrids: Arka Ananya, Arka Samrat and Arka Rakshak
- Varieties**:** Arka Ashish and Arka Vikas
- Cherry tomato lines: IIHR-2864 and IIHR-2866
- Wild species: *Solanum habrochaites* (LA-1777)
- Elite germplasm line: IIHR-249-1

Plants were raised in the field under irrigated conditions as per the standard package of practices. ‘B’ vitamins were assessed in fruits harvested at three different stages of ripening, viz., green stage (45 days post-anthesis), breaker stage (55 days post-anthesis) and red ripe stage (60 days post-anthesis, when the fruit is completely red or orange, yet firm). Samples were collected from three different plants in all the genotypes.

Reagents

The water-soluble vitamin standard thiamine hydrochloride (≥99%), riboflavin (99%), niacin (99.5%), calcium D-pantothenate (98%), pyridoxine hydrochloride (99%) and biotin (≥99%) were procured from Sigma Chemical Co., USA. Standard vitamin solutions were prepared in 0.01N HCl. Butylatedhydroxy toluene (BHT) and ammonium formate were procured from Himedia, India. Amino acid standard mixtures at a concentration 2.5µmoles per ml, *o*-phthalaldehyde (OPA) reagent and formic acid were obtained from Sigma. Amino acid standard solutions were prepared in 0.1N HCl. Sodium phosphate [monobasic and dibasic (anhydrous)], sodium hydroxide and boric acid were obtained from Merck, India. Organic solvents used as a mobile phase for liquid chromatography were of chromatographic/MS grade.

Equipment

Acquity UPLC-H class, coupled with Acquity TQD-MS/MS from Waters, USA with ESI source, was used for determinating water-soluble vitamins. The instrument was equipped with a degasser, quaternary pump, automatic injection system, with a diode array detector and a temperature control compartment for the analytical column. The detection system allowed simultaneous detection at various wavelengths and MRM for individual masses. The overall system-control and data acquisition were monitored by Mass Lynx™ software.

Extraction of water-soluble vitamins

Extraction of water-soluble vitamins was done as per methods previously reported, with some modifications (Santos *et al,* 2012; Zand *et al,* 2012). During extraction it was assumed, that the samples were protected from direct exposure to light, to avoid degradation of the vitamins. In brief, 3-4 fruits of tomato from each of the genotypes were homogenized for one minute in a mixer-blender. From the homogenized mixture, 10g were weighed and then extracted with 40ml of 10 mM ammonium formate/methanol 50:50 (v/v) containing 0.1% BHT. After shaking for 5 minutes to achieve good sample-dispersion in the extraction liquid, the samples were incubated in a water bath at 70°C for 40 min. After cooling down to room temperature, the samples were centrifuged at 14000g for 10 min and the volume made up to 50ml with 10mM ammonium formate. Finally, the supernatant was filtered through a 0.2µm nylon filter and injected into an UPLC-MS/MS system.

LC and MS-MS conditions

Separation, identification and quantification of the six ‘B’ vitamins was performed using UPLC coupled with tandem mass spectrometry detection, using Multiple Reaction Monitoring (MRM) mode. The column used was UPLC BEH C_{18} (2.1 x 50mm, 1.7µm; Waters, USA) with security guard column Vanguard BEH-C_{18} (2.1 x 5mm, 1.7µm; Waters, USA). The column oven was maintained at 25°C, with the sample injection volume being 3.0µl. Eluted vitamins were monitored using a PDA detector and TQD-MS/MS (Waters, USA), where LC-MS conditions were optimized for analysis of the vitamins. The binary mobile phase consisted of an aqueous phase of 0.1% formic acid in water (A) and organic phase of methanol (B). The initial flow was composed of 95% of A and 5% of B, and was held for 1.0 min. The gradient was gradually changed to 30% of A and 70% of B over a period of 6 min. then hold for 0.5 min. The system was then returned to 95% A and 5% B for 12 min. Flow rate was maintained at 0.1 ml/min. Simultaneous determination of the six ‘B’ vitamins through LC-MS/MS by MRM method is depicted in Fig. 1.

MS-MS method validation

Multiple reactions monitoring (MRM) detection mode was employed for analysis of ‘B’ vitamins. Details on precursor ions, collision induced product ions and the optimised cone voltage and collision energies for each of the vitamins under ESI^{+ve} mode are presented in Table 1.

MS-MS parameters such as capillary voltage, extractor voltage and RF lens volts were set at 3.2kV, 4V and 0.1V respectively. Nitrogen gas-flow for desolvation and cone were set at 550 and 50 l/h, with desolvation and ion-source temperatures at 350°C and 135°C respectively. Evaluation parameters used for validating the methodology for determination of the vitamins were: linearity, detection, quantification limits and repeatability. Regression line equations, correlation coefficients (r), LOD and LOQ for each vitamin are shown in Table 2. Linearity was determined by constructing calibration curves, and five injections (n= 5) were made at each level, resulting in mean coefficient of variation for the injections of 1.6 to 4.5%. Fresh fruits of tomato were used for validating the method of extraction and estimation of vitamins.

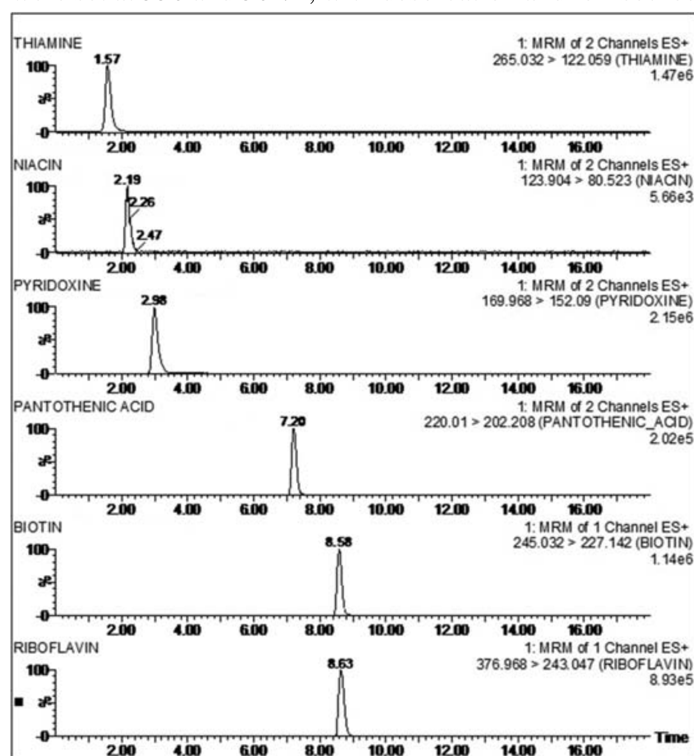

Fig. 1. LC-MS chromatograms of 'B' vitamins

Recovery obtained for all the six 'B' vitamins ranged between 85 and 90%. The recovery studies were carried out by estimating the six 'B' vitamins in the spiked and non-spiked samples. Recovery was calculated from the difference between the spiked and un-spiked samples, and was expressed as percentage.

HPLC analysis of free amino acids

Amino acids, viz., glutamic acid, alanine and valine, which are precursors for biosynthesis of most of the 'B' vitamins, were analyzed using high performance liquid chromatography (HPLC) (Bartolomeo and Maisano, 2006). The chromatograph used was LC-10A system (Shimadzu, Kyoto, Japan) connected to a UV-visible detector (10 A) with binary pump, and controlled by Shimadzu Class VP

Table 1. MRM of 'B' vitamin standards

'B' Vitamins	Formula mass	Parent ion (m/z) $[M+H]^+$	Daughter ions	Cone voltage	Collision energy (CE)	Ionisation mode
Thiamine (B_1)	264	265.03	122.06 (Q)[a]	20	16	ESI+
		265.03	144.02	20	14	ESI+
Riboflavin (B_2)	376	376.97	243.05	40	24	ESI+
Niacin (B_3)	123	123.9	80.523 (Q)[a]	34	20	ESI+
		123.9	77.47	34	18	ESI+
Pantothenic acid (B_5)	219.03	220.01	202.21	28	12	ESI+
		220.01	124.16 (Q)[a]	28	20	ESI+
Pyridoxine (B_6)	169	169.97	152.09 (Q)[a]	24	12	ESI+
		169.97	134.04	24	20	ESI+
Biotin (B_7)	244	245.03	222.14	26	14	ESI+

[a] "Q" taken as quantifier ion

Table 2. Calibration curves, LOD and LOQ for B vitamins

Compound	RT	Standard curve	Correlation coefficient (r)	LOD[a] (ng/µl)	LOQ[b](ng/µl)	Linear range tested (ng/µl)
Thiamine	1.60	Y = 4014X – 3229.6	0.999	0.50	1.53	1.92 - 15.6
Riboflavin	8.62	Y = 4493X + 1.3825	0.998	0.51	1.55	1.0 - 8.0
Niacin	2.15	Y = 212.14X + 27.2	0.997	0.62	1.87	2 - 16
Pantothenate	7.20	Y = 2593X - 66.54	0.999	0.19	0.59	1.6 - 12.8
Pyridoxine	3.02	Y = 47530X + 19652	0.996	0.5	1.50	2.14 - 17.12
Biotin	8.58	Y = 46818X + 1968.2	0.999	0.34	1.02	1.0 - 8.0

[a]LOD= Limit of detection (S/N=3)
[b]LOQ= Limit of quantitation (S/N=10)

Workstation software. The column used was Agilent Eclipse AAA (5µm, 4.6 X 150mm) with the guard column fitted with a C_{18} cartridge (Cat. no. 4287, Phenomenex). The binary mobile phase consisted of 40mM $Na_2H_2PO_4$/ Na_2HPO_4 (1:1) buffer at pH 7.8 (A) and Acetonitrile/ Methanol/Water (45:45:10) (Merck Ltd, India) (B) with flow-rate of 2.0ml/min.

Sample extraction and derivatization

Extraction of amino acids was done as per a previously reported method using Methanol:Chloroform (70:30 v/v) mixture (Marur *et al,* 1994; Pratta *et al,* 2011). Derivitization of amino acids was done using *o*-phthalaldehyde, before injection into the column, using a 20µL loop (Rheodyne, Rohnert Park, CA, USA). The column and guard column were thermostatically controlled at 32°C. The instrument was run in a gradient mode, and detection was monitored on UV absorbance at 338nm wavelength. Retention time for glutamate, alanine and valine were 5.13, 11.28 and 15.17 min respectively. Amino acids were quantified by running their standards under a gradient elution programme. The initial flow was composed of 100% of A, and was held for 1.9 min. The gradient was gradually changed to 43% of A and 57% of B, over a period of 2 min. The system was then changed to 100% B at 24 min; at 27 min, the system was returned to 100% A. The flow rate was maintained at 2.0 ml/min.

Statistical analysis

Analysis of Variance (ANOVA) was carried out for guaging the statistical significance of differences among genotypes. Results were analyzed by Two-way ANOVA (with replications), using Microsoft Excel software. Mean values were compared using least significant difference (LSD) at 1% probability. Mean values were calculated from three independent experiments in all the cases. Pearson correlation coefficient (r) among all the variables was calculated using Microsoft Excel software. Principal Component Analysis (PCA) using StatistiXL software (version 1.8) was made to assess the importance of each source of variation (genotype and ripening stage) in categorizing the results obtained to estimate metabolic changes occurring during fruit ripening.

RESULTS AND DISCUSSION

The B vitamins detected in tomato were: niacin, pyridoxine, pantothenic acid, riboflavin, thiamine and biotin (Table 3). Content of all the B vitamins differed significantly among genotypes and at different stages of fruit maturity, at $P \leq 0.01$.

Among the genotypes tested, highest values for niacin and pyridoxine were recorded at the ripe stage in Arka Vikas (0.350mg kg^{-1} fw) and Arka Rakshak (0.252mg kg^{-1} fw). Lowest pyridoxine content was recorded in the wild species, LA-1777 (0.010 mg kg^{-1} fw). However, pantothenic acid was highest at the ripe stage of the wild species LA-1777 (2.522 mg kg^{-1} fw), followed by IIHR-249-1 (1.295mg kg^{-1} fw); the other lines recorded this value in the range of 0.373 to 0.531mg kg^{-1} fresh weight. Maximum riboflavin content was recorded at the ripe stage in LA-1777 (0.621mg kg^{-1} fw), and was 15.2 times more than in IIHR-249-1 (0.041mg kg^{-1} fw), and 5.6 times more than that in the other seven lines (0.110mg kg^{-1} fw). Riboflavin content in the wild species was found to be 1.9 times more than the USDA reference value for tomato (0.034mg kg^{-1} fw). The highest value for thiamine was also observed at the ripe stage in LA-1777 (0.830mg kg^{-1} fw), followed by 'Arka Ananya' (0.326mg kg^{-1} fw), while, the lowest value was recorded in IIHR-249-1 (0.020mg kg^{-1} fw). The highest total biotin content was found at the ripe stage in IIHR-249-1 (0.564mg kg^{-1} fw), followed by 'Arka Vikas' (0.469mg kg^{-1} fw) and 'Arka Ashish' (0.427mg kg^{-1} fw). The wild species LA-1777 recorded the lowest biotin content (0.195mg kg^{-1} fw).

Some varieties of tomato have been earlier reported to contain a good quantity of pantothenic acid (0.42 to 0.54mg kg^{-1} fw), biotin (0.01 to 0.014mg kg^{-1} fw) and niacin (5.39mg kg^{-1} fw) (James, 1952). Thiamine content of 0.001 to 0.028mg per kg fresh weight was reported in fifteen commonly grown vegetables in southern Thailand (Taungbudhitham, 1995). However, in the present study, the highest value recorded for pyridoxine (0.252mg kg^{-1} fw) and niacin (0.350mg kg^{-1} fw) in tomato at ripe stage was less than the USDA reference value for both [pyridoxine (0.65 mg kg^{-1} fw), niacin (5.9 mg kg^{-1} fw)]. Riboflavin is naturally present in several foods and beverage, such as liver, cheese, milk, meat, eggs, peas, beans, whole-grain cereals, and wines (AMC, 2000; Capo-chichi *et al,* 2000). The variation in B vitamin content among the under study lines may be due to differences in their genetic background. Genotype IIHR-249-1, and the wild species LA-1777 were found to be rich in pantothenic acid, riboflavin, thiamine and biotin. These genotypes were also reported earlier to be rich in Vitamin C, lycopene, phenols and flavonoid with high TSS (Kavitha *et al,* 2013). These lines can be further used for improving vitamin content by introgression of wild species with lines having a good horticultural background.

Among the different stages of fruit ripening, niacin content increased with ripening in the case of IIHR-249-1

Table 3. 'B' vitamins in selected lines of tomato in (fruits harvested at three different stages of ripening, viz., Green stage (GS), Breaker stage (BS) and Ripe stage (RS)

Metabolite/ ripening stage	Niacin			Pantothenic acid			Pyridoxine			Riboflavin			Thiamine			Biotin		
	mg kg^{-1} fresh weight																	
Genotype	GS	BS	RS	GS	BS	RS	GS	BS	RS	GS	BS	RS	GS	BS	RS	GS	BS	RS
IIHR-249-1	0.042	0.112	0.127	0.260	1.005	1.295	0.097	0.223	0.124	0.010	0.041	0.041	0.036	0.104	0.020	0.292	0.450	0.564
	(0.737)	(0.783)	(0.792)	(0.872)	(1.227)	(1.340)	(0.773)	(0.850)	(0.790)	(0.714)	(0.735)	(0.735)	(0.732)	(0.777)	(0.721)	(0.890)	(0.974)	(1.032)
IIHR-2866	0.038	0.000	0.117	0.521	0.442	0.460	0.192	0.220	0.080	0.050	0.041	0.111	0.058	0.051	0.138	0.144	0.652	0.370
	(0.733)	(0.707)	(0.785)	(1.010)	(0.971)	(0.980)	(0.832)	(0.848)	(0.761)	(0.742)	(0.736)	(0.785)	(0.747)	(0.742)	(0.799)	(0.803)	(1.073)	(0.933)
IIHR-2864	0.040	0.206	0.083	0.894	0.631	0.491	0.150	0.218	0.071	0.086	0.115	0.120	0.049	0.067	0.202	0.139	0.538	0.459
	(0.735)	(0.840)	(0.763)	(1.181)	(1.063)	(0.995)	(0.806)	(0.847)	(0.755)	(0.766)	(0.784)	(0.787)	(0.741)	(0.753)	(0.838)	(0.799)	(1.019)	(0.979)
A. Rakshak	0.043	0.057	0.000	0.516	0.524	0.373	0.130	0.185	0.252	0.034	0.093	0.115	0.079	0.023	0.178	0.139	0.477	0.376
	(0.737)	(0.746)	(0.707)	(1.008)	(1.012)	(0.934)	(0.794)	(0.828)	(0.867)	(0.731)	(0.770)	(0.784)	(0.761)	(0.724)	(0.823)	(0.800)	(0.988)	(0.936)
A. Ashish	0.000	0.000	0.067	0.168	0.864	0.416	0.078	0.264	0.125	0.010	0.053	0.123	0.029	0.032	0.262	0.224	0.617	0.427
	(0.707)	(0.707)	(0.753)	(0.817)	(1.168)	(0.957)	(0.760)	(0.874)	(0.791)	(0.714)	(0.744)	(0.790)	(0.727)	(0.729)	(0.873)	(0.851)	(1.057)	(0.963)
A. Ananya	0.062	0.040	0.044	1.058	0.371	0.532	0.181	0.152	0.169	0.026	0.059	0.108	0.278	0.033	0.326	0.042	0.582	0.315
	(0.750)	(0.735)	(0.738)	(1.248)	(0.933)	(1.016)	(0.825)	(0.808)	(0.818)	(0.726)	(0.748)	(0.780)	(0.882)	(0.730)	(0.909)	(0.737)	(1.036)	(0.710)
A. Vikas	0.025	0.073	0.350	0.314	0.905	0.454	0.058	0.180	0.066	0.008	0.034	0.098	0.040	0.031	0.185	0.057	0.221	0.469
	(0.724)	(0.757)	(0.922)	(0.902)	(1.186)	(0.977)	(0.747)	(0.825)	(0.752)	(0.713)	(0.731)	(0.773)	(0.735)	(0.729)	(0.828)	(0.746)	(0.849)	(0.984)
A. Samrat	0.054	0.061	0.045	0.854	0.364	0.473	0.138	0.182	0.100	0.025	0.098	0.113	0.009	0.037	0.168	0.031	0.435	0.391
	(0.745)	(0.749)	(0.738)	(1.164)	(0.929)	(0.986)	(0.799)	(0.826)	(0.775)	(0.724)	(0.773)	(0.783)	(0.714)	(0.733)	(0.817)	(0.729)	(0.967)	(0.944)
LA-1777	0.027	0.000	0.193	2.208	0.000	2.522	0.014	0.000	0.010	0.110	0.000	0.621	0.079	0.000	0.830	0.143	0.000	0.195
	(0.726)	(0.707)	(0.833)	(1.646)	(0.707)	(1.738)	(0.717)	(0.707)	(0.714)	(0.781)	(0.707)	(1.059)	(0.761)	(0.707)	(1.153)	(0.802)	(0.707)	(0.834)
Mean	0.036	0.061	0.114	0.755	0.567	0.780	0.115	0.180	0.111	0.040	0.059	0.161	0.073	0.042	0.257	0.130	0.377	0.362
CD ($P \leq 0.01$)																		
Genotype (G)		0.0095			0.0231			0.0061			0.0033			0.0057			0.0232	
Stage (S)		0.0055			0.0133			0.0035			0.0019			0.0032			0.0134	
G x S		0.0164			0.0400			0.0106			0.0056			0.010			0.0402	

*Values in parentheses are square root transformed

(0.042 to 0.127mg kg^{-1} fw), IIHR-2866 (0.038 to 0.117mg kg^{-1} fw), 'Arka Vikas' (0.025 to 0.350mg kg^{-1} fw) and LA-1777 (0.027 to 0.193mgkg^{-1} fw); but, it was higher in the breaker stage, and decreased with ripening in the other lines. Among the different stages of ripening, pyridoxine content was high in the breaker stage in all the lines studied, except 'Arka Rakshak' which recorded highest pyridoxine content at the ripe stage (0.252mg kg^{-1} fw). Pantothenic acid content increased with ripening in the line IIHR-249-1 (0.260 to 1.295mg kg^{-1} fw), and LA-1777 (2.208 to 2.522mg kg^{-1} fw), whereas, it was higher at the green stage, and decreased with ripening in cherry tomato lines IIHR-2866 (0.521 to 0.460mg kg^{-1} fw) and IIHR-2864 (0.894 to 0.491mg kg^{-1} fw). A similar trend was observed in IIHR hybrids 'Arka Ananya' (1.058 to 0.532mg kg^{-1} fw), 'Arka Rakshak' (0.516 to 0.373mg kg^{-1} fw) and 'Arka Samrat' (0.854 to 0.473mg kg^{-1} fw). In the case of varieties, it was high in the breaker stage, decreasing thereafter. Among the different stages analyzed, riboflavin increased with ripening in all the lines, while thiamine content increased with ripening in all the lines except IIHR-249-1 (where it decreased from green stage to ripe stage) (0.036 to 0.020mg kg^{-1} fw). Biotin content increased with ripening in IIHR-249-1 (0.292 to 0.564mg kg^{-1} fw) and in other lines; whereas, it decreased from the breaker to ripe stage in IIHR-2866 (0.652 to 0.370mgkg^{-1} fw), 'Arka Rakshak' (0.477 to 0.376mg kg^{-1} fw), 'Arka Samrat' (0.435 to 0.391mg kg^{-1} fw) and 'Arka Ashish' (0.617 to 0.427mg kg^{-1} fw).

In general, 'B' vitamin content in the ripe fruit was considerably higher than in the unripe fruit, which may be related to higher availability of carbohydrate precursors during fruit ripening (Carrari and Fernie, 2006). However, very few reports are available on accumulation of vitamins at different stages of fruit maturity. An increase in Vitamin C content as pepper fruits mature has been reported earlier (Osuna-Garcia *et al,* 1998; Bae *et al*, 2014).

Relation between 'B' vitamins and their amino acid precursors

Glutamate, alanine and valine precursors for biosynthesis of some of the 'B' vitamins were analyzed at different stages of fruit ripening. Highest glutamate content was recorded at the ripe stage in 'Arka Ashish' (417.86mg kg^{-1} fw), and lowest in 'Arka Samrat' (221.67mg kg^{-1} fw). There was an increase in accumulation of glutamate from the green to the ripe stage in most of the genotypes. Higher value for alanine was recorded at the ripe stage in LA-1777 (1505.4 mg kg^{-1} fw), which was about 5 times more than in the elite germplasm line IIHR-249-1 (317.05mg kg^{-1} fw). Alanine content increased with ripening in almost all the genotypes. Among the genotypes, highest valine content was recorded at the ripe stage in the wild species LA-1777 (159.02mg kg^{-1} fw), and the lowest in the hybrid 'Arka Samrat' (5.24mg kg^{-1} fw). Accumulation of valine was higher in the breaker stage compared to that in ripe or green stages (Fig. 2).

Correlation coefficients run between 'B' vitamins and the three precursor amino acids indicated that alanine and valine were strongly correlated to pantothenic acid ($r = 0.97$, $r = 0.91$ respectively, $P \leq 0.01$), whereas, alanine did not show any significant relationship with biotin. Glutamate showed significant correlation with pyridoxine ($r = 0.86$, $P \leq 0.01$). However, it did not show any significant relationship with niacin. In the present study, the higher levels of pantothenic acid and pyridoxine observed may be directly related to higher supply of precursor amino acids valine, alanine and glutamate.

As previously reported, glutamate is the principal free-amino-acid in ripe fruits of cultivated varieties of tomato, and free amino acids increase dramatically during fruit ripening, with their abundance changing differentially (Sorrequieta *et al*, 2010). Total amino acid content at red ripe stage was higher than in the mature green stage in tomato germplasm lines, and their relative content increased from mature-green to ripe stage (Forde and Lea, 2007; Pratta *et al,* 2011). Significant increase in glutamic acid and reduced levels of alanine and valine, throughout maturation and ripening was reported in various lines of tomato (Omas-Oliu *et al,* 2011). Increased glutamate content towards the end of ripening is also reported in tomato, which could be due to a cessation of chlorophyll biosynthesis, since, glutamate is also a precursor of chlorophyll (Carrari and Fernie, 2006).

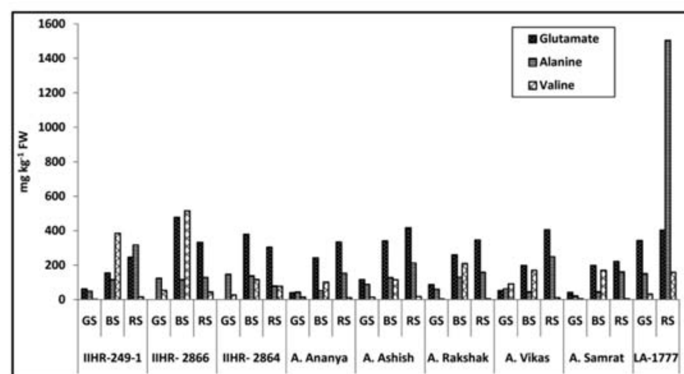

Fig. 2. Glutamate, alanine and valine in various genotypes of tomato at different stages of fruit maturity (GS: Green Stage, BS: Breaker Stage, RS: Ripe Stage)

Principal component analysis (PCA) for distinguishing genotypes and stages of fruit maturity

Principal Component Analysis (PCA) was done to understand the pattern of accumulation of 'B' vitamins at different stages of fruit maturity. As the selected genotypes were of different genetic backgrounds, PCA allowed us to understand the relation between distribution of 'B' vitamins across genotypes, and at different stages of fruit maturity.

Data on vitamins at the ripe stage in all the genotypes were subjected to PCA. PC 1 and PC 2 contributed to the wide variability of 97.4% among genotypes (Fig. 3). A biplot of PC1 and PC 2 revealed that the wild species LA-1777, and the germplasm line IIHR-249-1 were completely different from the other genotypes (hybrids, varieties or cherry tomato lines). LA-1777 was distinct from IIHR-249-1, but these two genotypes were characterized by high levels of niacin, thiamine, riboflavin and pantothenic acid. All the other genotypes grouped along with PC2 were found to contain higher levels biotin and pyridoxine, but lower levels of the other 'B' vitamins.

A second PCA was done to study accumulation of 'B' vitamins at different stages of fruit maturity. Out of six principal components (PC's), two, viz., PC 1 and PC 2, accounted for 87.8% variability among the different stages of fruit maturity (Fig. 4). In a biplot of PC 1 and PC 2, the three stages in the genotypes appeared as separate groups. The ripe stage of the genotypes was characterized by higher amounts of 'B' vitamins, particularly biotin, thiamine and riboflavin. Breaker stage was found to possess the highest amount of niacin, pyridoxine and pantothenic acid, while, the green stage was found to be associated with low levels of all the 'B' vitamins studied. A wide variability was observed between the green and the breaker stage than in the ripe stage in all the genotypes studied, indicating that biosynthesis of 'B' vitamins was primarily ripening-regulated.

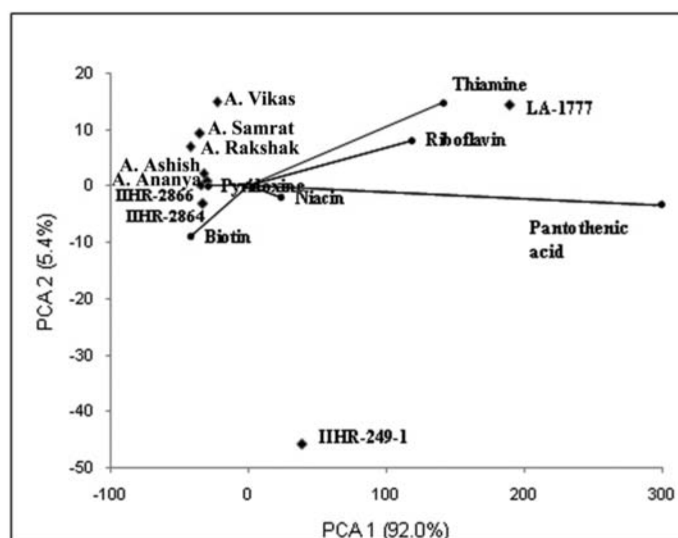

Fig. 3. Distribution of 'B' vitamins (circles) and tomato genotypes (rhombus) at ripe stage in the coordinates of Principal Components 1 and 2 (PC1 and PC2, respectively)

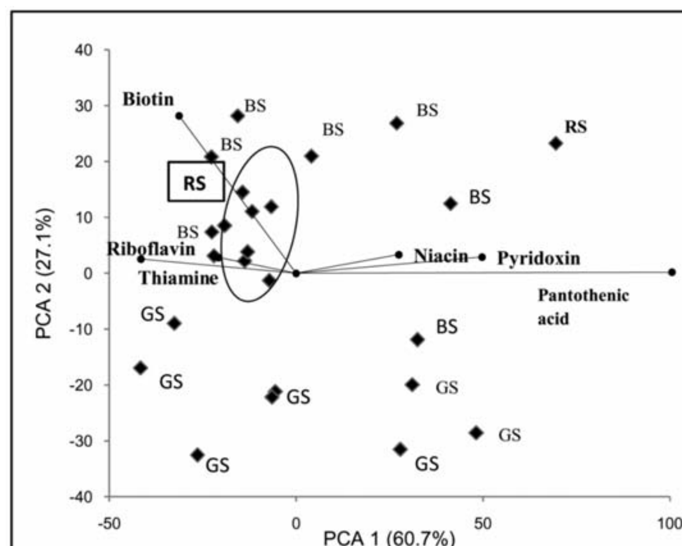

Fig. 4. Distribution of B vitamins (circles) at Green Stage (GS), Breaker Stage (BS) and Ripe Stage (RS) in the coordinates of Principal Components 1 and 2 (PC1 and PC2, respectively)

CONCLUSION

LCMS-MRM as a technique has proved to be sensitive, selective and a reliable method for individual determination of six 'B' vitamins in tomato. The elite germplasm line IIHR-249-1, and the wild species LA-1777 with high pantothenic acid, riboflavin, thiamine and biotin content; the hybrid 'Arka Rakshak', with fairly high pyridoxine serve as a good source material for improving nutritive value of the tomato for use in crop breeding or biotechnological approaches. Higher levels of alanine and valine may be indicative of higher accumulation of pantothenic acid in tomato. Ripe stage was found to be a rich source of vitamins. However, in some genotypes, niacin, pyridoxine and biotin levels remained nearly the same at breaker and ripe stages.

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Changes in fruit quality and carotenoid profile in tomato (*Solanum lycopersicon* L.) genotypes under elevated temperature

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ABSTRACT

Tomato (*Solanum lycopersicon* L.) is a rich source of carotenoids, especially lycopene, and is affected severely by high temperatures under tropical conditions. To study the effect of elevated temperature on lycopene content and other quality parameters, five tomato genotypes, viz., RF4A, Abhinava, Arka Saurabh, IIHR 2195 and Arka Vikas, were grown in a temperature gradient tunnel (TGT) facility under 33.4 and 35.4ºC temperature conditions. Fruits were analyzed for total carotenoids, total phenols, total flavonoids, total sugars, TSS, acidity, Vitamin C besides carotenoids profile (β-carotene, lycopene, phytoene and luteoxanthin content). Results revealed that all the quality parameters studied were superior at 33.4ºC, compared to 35.4ºC in all the genotypes. 'IIHR 2195' recorded highest total phenols (479.28mg/100g dw), total flavonoids (70.27mg/100g dw), ferric reducing antioxidant potential (FRAP) (310.53mg/100g dw), diphenyl picryl hydrazyl (DPPH) radical (487.89mg/100g dw), Vitamin C content (292.25mg/ 100g dw) and total sugars (606.88mg/g dw) at 33.4ºC and at 35.4ºC. 'RF4A' and 'Arka Vikas' were found to have better total carotenoids content and lycopene at higher temperature than other genotypes. 'Arka Vikas' recorded highest total soluble solids (TSS) (8.9ºBrix) and acidity (0.80%) at 35.4ºC. Higher TSS and acidity were recorded at 35.4ºC than at 33.4ºC in all the five genotypes. Genotypic variation was observed in the above stated biochemical parameters in response to elevated temperatures.

Key words: Tomato, TGT, antioxidants, elevated temperature, UPLC

INTRODUCTION

Global warming is an important issue threatening most horticultural crops, and can lead to serious consequences in food production. Tomato, being sensitive to temperature, is likely to be influenced by elevated temperatures under a climate change scenario (Laxman *et al,* 2013). Increase in temperature under climate-change circumstances affects crop yield, in turn affecting sustained supply for meeting a growing demand.

Tomato, an important horticultural crop in India, is currently the second largest vegetable in terms of production. It is one of the most consumed vegetables in the world. Tomatoes are rich in bioactive compounds, including carotenoids (lycopene, β-carotene, phytoene and luteoxanthin), ascorbic acid, flavonoids and phenolic compounds (Kaur *et al,* 2013). Along with phenols, higher intake of flavonoids, Vitamin C and carotenoids has been reported to reduce the risk of many degenerative diseases (Agarwal and Rao, 2000).

Optimal mean daily temperatures for tomato lie between 21 and 24°C, depending on the developmental stage (Geisenberg and Stewart, 1986). Supra-optimal temperatures cause a series of complex morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity (Wang *et al*, 2003). Temperature has a significant influence on many aspects of growth and development in tomato. Temperature below 16ºC can cause flower abscission, while temperature above 30ºC can cause fruit cracking and blotchy ripening (Islam, 2011). Impact of high temperature on the plant is not limited to flowering and fruit-set, but also subsequent development and maturity of the fruit, and fruit quality. Lee and Kader (2000) reported higher Vitamin C content in tomato grown under low temperatures than that under high temperature. High temperature also affects biosynthesis of carotenoids,

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especially lycopene (Kaur *et al,* 2013). Environmental factors other than temperature, like, plant nutrition and light, can also considerably affect biosynthesis of carotenoids. Phenolic acids and flavonols are reported to increase under high temperature conditions in strawberry (Wang and Zheng, 2001). Although sufficient literature is available on fruit quality parameters in different tomato genotypes, studies on varietal response to elevated temperature in terms of fruit quality are scanty and this information is essential to identify varieties suited to a changing climate. Therefore, the present experiment was set in a temperature gradient tunnel to study the effect of temperature on fruit quality parameters and carotenoid profile in five tomato genotypes.

MATERIAL AND METHODS

The experiment was carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru, in a temperature gradient tunnel during the months of October 2011 to February 2012. Bengaluru is located at 13º58' N latitude, 78ºE longitude and 890m above mean sea level. Five genotypes of tomato (*Solanum lycopersicon* L.), viz., RF4A, Abhinava, Arka Saurabh, IIHR 2195 and Arka Vikas, were selected for the study. Twenty-five day old seedlings were transplanted into 20 litre capacity plastic containers filled with soil, FYM and sand, in the ratio of 2:1:1. Temperature gradient tunnel (TGT) measuring 18m length, 4.5m width and 3m height, covered with a polycarbonate sheet was used in the study. One week after transplanting, the containers were shifted to TGT for imposition of temperature treatments. One set comprising six plants each of the five genotypes was placed near the cooling pad and another set with the same number of plants was placed towards the fan (where the average air temperature was about 2°C higher than at the cooling-pad end). Daily temperatures and relative humidity (RH) during fruit growth period recorded inside TGT are shown in Fig. 1. The gradient inside TGT was maintained only during daytime, as TGT worked on the pad-and-fan system. Since there was no

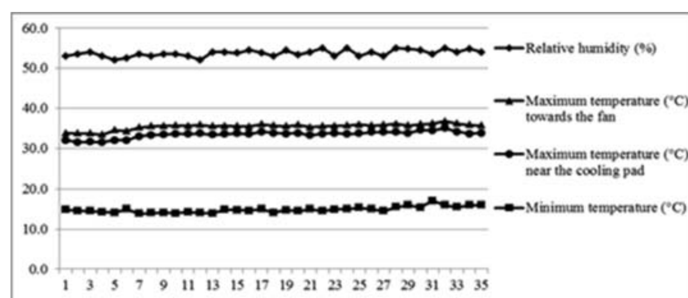

Fig. 1. Daily maximum/minimum temperature (°C) and relative humidity (%) during the last 35 days of fruiting season

gradient in the night-time minimum temperature, only one value for temperature is indicated (Fig. 1). Photosynthetically active radiation (PAR) inside the TGT was about 85% of that of the light outside. The plants were provided with recommended dose of fertilizer, and suitable crop protection measures were applied when required.

Freshly-harvested, fully ripe fruits were used for analysis. Fruits were crushed in a blender and a known quantity of the homogeneous mass was set apart for analysis. Quality parameters like TSS, % acidity, Vitamin C content, total phenols, total flavonoids, FRAP, DPPH, total carotenoids and total sugars were analyzed.

Total soluble solids (TSS) were recorded using a digital refractometer (ARKO India Ltd., Model DG-NXT) and expressed in ºBrix. Acidity was determined by the titration method (AOAC, 942.15) using phenolphthalein as the indicator. Acidity was expressed in per cent citric acid equivalent. Vitamin C content was determined using 2,6-dichlorophenol indophenol (DCPIP) method (AOAC, 967.21) and calculated as mg ascorbic acid equivalent per 100g dry weight. Total phenols present in 80% methanol extract were estimated by Folin-ciocalteu method (Singleton and Rossi, 1965). Methanol extract was mixed with FCR reagent and the color developed with 20% sodium carbonate reagent. Intensity of color developed was read at 700nm using a spectrophotometer (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd., UK). Results were expressed in mg gallic acid equivalent per 100g dry weight. Total flavonoids content was estimated as per Chun *et al* (2003). Flavonoids present in the 80% methanol extract were estimated using 5% $NaNO_2$ and 10% $AlCl_3$. Absorbance of the pink mixture was read at 510nm and expressed as mg catechin equivalent per 100g dry weight. Antioxidant capacity was measured as FRAP, using a modified method of Benzie and Strain (1996). Methanol extract (0.2ml) was mixed with 1.8ml FRAP reagent. Intensity of the blue colour that developed was measured at 593nm. Total antioxidant capacity (as ferric reducing antioxidant potential) was calculated and the antioxidant capacity was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) per 100g dry weight. Radical-scavenging ability was measured using DPPH radical assay of Kang and Saltveit (2002). A 0.2ml aliquot of methanol extract was mixed with 0.3ml of 10mM acetate buffer (pH 5.5) and 2.5ml methanolic 0.2mM DPPH solution. Reduction in color due to scavenging of DPPH radicals by antioxidants was estimated by reading the absorbance at 517nm. Radical-scavenging ability was expressed as weight of the sample required for 50%

reduction in DPPH radicals. Total sugars present in the 80% ethanol extract were estimated using dinitrosalicylic acid method (Miller, 1959). A 0.2ml aliquot of extract was mixed with DNS reagent and the absorbance read at 540nm, expressed as mg glucose equivalent per gram dry weight using a standard curve. Total carotenoids and lycopene content were analyzed by spectrophotometric method (Lichtenthaler, 1987). Carotenoids were estimated by extracting with acetone, partitioned to hexane, and their absorbance read at 470 and 503nm. Standards were used for calibration, and results were expressed as mg per 100g dry weight.

Carotenoid profile by UPLC

Carotenoid profile was estimated by UPLC as per Serino *et al* (2009) with minor modifications. Acquity-UPLC system from Waters (Milford, MA, USA) consisting of a quaternary pump, auto sampler injector and PDA detector equipped with Acquity-UPLC BEH-C18 column (1.7µm, 2.1X50mm) with BEH-C18 (1.7µm, 2.1X5mm) guard column was used. Instrument control and data processing were done using Mass Lynx software. The mobile phase consisted of phase-A acetonitrile:methanol:ethyl acetate (53:7:40) and phase-B methanol. Isocratic flow rate of 0.2ml/min was used in the ratio of A:B (95:5) for 6 min with PDA scanning from 200 to 650nm. Individual carotenoids were identified by diode array spectral characteristics, retention time and relative elution order compared to standards and values in literature. Carotenoids were quantified as β-carotene equivalents. A known quantity (1ml) of hexane extract was evaporated to dryness, and residual carotenoids were dissolved in the mobile phase and filtered through 0.2µm nylon filter prior to ion injecting in UPLC for further analysis. The detection was monitored at 450nm for β-carotene, 470nm for lycopene, 286nm for phytoene and 420nm for luteoxanthin.

Statistical analysis

Data were subjected to Analysis of Variance using ANOVA, and, means were compared, with critical difference at $P \leq 0.05$.

RESULTS AND DISCUSSION

Changes in fruit quality parameters in five tomato genotypes at two temperature conditions are presented in Table 1. TSS increased with increase in temperature in all the genotypes (5.6 to 7.2ºBrix) and ranged from 3.8 to 7.1ºBrix at 33.4ºC, and at 35.4ºC, it ranged from 4.5 to 8.9ºBrix. Similar trend was observed in per cent acidity too. Acidity ranged from 0.34 to 0.68% at 33.4ºC, whereas, at 35.4ºC, the acidity ranged from 0.39 to 0.80%. Sugars and acids are important components in tomato fruit flavor (George *et al*, 2004; Kaur *et al*, 2013). Increase in titratable acidity with increase in temperature has been reported (Khanal, 2012). Among genotypes, Arka Vikas recorded the highest TSS (8.9ºBrix) and acidity (0.80%) at 35.4ºC. Higher temperature is known to enhance soluble solids level in relation to ambient temperature conditions (Gunawardhana and De Silva, 2011; Khanal, 2012).

Table 1. Changes in fruit quality parameters of five tomato genotypes at two temperature conditions

Genotype	Mean temperature during fruiting stage	TSS (ºBrix)	Acidity (%)	Vitamin C (mg/100g dw)	Total phenols (mg/100g dw)	Total flavonoids (mg/100g dw)	FRAP (mg/100g dw)	DPPH (mg/100g dw)	Total carotenoids (mg/100g dw)	Total sugars (mg/g dw)
RF4A	33.4ºC	5.9	0.53	265.68	344.51	44.63	209.34	321.56	270.36	375.91
	35.4ºC	7.1	0.66	272.49	378.99	45.06	160.37	306.32	191.97	366.21
Abhinava	33.4ºC	6.1	0.46	272.06	361.73	42.83	202.06	310.16	228.98	423.03
	35.4ºC	8.6	0.66	263.70	377.48	43.21	168.35	293.08	136.14	221.03
Arka Saurabh	33.4ºC	3.8	0.34	225.94	336.88	33.03	198.39	315.28	269.14	403.99
	35.4ºC	4.5	0.39	258.59	419.68	45.52	191.93	318.73	176.74	264.38
IIHR 2195	33.4ºC	4.9	0.46	292.25	479.28	70.27	310.53	487.89	205.13	606.88
	35.4ºC	6.8	0.64	271.63	461.61	66.88	231.92	415.20	155.45	379.79
Arka Vikas	33.4ºC	7.1	0.68	226.19	352.64	49.66	208.72	343.55	197.33	347.23
	35.4ºC	8.9	0.80	212.87	436.62	64.40	192.90	377.65	158.24	254.68
Mean	33.4ºC	5.6	0.49	256.42	375.01	48.08	225.81	355.69	234.19	431.41
	35.4ºC	7.2	0.63	255.86	414.88	53.01	189.09	342.20	163.71	297.22
CD for varieties (V) ($P \leq 0.05$)		0.02	0.01	1.98	1.67	0.82	1.36	1.40	0.81	1.86
CD for temperature (T) ($P \leq 0.05$)		0.01	0.01	NS	0.67	0.33	0.55	0.56	0.32	0.74
CD for V x T ($P \leq 0.05$)		0.05	0.03	3.96	3.34	1.64	2.73	2.80	1.62	3.72

NS=Non-Significant

Vitamin C content did not show significant differences among the two temperature treatments. However, among genotypes, IIHR 2195 and RF4A recorded higher Vitamin C content at 33.4ºC and 35.4ºC respectively compared to other genotypes. Total phenols and flavonoids increased with increase in temperature in all the genotypes (375.01 to 414.88 and 48.08 to 53.01 mg/100g dw for total phenols and total flavonoids respectively). Higher total phenols and flavonoids were observed in cv. IIHR 2195. Phenolic substances are reported to have a protective effect on ascorbic acid (Toor and Savage, 2006). Therefore, presence of phenolics and flavonoids in tomato cells may have helped maintain ascorbic acid level. Ferreyra *et al* (2007) also reported ascorbic acid level to be not significantly affected by temperature during the growth season. Wang and Zheng (2001) found elevated growth temperature of 30ºC to significantly enhance the content of phenolic acids and flavonols in strawberry. Accumulation of phenolics at higher growth temperature has been reported in other crops too (Wang, 2006; Toor *et al*, 2006).

Total antioxidant capacity and radical-scavenging ability were assessed using FRAP and DPPH methods respectively. All the genotypes recorded significantly higher FRAP and DPPH at 33.4ºC. Among genotypes, higher FRAP and DPPH values were recorded in IIHR 2195 at both 33.4ºC and 35.4ºC. 'RF4A' and 'Abhinava' recorded lower FRAP values at 35.4ºC compared to other genotypes. 'Abhinava' recorded lower DPPH values at both 33.4ºC and 35.4ºC.

All the genotypes recorded higher total sugars at 33.4ºC than at 35.4ºC. IIHR 2195 recorded the highest total sugars (606.88mg/g dw) at 33.4ºC. Temperature changes are known to affect fruit maturation and growth through influencing regulation of the enzymes acid invertase and sucrose synthase or cell-expansion and division and regulation of sugar transport into the fruit (Fleisher *et al*, 2006). Gautier *et al* (2005) reported decrease in sugar content in cherry tomato when fruit temperatures increased. Sugar content in purple passionfruit juice was highest at low growth temperature, and lowest at high growth temperature. More sucrose accumulated at low temperature, while glucose and fructose content increased at higher temperature (Utsunomiya, 1992). All these studies support our observations in tomato.

In the present study, all the genotypes studied recorded higher total carotenoids and lycopene content at 33.4ºC than at 35.4ºC. Carotenoid profiles indicated that β-carotene, lycopene, phytoene and luteoxanthin content was greater at 33.4ºC in all genotypes. Temperature had a significant influence on total carotenoids and lycopene content. High temperature may lead to degradation of lycopene (Demiray *et al,* 2013), in addition to a reduced synthesis (Helyes *et al,* 2007). Temperatures greater than 30ºC lead to inhibition of lycopene synthesis in normal red cultivars of tomato and synthesis is restored when the temperature drops below 30ºC. These temperature effects vary with the tomato cultivar (Garcia and Barrett, 2006). Temperatures below 12ºC strongly inhibit lycopene biosynthesis, while temperatures over 32ºC stop this process altogether (Dumas *et al*, 2003). Temperature during fruit ripening plays a more important role in lycopene biosynthesis than it does during fruit growth period. Fig. 2 shows chromatograms of tomato carotenoids at 33.4ºC and 35.4ºC. All the carotenoid pigments under study diminished at 35.4ºC in all five genotypes (Table 2). Greatest reduction was observed in two major pigments, lycopene and phytoene. However, reduction was lower in 'RF4A' and 'Arka Vikas'. Higher reduction was noticed in 'Abhinava'.

In conclusion, Changes in fruit quality parameters in five tomato genotypes under elevated temperature were studied under TGT. Variations were observed among tomato genotypes for fruit quality parameters at elevated temperature. Increase in temperature improved TSS and per cent acidity, but decreased total carotenoids, lycopene

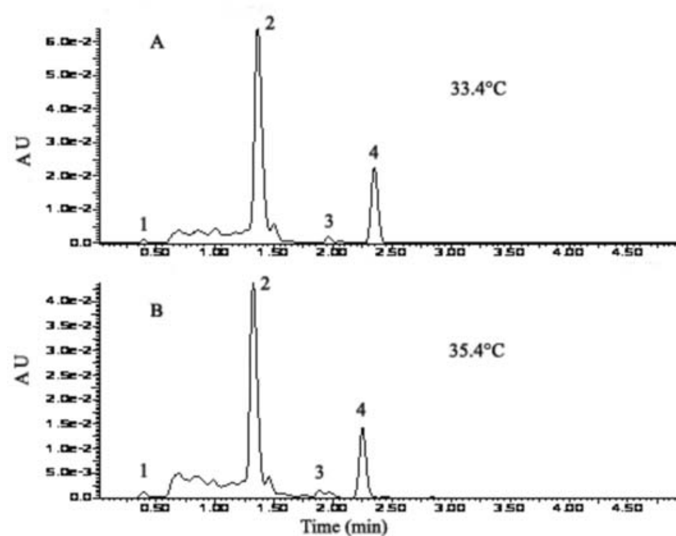

Fig. 2. UPLC chromatograms of carotenoids in tomato fruit extract. Chromatogram A represents carotenoid profiling at 33.4°C (maximum temperature near the cooling pad) and chromatogram B represents carotenoid profiling at 35.4°C (maximum temperature towards the fan). Peaks identified are: (1) Luteoxanthin, (2) Lycopene, (3) β-Carotene and (4) Phytoene

Table 2. UPLC data on phytoene, lycopene, β-carotene and luteoxanthin in five tomato genotypes at two different temperature conditions

Genotype	Phytoene (mg/100g dw)			Lycopene (mg/100g dw)			β-Carotene (mg/100g dw)			Luteoxanthin (mg/100g dw)		
	33.4°C	35.4°C	% increase/ decrease over 33.4°C	33.4°C	35.4°C	% increase/ decrease over 33.4°C	33.4°C	35.4°C	% increase/ decrease over 33.4°C	33.4°C	35.4°C	% increase/ decrease over 33.4°C
RF4A	29.20	27.05	-7.35	174.38	130.43	-25.20	11.38	5.95	-47.70	4.48	4.94	10.36
Abhinava	67.02	35.10	-47.64	150.65	88.91	-40.98	8.11	6.20	-23.52	3.88	2.17	-44.16
Arka Saurabh	29.74	17.67	-40.58	175.54	121.34	-30.87	5.74	3.67	-36.09	5.01	2.95	-41.20
IIHR 2195	36.67	19.25	-47.49	131.46	88.38	-32.77	7.69	3.69	-52.06	4.03	2.23	-44.57
Arka Vikas	30.08	17.64	-41.37	146.46	122.61	-16.29	4.39	5.22	18.67	3.43	1.61	-53.19
Mean	38.54	23.34		170.78	122.65		7.46	4.94		4.17	2.78	
CD for varieties (V) ($P \leq 0.05$)	0.40			2.68			0.46			0.13		
CD for temperature (T) ($P \leq 0.05$)	0.16			1.07			0.18			0.05		
CD for V x T ($P \leq 0.05$)	0.80			5.36			0.92			0.27		

and total sugars in all the genotypes studied. Among the genotypes, IIHR 2195 was found to be better in terms of total phenols, total flavonoids, FRAP, DPPH and total sugars at 33.4°C, as also at 35.4°C. ‘Arka Vikas’ was found to be high in TSS and per cent acidity at 33.4°C. RF4A and Arka Vikas were found to be good at maintaining lycopene level at elevated temperature, compared to the other genotypes. Genotype IIHR 2195 is recommended for cultivation at elevated temperatures.

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Direct nutrient-feeding to 'Ney Poovan' banana (*Musa* sp. AB) bunch under organic or conventional farming for yield, fruit quality and profitability

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ABSTRACT

Three types of direct nutrient-feeding methods [applying 500g fresh cow-dung and 100ml water enriched with (i) 2.5g each of urea + SOP; (ii) 100ml of *panchangavya*; and (iii) 100ml of cow urine] were evaluated in 'Ney Poovan' banana grown under organic or conventional farming. Plants grown under conventional farming were more robust in girth and length of their pseudostem and number of leaves retained on the plant at harvest. Conventional farming produced 62.6% and 59.0% higher fruit and bunch weight than plants grown under organic farming. Quality-wise, fruits from organic farming were superior in pulp:peel ratio and pulp total soluble solids (TSS). Conventional farming significantly increased P, S, Fe, Mn and Zn content of the pulp over organic farming. Benefit:cost ratio was significantly higher at 3.61 under conventional farming, while, it was 2.15 under organic farming. All the methods of direct nutrient-feeding of banana bunch tested were significantly superior to 'Control' where the male bud was retained on the bunch until harvest. Increase in fruit and bunch weight was in the following order of blend: urea + SOP > *panchangavya* > cow urine, with fresh cow dung. Improvement in pulp:peel ratio and benefit:cost ratio was maximum when direct nutrient-feeding was done using cow-dung blended with urea + SOP, while, TSS of the pulp declined to 24.0°B from 25.1°B when *pancahgavya* was used. Results indicated that conventional farming with adequate organic manuring, and, adopting direct nutrient-feeding of the banana bunch using cow-dung slurry enriched with 2.5g each of urea and SOP, achieved high bunch yield, pulp:peel ratio, and was profitable.

Key words: Bunch size, direct nutrient-feeding, 'Ney Poovan' banana, *Musa* sp. AB, organic farming, conventional farming, fruit quality, benefit:cost ratio

INTRODUCTION

Development of uniform-sized fingers in a banana bunch is important for meeting market demands. In all varieties of banana, the lower hands invariably develop poorly, reducing the bunch weight, yield and marketability. Despite nutrient supplementation through soil/ foliage, the phenomenon persists. Direct nutrient-feeding of the bunch has succeeded in overcoming this shortcoming (Venkatarayappa *et al,* 1976; Prasanna Kumari Amma *et al,* 1986; Ancy *et al,* 1998). However, the technique suffered from blackening and rotting of fruits when fed with urea at >50g (Ancy and Kurien, 2000), and, was therefore not accepted by growers. However, in 'Robusta' (*Musa* sp. AAA, Kotur and Keshava Murthy, 2008), and in 'Ney Poovan' banana (Kotur and Keshava Murthy, 2010), this technique was refined using enriched cow-dung slurry. This needed lesser quantity of urea and was augmented with sulphate of potash (SOP). Currently, the technology is widely accepted across the country. In view of the increasing market-demand for organically grown banana, it is timely to compare direct nutrient-feeding technique in banana raised under organically *vis-a vis* conventionally grown 'Ney Poovan' banana.

MATERIAL AND METHODS

'Ney Poovan' banana (*Musa* sp. AB) was grown on red sandy-loam (alfisol) in the farm of Shri H.Y. Ramaiah of Udarahalli village, Ramanagar district, Karnataka, India. The soil was maintained organically by repeated green-manuring with horse gram and sun hemp. Tissue culture grown plants of banana were planted at 1.8×1.8m spacing, with each pit receiving 10kg FYM and 500g *neem* cake. *Panchagvya* was prepared by mixing 500g of *ghee* (clarified butter), 5kg fresh cow-dung and 1kg black *gur* (jiggery/ molasses) in five litres of water. The blend was

stirred daily for five days and supplemented with five litres of cow-urine, 2 litres of sour curd, 2 litres of milk and tender coconut water of one nut. The slurry was stirred thrice daily and cured for a month. Each plant received 1 litre of dilute *panchagavya* (1:40 *Panchagavya*:water) applied at fortnightly intervals. The experiment was laid out in split-plot design, with two main treatments: (i) organic farming and (ii) conventional farming. In the latter treatment, N:P:K dose of 110:25:250g/plant was applied (in four equal splits of N and K, and two splits of P, along with 1st and 2nd split applications made at 50,100,150 days after planting, and at shooting). There were four sub-treatments: (i) 'Control', in which the male bud was retained on the bunch until harvest; (ii) direct feeding of the bunch with nutrients using 500g fresh cow-dung and 100ml cow-urine; (iii) direct feeding of nutrients with 500g fresh cow-dung and 100ml *panchagavya*; and, (iv) direct feeding of nutrients with 2.5g each of urea (blended with 100ml water) and 500g fresh cow-dung. There were six replications of one plant each, forming a treatment unit. Direct nutrient-feeding was done by de-navelling the bunch after 15-20 bracts/spathes dropped off in the male bud, leaving a distal rachis of about 15cm length beneath the youngest hand of the bunch. At the time of harvest (about 100 days after flowering), girth of the pseudostem at the base, height of the pseudostem, and number of green leaves, were recorded as a measure of plant vigour. Pulp:peel ratio and total soluble solids in the pulp (TSS, determined using a refractometer) were recorded in uniformly edible-ripe fruits. Pulp samples drawn at quality-determination were sliced, dried in an oven at 70°C, and powdered for chemical analysis using standard procedures. Soil samples were drawn at harvest from the top 22.5cm length and analyzed for chemical properties using standard procedures (Table 1). For calculating benefit:cost ratio, all the costs (including fixed and variable costs) were taken into account (which amounted to Rs. 72.50/plant under organic farming and Rs. 84.50 under conventional farming) in banana. The prevailing wholesale price was Rs. 26.25/kg of fruit.

RESULTS AND DISCUSSION

Yield, fruit quality and profitability

Conventional farming produced distinctly robust banana plants compared to organic farming. Diameter at the base, and height of the pseudostem, as also number of green leaves present at harvest, were higher (22.2 ± 1.52cm; 345.0 ± 28.42cm and 10.8 ± 1.04 leaves, respectively). Corresponding values under organic farming were lower: 19.8 ± 0.72cm; 298.0 ± 18.91cm and 7.5 ± 1.16 leaves, respectively. As a result, fruit and bunch weight were significantly higher under conventional farming (by 62.6% fruit weight and 59.0% bunch weight) (Table 2). Quality-

Table 1. Composition of soil, cow dung, urine, *panchagavya* and their contribution in direct nutrient feeding of 'Ney Poovan' banana bunch

Property*	Soil properties		Cow dung	Cow urine	*Panchagavya*
	Organic farming	Conventional farming			
Moisture (%)	-	-	22.0	95.5	82.5
pH	7.32	7.15	5.8	5.7	5.2
Organic carbon (%)	0.65	0.45	-	-	-
Cation exchange capacity	13.5	12.9	-	-	-
Nitrogen	348	215	1.50	3.11	2.51
Phosphorus	30	28	0.089	0.076	0.058
Potassium	84	86	1.10	0.32	1.20
Calcium	3.6	4.06	0.211	0.156	0.194
Magnesium	1.12	1.36	0.045	0.076	0.036
Sulphur	41	18	0.45	0.83	0.57
Iron	15	14	233	68	31
Manganese	27	21	56	29	312
Zinc	2.0	1.7	0.541	0.029	0.679
Copper	1.6	1.4	0.149	0.077	0.141

*Properties (unit): Soil, pH (1:2.5 soil:water); organic carbon (%); cation exchange capacity (cmol kg^{-1}): available N (mg kg^{-1}); available (Bray-1) P (mg kg^{-1}); available K (mg kg^{-1}); exchangeable Ca (cmol kg^{-1}); exchangeable Mg (cmol kg^{-1}); available S (kg ha^{-1}); DTPA extractable Fe, Mn,Zn and Cu (ìg g^{-1}); other materials, moisture (%); pH, whole; N, P, K, Ca, Mg and S (total, %), Fe, Mn, Zn and Cu (total, ìg g^{-1}), on oven dry basis

Table 2. Effect of direct nutrient feeding of bunch on yield and quality in 'Ney Poovan' banana raised under conventional or organic farming

Treatment	Fruit weight (kg)	Bunch weight (kg)	Pulp:peel ratio	TSS (Brix, %)	Benefit: Cost ratio
Type of farming					
Organic farming	7.805	8.641	5.54	24.9	2.15
Conventional farming	12.694	13.737	4.60	24.5	3.61
SEM (±)	0.2057	0.2076	0.099	0.12	0.025
CD (p=0.05)	0.5956	0.6013	0.289	0.34	0.071
Type of direct nutrient feeding					
Control	9.200	10.074	4.23	24.8	2.52
Cow dung + Cow urine	10.210	11.140	4.67	24.7	2.91
Cow dung + *Panchagavya*	10.412	11.347	5.32	25.1	2.98
Cow dung + Urea + SOP	11.179	12.194	6.05	24.0	3.25
SEM (±)	0.2909	0.2940	0.140	0.17	0.028
CD (P=0.05)	0.8423	0.8503	0.406	0.48	0.080

wise, fruits from organic farming showed significantly superior pulp:peel ratio and pulp TSS. Benefit:cost ratio of banana cultivation under conventional farming was 3.61, which was significantly and substantially higher due to 62% increase in fruit yield under the former, compared to that in organic farming (2.15).

All modes of direct nutrient feeding of the banana bunch tested caused significant increase in fruit yield and bunch weight, in the order of blend: urea + SOP > *panchagavya* > cow-urine with cow-dung. Increase observed due to blending cow-urine and *panchagavya* was *at par,* just as was blending *panchagavya* with urea + SOP. Pulp:peel ratio indicates the relative edible-portion of the banana fruit indicating fruit quality. Higher value is preferred in fruits. Pulp:peel ratio increased significantly owing to direct nutrient-feeding compared to 'Control' due to enhanced pulp growth over that of the peel. Best improvement in pulp:peel ratio was observed when direct nutrient-feeding was done with cow-dung blended with urea + SOP. Direct nutrient-feeding with cow-dung enriched with urea + SOP reduced TSS to 24.0°Brix compared to that in the other methods. As for profitability, direct nutrient-feeding increased benefit:cost ratio significantly from 2.52 in 'Control' to 2.91 and 2.98 under nutrient-feeding with cow-dung blended with cow urine or *panchagavya,* respectively. Blending urea + SOP with cow-dung, however, showed highest benefit:cost ratio (3.25).

Nutrient composition of banana pulp

Conventional farming significantly increased S, Fe Mn and Zn content in the pulp compared to that in organically cultivated banana fruits (Table 3). Among various methods of direct nutrient-feeding, two contrasting trends were observed. As for N, P, K, Ca, Mg and S, direct nutrient-feeding increased the content of these nutrients significantly in the pulp compared to that in 'Control'. Maximum increase was seen in direct nutrient-feeding with cow-dung enriched with urea + SOP. Perhaps, N, K, and S contained in the fertilizers, in addition to nutrients inherently present in the cow-dung (as presented in Table 1), caused this improvement. As regard micronutrients, the opposite was true, and maximum reduction was observed in the direct nutrient-feeding with urea + SOP. This may be attributed to dilution of the nutrients by improved pulp development in fruits under direct nutrient-feeding.

Substantial response of fruit and bunch yield may be attributed to notable amounts of N, K, S and other mineral nutrients (Table 1) present in cow-dung, cow-urine and *panchagavya* besides other biochemicals. For instance, 500g fresh cow-dung and 100ml cow-urine used in direct nutrient-feeding contained 1.79g N, 1.22g K and 0.54g S. Contribution from 100ml *panchagavya* was 2.08g N, 1.42g K and 0.60g S. Inclusion of 2.5g each of urea and SOP, however, increased the levels of these nutrients to 2.8, 2.34 and 0.95g, respectively, and resulted in maximum development of fruit and bunch. Unorthodox movement of nutrients from the distal stalk-end into the bunch may be attributed to the fact that a developing bunch is a strong sink for nutrients available in the cow-dung slurry acting as a source in source-sink relationships. This was conclusively demonstrated by a significant movement of ^{15}N from the cow-dung slurry to fruits, by Kotur and Keshava Murthy (2008). This was to an extent of 44.1% of applied N in 'Robusta' and 41.5% in 'Ney Poovan' banana (Kotur and Keshava Murthy, 2010). Inclusion of urea in the slurry has been reported to enhance urease activity, which may facilitate hydrolysis of urea to NH_3, for easy absorption and

Table 3. Effect of different types of direct nutrient-feeding of bunch on composition of 'Ney poovan' banana pulp under organic and conventional farming

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Fe (μg g⁻¹)	Mn (μg g⁻¹)	Zn (μg g⁻¹)	Cu (μg g⁻¹)
Type of farming										
Organic farming	1.25	0.12	1..27	0.54	0.19	0.04	30.5	7.6	8.6	3.7
Conventional farming	1.25	0.11	1.39	0.56	0.18	0.16	102.9	38.2	11.2	3.4
SEM (±)	0.045	0.001	0.031	0.010	0.004	0.007	2.47	2.01	0.26	0.12
CD (p=0.05)	NS	0.003	0.896	0.030	0.012	0.020	7.14	5.83	0.75	NS
Type of direct nutrient feeding										
Control	1.19	0.10	1.16	0.29	0.15	0.14	83.3	30.7	12.0	3.2
Cow dung + Cow urine	1.04	0.11	1.39	0.62	0.19	0.09	81.8	19.8	10.3	3.6
Cow dung + *Panchagavya*	1.31	0.11	1.41	0.64	0.19	0.09	74.7	27.4	9.9	3.3
Cow dung + Urea + SOP	1.46	0.12	1.37	0.65	0.21	0.07	27.1	14.0	7.3	4.1
SEM (±)	0.185	0.002	0.044	0.015	0.06	0.010	3.49	2.84	0.37	0.17
CD (p=0.05)	0.398	0.005	0.127	0.042	0.016	0.029	10.11	8.24	1.06	0.50

assimilation of N, thereby enhancing bunch yield (Ancy *et al,* 1998). De-navelling *per se* saves the plant from unnecessary expense of energy and nutrients when male buds are retained on plants until harvest. Direct nutrient feeding through the distal-end after de-navelling adds further to bunch development (Singh, 2001). Improvement in the composition of fruit pulp with regard to P, K, Ca and Mg may be attributed to similar translocation of nutrients present in the slurry. Decrease in the content of micronutrients in pulp may be due to a dilution caused by an enhanced biomass. Soil used for organic farming showed a relatively high pH, organic carbon, available N and S than did soil from conventional farming, while, differences between the rest of the nutrients was negligible (Table 1). However, additional nutrients from fertilizer received by the crop in conventional farming led to a significantly higher plant, fruit and bunch growth. Supply of nutrients and other biochemicals contained in *panchagavya* besides maintenance of a good organic regime further facilitated superior crop performance under conventional farming. Significance of the variation seen in TSS (between 24.0 and 25.1°Brix) needs to be studied organoleptically. Improved nutrient content in the pulp may have beneficial nutraceutical consequences of relevance in promoting nutritional security of the fruit, in general. Results show that it is remunerative to grow high quality 'Ney Poovan' banana under conventional farming by adopting green-manuring, applying adequate amount of farm yard manure and *panchagavya,* supplemented by fertilizer application and, above all, direct nutrient feeding of banana bunch with 2.5g each of urea and SOP blended in fresh cow-dung slurry. For growers practicing organic banana production, direct nutrient-feeding by a blend of *panchagavya* with cow-dung slurry after de-navelling, is profitable.

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Effect of polyamines on storability and quality of pomegranate fruit (*Punica granatum* L.) cv. Bhagwa

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ABSTRACT

Pomegranate cv. Bhagawa fruits harvested at adequate stage of maturity were dipped in aqueous solutions containing various concentrations of the polyamines putrescine (1mM, 2mM and 3mM) and spermidine (0.5mM, 1mM and 1.5mM), along with Tween-20 as a surfactant, for 5 minutes. The fruits were then stored at 5°C and 8°C temperature with under 90-95% relative humidity. Polyamine-treated fruits showed reduced chilling-injury, weight loss and respiration rate during storage at these 5°C and 8°C temperatures. An increasing trend in total soluble solids (TSS) content, and a decreasing trend in acidity were found in polyamine-treated fruits during storage at 5°C and 8°C temperature. Maximum reduction in chilling-injury was obtained with putrescine (2mM) at both the storage temperatures. Control fruits stored at 5°C and 8°C temperature rapidly developed chilling-injury developed symptoms of brown discoloration of skin and weight-loss in pomegranate fruits.

Key words: Pomegranate, polyamines, shelf-life, storability, chilling injury

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a widely grown fruit crop in almost all the tropical and subtropical countries. It is classified as a non-climacteric fruit, and, in spite of the low respiration rate reported (Ben-Arie *et al*, 1984), it is a highly perishable commodity. Pomegranate, when stored at room temperature, suffers reduction in shelf-life by accelerated desiccation and decay, which makes it necessary to store fruits at low temperatures. However, when stored below 5°C, pomegranate fruits develop chilling-injury (CI), resulting in reduced internal and external fruit quality (Mirdehghan and Rahemi, 2005). To reduce occurrence of CI, several technologies have been tested, including chemical treatment with thiabendazole (McDonald *et al*, 1991), controlled and modified atmosphere storage, intermittent warming (Artés *et al*, 1996), shrink-film wrapping and coatings (Nanda *et al*, 2001). Polyamines (PAs) are low molecular-weight, small, aliphatic amines ubiquitous in living organisms, and have been implicated in a wide range of biological processes, including plant growth, development and response to stress (Smith, 1985). The most common PAs, putrescine (PUT), spermidine (SPD) and spermine (SPM) are found in every plant cell. It is believed that PAs have anti-senescence function (Kumar *et al*, 1997), but their levels usually decrease during ripening in most fruits. This general diminution affects textural attributes of the fruit and its shelf-life. Thus, exogenous application of PAs has been reported to enhance shelf-life and textural attributes in fruits like plum (Pérez-Vicente *et al*., 2002) and mango (Malik and Singh, 2005). Scanty information is available on the effect of polyamines on extending shelf-life, alleviating CI, and maintaining quality attributes of pomegranate fruits. In view of the importance of pomegranate cv. Bhagwa, and problems faced by growers/traders in cold-storing, this experiment aimed to study the effect of polyamines on storage-life and quality attributes of pomegranate fruits under low-temperature storage.

MATERIAL AND METHODS

Sample preparation: Pomegranate cv. Bhagawa fruits were procured from a commercial orchard in Solapur (Maharashtra). Fruits were harvested at commercial maturity stage and transported immediately to the laboratory. Uniform-sized fruits, free from sunburn, cracks or bruises were selected. The experiment was conducted in Completely

Randomized Design, including seven treatments, viz., T_1–1mM putrescine, T_2–2mM putrescine, T_3–4mM putrescine, T_4–0.5mM spermidine, T_5–1mM spermidine, T_6–1.5mM spermidine and T_o–Control, with three replications. Fruits were treated with various concentrations of putrescine (PUT) (1mM, 2mM and 3mM) and spermidine (SPD) (0.5mM, 1mM and 1.5mM), along with Tween-20 as a surfactant, for 5 min and washed in distilled water (Control). After treatment, fruits were air-dried and kept in ventilated, corrugated fiber-board boxes. Fruits packed in boxes were kept in the laboratory at room temperature, and at low temperatures of 5°C and 8°C. After 15, 30, 45, 60 and 75 days of storage, fruits from each treatment were sampled. Fruit peel was carefully cut at the equatorial zone with sharp knives and arils were taken out from which juice was extracted, manually, for further analysis.

Weight-loss in fruits was determined during storage at different sampling intervals of 15, 30, 45, 60 and 75 days after treatments and expressed as percentage. Respiration rate was measured using auto gas analyzer (Model: Checkmate 9900 O_2/CO_2, PBI Dansensor, Denmark). Respiration rate was expressed in milliliters of CO_2 released per kg of fruit per hour (mL CO_2 kg^{-1} h^{-1}). Pomegranate fruits for studies on chilling-injury were rated on a scale of 0–4 (Wild and Hood, 1989). For juice recovery, arils were removed from the fruit and weighed using an electronic balance. Juice was extracted by a hydraulic juice press and weighted. Juice recovery was expressed as percentage of total aril weight at the time of measurement. Total soluble solids content was determined using Erma hand refractometer at 20°C and results expressed as percentage. Titrable acidity was estimated as per Ranganna (1986). Data were subjected to ANOVA in Completely Randomized Design, and, the means were separated by LSD test.

RESULTS AND DISCUSSION

Effect of polyamines on physiological changes in fresh pomegranate fruit

Physiological loss of weight: Physiological loss in weight was found to increase with advancement in storage period at room temperature. All the treatments led to loss in fruit weight during the entire storage period up to 75 days (Table 1). At 30 days of storage, highest (18.5%) physiological loss in weight was found in Control treatment T_0, while, the lowest was recorded in treatments T_1 and T_5 (8.8 and 9.0%, respectively) in fruit stored at room temperature. At 5⁰C, the highest percentage of weight loss (8.9%) was recorded in Control fruits, and the lowest (2.22%) in treatment T_1. At 30 days of storage, a similar trend in physiological loss of weight was observed at 8⁰C storage. At 30 days of storage, end of the shelf-life of fruit was observed in Control treatment T_0. At 75 days of storage, the lowest (11.00%) physiological loss in weight was recorded in treatment T_1, followed by that in treatment T_5 (11.12%) at 5⁰C. At 75 days of storage, a similar trend of physiological loss in weight was observed at 8⁰C. Loss of weight in the stored pomegranate fruit is mainly due to transpiration of water from the fruit, and is apparent as shrivelling. Loss in weight was found reduced with application of PUT. Lower weight-loss in PUT treated fruits can be attributed to stabilization or consolidation of cell integrity and permeability of tissues, and amelioration of CI. The CI induces tissue disruption and the connection between fruit skin and the external atmosphere, allowing transfer of water vapour. Besides this, lower respiration rate in PUT treated fruits may also contribute to lower rate of weight-loss (Valero *et al*, 1998). Elyatem and Kader (1984) also established a strong relation between respiration rate in pomegranate and loss in weight.

Table 1. Effect of polyamines on physiological loss in weight (PLW %) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)											
	Room temperature		5°C temperature					8°C temperature				
	15	30	15	30	45	60	75	15	30	45	60	75
T_1	2.18	8.8	1.35	2.21	6.12	9.84	11.00	1.40	2.40	6.19	9.84	11.07
T_2	3.31	11.23	2.22	3.70	9.10	12.16	14.13	2.29	3.82	9.13	12.17	14.21
T_3	2.91	12.23	2.74	3.97	8.90	11.44	13.86	2.91	3.94	8.90	11.49	14.07
T_4	2.87	11.80	2.83	3.98	9.12	12.20	14.16	2.86	4.01	9.16	12.21	14.24
T_5	2.27	9.00	1.41	2.74	7.14	10.04	11.12	1.64	2.80	7.16	10.08	11.26
T_6	2.92	12.00	2.87	4.01	8.89	11.05	13.92	2.90	3.98	9.10	11.85	14.04
T_0	9.34	18.50	4.30	8.90	00	00	00	4.42	9.09	00	00	00
SE±	0.36	0.12	0.026	0.157	0.03	0.06	0.03	0.01	0.08	0.01	0.01	0.04
CD at 5%	1.11	0.38	0.08	0.476	0.09	0.09	0.09	0.03	0.26	0.04	0.03	0.12

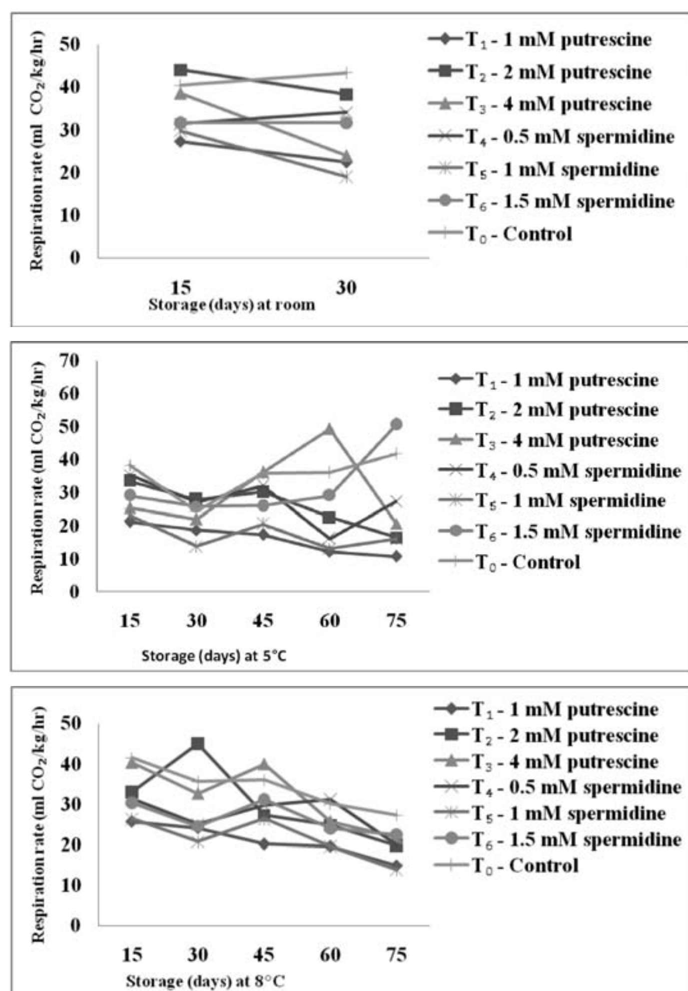

Fig. 1 Effect of polyamines on respiration rate in pomegranate fruit stored at room temperature and low temperature

Respiration rate: Respiration rate of fruit increased with advancement in storage period under all treatments tested (Fig. 1). Up to 15 days of storage, no significant difference in respiration rate was seen in fruits treated with PUT and SPD, at room temperature, 5°C and 8°C. However, a marked difference was recorded in respiration rate at 45 and 60 days of storage under all the treatments used. At 60 days of storage, highest respiration rate (41.80mL CO_2 kg^{-1} h^{-1}) was recorded under Control, while, the lowest (10.76mL CO_2 kg^{-1} h^{-1}) in T_1 treatment at 5°C. Comparatively higher respiration rate in the Control fruits was mainly due to CI. Chilling injury is known to abrade cell membrane and other cell organelles, which leads to higher cell-respiration rate. The above findings are in agreement with MacRae (1987) in persimmon.

Chilling injury: CI developed in pomegranate fruits from 45th day of storage at 5°C and 8°C (Table 2). Symptoms appeared as skin-browning, and its intensity increased with storage duration prolonged to 75 days. Highest CI was recorded in the Control fruits (40.00%). However, application of PUT and SPD led to significant reduction in CI and skin-browning. At the end of the experiment, fruits treated with T_1 showed 55% lower symptoms of CI compared to the Control fruits. Chilling injury could be considerably reduced by the sole application of PUT or SPD. In addition, adaptation or cold acclimation has been proposed to cause an increase in the proportion of unsaturated membrane lipid, and, this is considered to be a critical factor for maintenance of cellular integrity under chilling conditions (Campos *et al*, 2003). Here, the Control fruits failed at cold-acclimation/adaptation, thus leading to CI. Polyamines play a very significant role in alleviating chilling injury symptoms in fruits. When polyamines are applied exogenously, they seem to induce cold-acclimation, which could help maintain membrane fluidity at low temperatures, and in thus, responsible for reducing electrolyte-leakage and skin-browning. Polyamines, due to their antioxidant property, prevent mainly lipid peroxidation, thus protecting membrane lipids from conversion in physical state (Mirdehghan *et al*, 2007).

Table 2. Effect of polyamines on chilling injury (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)							
	5°C temperature				8°C temperature			
	30	45	60	75	30	45	60	75
T_1	00	-	14.06 (16.97)	21.20 (12.23)	00	27.20 (15.78)	29.20 (16.98)	39.22 (23.08)
T_2	00	21.73 (12.55)	29.20 (8.085)	39.40 (23.20)	00	30.20 (17.75)	34.06 (19.91)	45.50 (27.06)
T_3	00	19.22 (11.08)	31.05 (18.08)	37.06 (21.75)	00	30.32 (17.68)	32.22 (18.79)	45.30 (26.93)
T_4	00	24.66 (14.29)	32.33 (18.86)	39.33 (22.86)	00	30.22 (17.52)	32.06 (18.70)	44.19 (26.22)
T_5	00	-	18.30 (18.54)	26.60 (15.42)	00	28.30 (16.44)	30.10 (17.51)	41.03 (24.22)
T_6	00	17.32 (19.76)	18.40 (10.60)	39.06 (22.99)	00	-	34.20 (20.00)	44.07 (26.23)
T_0	00	28.30	35.00	40.00	00	30.25	36.00	46.00
SE±		0.75	0.011	0.015		0.010	0.029	0.37
CD at 5%		2.28	0.034	0.046		0.031	0.088	0.14

*Figures in parentheses indicate transformed value

Juice recovery: Juice recovery decreased in all the treatments (Fig. 2). However, the decline was much higher in Control (arils from untreated fruit) compared to treatment with PUT and SPD. At 30 days of storage, this trend was found to be reverse, where, juice recovery increased in Control fruits. But, this surge was observed much later, at 45 days of storage in PUT- and SPD- treated fruits. Regardless of the treatment, juice recovery depleted after 45 days of storage. However PUT treatment proved to be better over the Control. In the present investigation, application of PUT and SPD gave positive results going by the higher juice recovery over Control. Owing to its anti-senescence property, PUT retards respiration rate and activities of enzymes responsible for cell-wall degradation. Further, the role of PAs in reducing CI and associated activities of cell-wall degrading enzymes have been reported by several workers (Fernández-Trujillo *et al*, 1998). Thus, in the Control pomegranate fruits, increase in juice recovery after 30 days of storage may be attributed to CI-mediated activity of cell-wall degrading enzymes such as pectinmethylesterase and polygalacturonase.

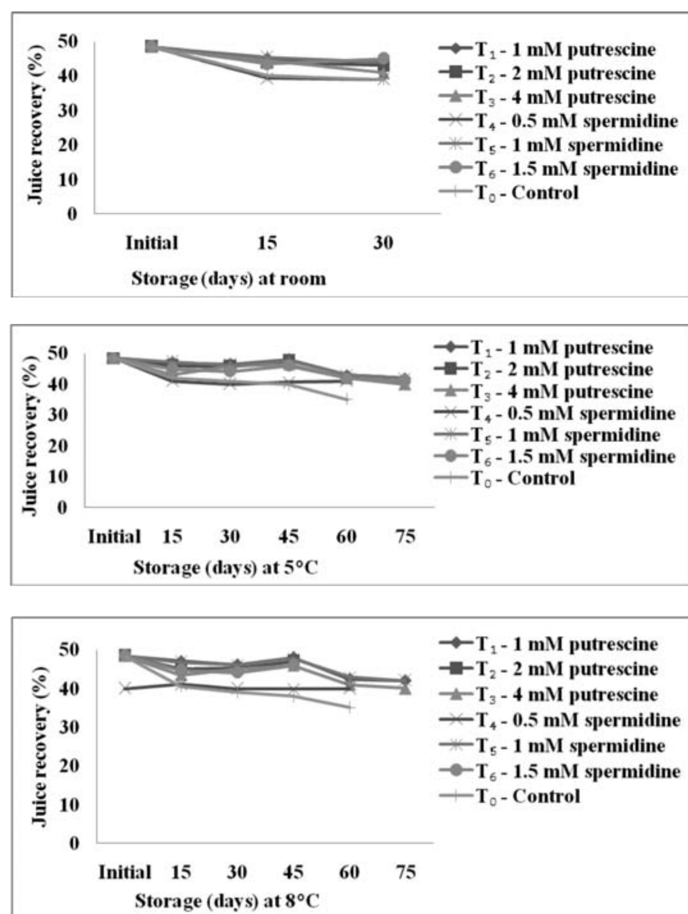

Fig. 2. Effect of polyamines on juice recovery in pomegranate fruit stored at room temperature and low temperature

Effect of polyamines on chemical composition of fresh pomegranate fruit

Total soluble solids (TSS): The total soluble solids increased with increase in storage period (Table 3). At 15 days of storage, the lowest (15.37%) TSS was recorded in treatment T_4, at room temperature. The highest (15.49%) TSS was recorded in treatment T_1, followed by that in treatment T_5 (15.47%). At 60 days of storage, a similar trend was observed too. At 75 days of storage, highest TSS was recorded in Control treatment T_0 (17.01%), at 5^0C, while, the lowest was recorded in treatment T_4 (16.17%), followed by treatment T_1 (16.18%).

Titrable acidity: Titrable acidity decreased with increase in storage period (Table 4). At 15 days of storage, highest (0.61%) titrable acidity was recorded in Control treatment T_0. The lowest (0.36%) titrable acidity was recorded in treatment T_1, followed by treatment T_5 (0.37%). At 60 days of storage, a similar trend was observed. At 75 days of storage, highest titrable acidity was recorded in Control treatment T_0 (0.39%), while, lowest titrable acidity was recorded in treatment T_1 (0.29%), followed by treatment T_5 (0.33%). Previous work on plum (Serrano *et al*, 2003)

Table 3. Effect of polyamines on total soluble solids (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)														
	Room temperature			5^0C temperature						8^0C temperature					
	0	15	30	0	15	30	45	60	75	0	15	30	45	60	75
T_1	15	15.49	15.74	15	15.5	15.76	16.21	16.41	16.18	15	15.50	15.71	16.17	16.37	16.37
T_2	15	15.37	15.73	15	15.42	15.62	15.84	15.95	16.16	15	15.42	15.60	15.81	15.90	15.90
T_3	15	15.36	15.72	15	15.41	15.58	15.64	15.90	16.15	15	15.41	15.62	15.71	15.81	15.81
T_4	15	15.37	15.70	15	15.41	15.68	15.54	15.85	16.10	15	15.41	15.56	15.61	15.71	15.75
T_5	15	15.47	15.77	15	15.46	15.71	16.14	16.35	16.17	15	15.46	15.66	16.12	16.35	16.35
T_6	15	15.39	15.70	15	15.40	15.70	16.17	16.20	16.07	15	15.40	15.65	16.11	16.16	16.16
T_0	15	15.42	15.60	15	15.39	15.56	16.23	16.50	17.01	15	15.39	15.53	16.22	16.47	16.60
SE±		0.007	0.005	0	0.005	0.008	0.005	0.005	0.005	0	0.005	0.01	0.006	0.005	0.005
CD at 5%		0.023	0.015	0	0.015	0.026	0.017	0.17	0.17	0	0.015	0.03	0.019	0.017	0.017

Table 4. Effect of polyamines on titrable acidity (%) in pomegranate fruit stored at room temperature and low temperature

Treatment	Storage period (days)														
	Room temperature			5°C temperature						8°C temperature					
	0	15	30	0	15	30	45	60	75	0	15	30	45	60	75
T_1	0.36	0.36	0.36	0.36	0.32	0.31	0.30	0.30	0.29	0.36	0.34	0.33	0.33	0.32	0.30
T_2	0.36	0.40	0.39	0.36	0.37	0.36	0.35	0.35	0.33	0.36	0.36	0.35	0.34	0.33	0.32
T_3	0.36	0.51	0.50	0.36	0.36	0.36	0.35	0.35	0.34	0.36	0.70	0.36	0.35	0.34	0.34
T_4	0.36	0.56	0.55	0.36	0.37	0.40	0.39	0.39	0.38	0.36	0.43	0.42	0.41	0.40	0.35
T_5	0.36	0.37	0.36	0.36	0.36	0.35	0.34	0.34	0.33	0.36	0.30	0.35	0.34	0.30	0.32
T_6	0.36	0.46	0.45	0.36	0.41	0.36	0.35	0.35	0.35	0.36	0.36	0.36	0.35	0.34	0.31
T_0	0.36	0.61	0.58	0.36	0.42	0.41	0.40	0.40	0.39	0.36	0.38	0.37	0.36	0.36	0.36
SE±	—	0.008	0.008	—	0.007	0.006	0.007	0.006	0.006	—	0.007	0.007	0.007	0.007	0.003
CD at 5 %	—	0.027	0.0027	—	0.02	0.02	0.022	0.02	0.02	—	0.021	0.022	0.0023	0.024	0.012

and pomegranate (Mustapha *et al,* 1995) also confirm these findings.

CONCLUSION

Exogenous application of polyamines like putrescine and spermidine showed improvement in storability of pomegranate at 5°C, which otherwise would lead to chilling injury and compromised quality. Treatment with putrescine reduced respiration rate and physiological loss of weight, and enhanced total soluble solids content, amount of reducing sugars, and decreased acidity of the fruit. Thus, shelf-life can be extended in pomegranate fruits stored at low temperatures (5°C) for upto 75 days. As demonstrated by us, application of 1mM putrericine could be effective in alleviating chilling injury symptoms during long duration, low-temperature storage of pomegranate fruits. However, further studies are necessary on combined use of putrescine with other treatments in alleviating chilling injury and possible mechanisms of action for increasing post-harvest life of the fruit.

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Nutraceutical composition of *ber* (*Zizyphus mauritiana* Lamk.) juice: effect of enzyme-assisted processing

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ABSTRACT

An investigation was undertaken to study the effect of pre-press maceration treatment with cell-wall degrading enzyme, pectinase, on antioxidant composition of *ber* juice, during 2011-2012. Enzyme-assisted processing significantly ($p<0.05$) improved antioxidant composition of *ber* juice. *Ber* juice extracted using pectinase had richer nutraceutical composition than in the Control. There was an overall increase of 43% in juice yield, 30% in total phenolics and 37% in total flavonoids with use of pectinase. *In vitro* total antioxidant activity (AOX) in *ber* juice was 19.58µmol Trolox/ml in Ferric Reducing Antioxidant Power (FRAP) and 13.44µmol Trolox/ml in Cupric Reducing Antioxidant Capacity (CUPRAC) assay. There was 41-65% increase in total AOX of *ber* juice extracted with the enzyme over-straight pressed juice. Results indicated that tailoring of the enzyme can yield antioxidant-rich juice products.

Key words: *Ber*, enzyme assisted processing, pectinase and antioxidant activity

INTRODUCTION

Ber (*Zizyphus mauritiana* Lamk.) grows excellently in arid and semi-arid regions of the world and is considered the poor man's apple, being an excellent source of several polyphenols including caffeic acid, *p*-hydroxybenzoic acid, ferulic acid and *p*-coumaric acid (Dahiru and Obidoa, 2008). As phenolics are known for their wide ranging health-protecting properties as anti-atherogenic, anti-inflammatory and anti-microbial, commercial processing of *ber* into juice rich in phenolics could prove useful. However, extraction of juice from *ber* is difficult and protracted because of its pulpy nature and high pectin content. Enzyme-assisted processing using pectinolytic enzyme is an effective approach for degrading pectineous material to yield free-flowing juice. In addition, the enzyme-catalyzed degradation also helps release phenolics and flavonoids that would otherwise be lost in press residues (Sowbhagya and Chitra, 2010). Several researchers have reported pectinase and cellulase enzyme treatments to significantly enhance recovery of phenolics and to improve functional properties of the juice. In view of the enormous potential of *ber* as a source of phenolics, the current study was undertaken to examine the effect of enzyme-assisted processing on nutraceutical composition of *ber* juice.

MATERIAL AND METHODS

The present study was carried out on antioxidant composition of *ber* juice as affected by enzyme-assisted processing, during the year 2010-2011. Mature, ripe fruits of *ber* (cv. Umran), free from blemishes and mechanical injury were obtained from the local market of Parbhani and processed at Post Harvest Technology Laboratory of Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Fruits were washed thoroughly in tap water to remove any adhering dirt or dust. Whole fruits were then subjected to hot-breaking at 90°C for 20 min to soften them. These were then macerated in a Waring blender and, subsequently, passed through a laboratory-scale pulper for extracting a homogeneous pulp and to separate seeds. Pulp samples were weighed out into 500ml glass bottles and the enzyme preparation (pectinase EC 3.2.1.1 from *Aspergillus niger,* 1 U/mg from *Aspergillus* sp.) was added at four levels of dose: 0.10, 0.15, 0.20 and 0.25% E/S. Control (straight-pressed) juice samples were incubated without the enzyme under the same conditions. For each concentration, 500ml pulp was taken in three replicates. The bottles were capped and incubated at 50°C in a thermostatically controlled water bath for 1 h. The macerate was then pressed using a hydraulic press with a nylon filter

bag to extract the juice. Juice yield was determined by weighing the juice extracted, which was subsequently heat-processed at 90°C for 1min, and packed in clean, sterilized glass bottles, upturned and sealed. This juice was then used for analysis.

Determination of total amount of phenolics, flavonoids and total antioxidant activity

Total phenolic content of the juice (80% ethanol extract) was estimated spectrophotometrically using Folin–Ciocalteu reagent, as per Singleton *et al* (1999). Results were expressed as mg gallic acid equivalents (GAE/100ml). Total amount of flavonoids was estimated by the method of Zhishen *et al* (1999) and the results were expressed as catechin equivalents/100 ml. Antioxidant activity was measured using two *in vitro* assays: ferric-reducing antioxidant power (FRAP), and cupric-reducing antioxidant capacity (CUPRAC). FRAP assay was performed as per Benzie and Strain (1996), and CUPRAC assay was performed as per Apak *et al* (2004). Results were expressed in mmol Trolox/ml (TE/ml).

Statistical analysis

Each experimental unit was replicated three times. Data were subjected to Analysis of Variance, using Completely Randomized Design.

RESULTS AND DISCUSSION

Juice yield

Data on effect of pectinase enzymes at different doses (0.1–0.25%) on *ber* juice yield is presented in Fig. 1. The pulpy macerate of *ber* was highly viscous and difficult to press. With conventional straight-pressing (Control), the yield averaged 27%, while, with increasing concentrations of pectinase enzyme, juice-yield increased to 70%. Enzyme-assisted processing accelerated liquefaction of the pulpy macerate, resulting in an 43% increase in juice yield.

Total amount of phenolics, flavonoids and antioxidant (AOX) composition of *ber* juice

Enzyme-assisted processing had a significant impact on recovery of total phenolics and flavonoids too in *ber* juice. Compared to the Control, percentage increase in recovery of total phenolics was higher in pectinase treatments. Total phenolics content increased to 314.36mg GAE/100ml at 0.25% pectinase, from an initial 240.48mg GAE/100ml (Fig. 2). Phenolics contained in the vegetable and fruit matrix appear to be entangled with the plant cell wall polysaccharides via tight hydrophilic and hydrophobic bonds. The release of those phenolics can be enhanced via enzyme

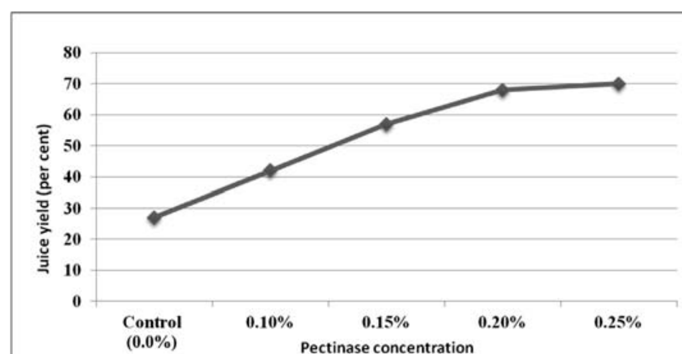

Fig 1. Effect of pectinase treatment on juice yield in *ber* cv. Umran

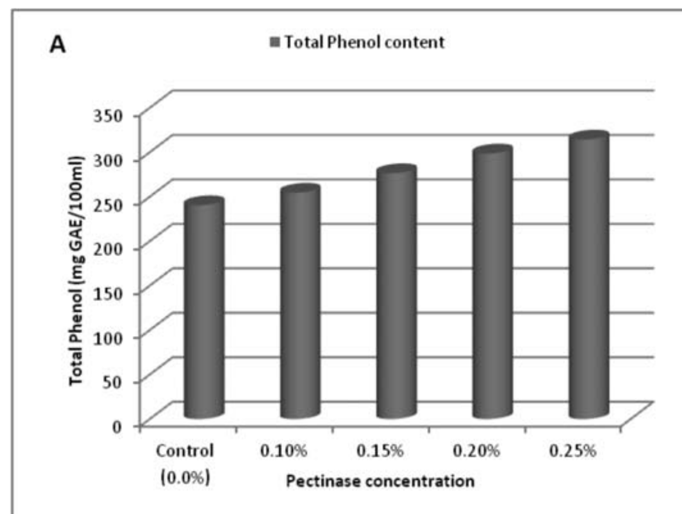

Fig 2. Effect of pectinase treatment on total phenol content in *ber* juice cv. Umran

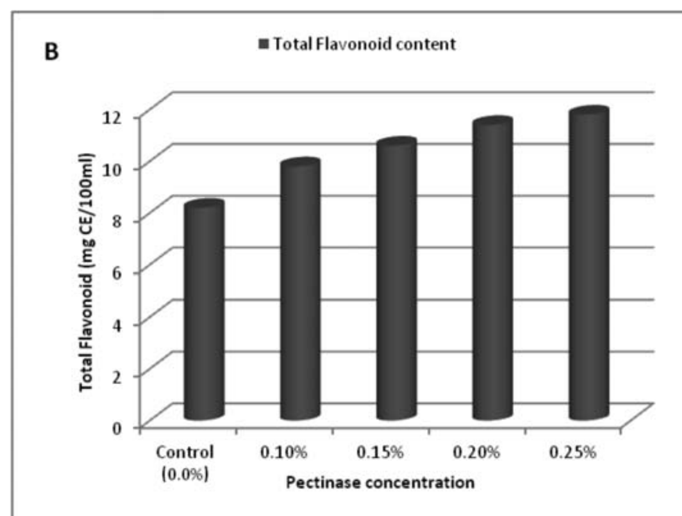

Fig 3. Effect of pectinase treatment on total flavonids in juice of *ber* cv. Umran

catalyzed degradation of the cell wall polysaccharides. Enzyme facilitated polysaccharide helps in exposing possible cell wall sites for phenolics, resulting in enhanced recovery (Pinelo and Meyer, 2008).

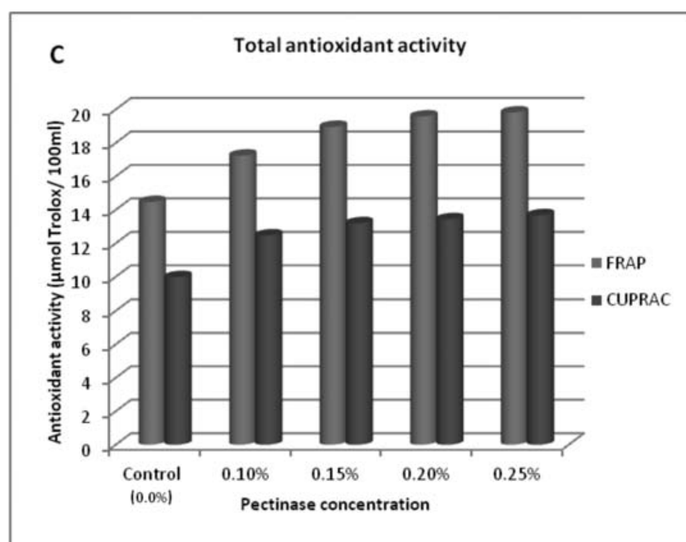

Fig 4. Effect of pectinase treatment on antioxidant activity in juice of *ber* cv. Umran

Total flavonoids content in juice also showed progressive increase with various pectinase treatments (Fig. 3). Antioxidant activity of *ber* juice, too, improved dramatically upon enzyme-assisted processing. Values for this ranged from 14.47 to 19.82mmol/ml, respectively, in the Control and in the juice treated with the enzyme pectinase (Fig. 4). Overall, there was a significant increase in total AOX in the juice over Control. An almost identical trend was observed in CUPRAC assay (Fig. 4). High AOX in enzyme-assisted juice may be attributed to a high recovery of phenolic compounds observed in the juice. Similar results on high phenolic content and antioxidant activity have been reported in bilberry by previous workers (Puupponen-Pimia *et al,* 2008).

Enzyme-assisted processing of *ber* significantly enhanced nutraceutical composition of the juice, in contrast to straight-pressing. These results could lead to tailoring of the enzyme for obtaining optimum levels of antioxidants in the juice products. The study also indicated that *ber*, a fruit rich in nutrceuticals, can be commercially processed into juice rich in phenolics.

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Evaluation of potato genotypes for processing traits in late autumn

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ABSTRACT

The present study was undertaken to evaluate potato genotypes for their suitability for processing when grown under different crop durations, i.e., E_1 (80 days' crop duration), E_2 (100 days' crop duration) and E_3 (120 days' crop duration). Among the three durations studied, E_3 yielded the highest processing-grade yield (q/ha), followed by E_2 and E_1. The crop in E_3 also exhibited high dry-matter content and low levels of reducing sugars compared to that in the other crop durations, which is desirable for processing. Among the cultivars under study, Kufri Badshah, Kufri Anand, Kufri Bahar, Kufri Chipsona-1, Kufri Chipsona-2, Kufri Ashoka and Kufri Jawahar gave the highest total tuber-yield. However, for processing-grade yield, cvs. Kufri Badshah, Kufri Chipsona-1 and Kufri Jawahar yielded significantly better than the mean, but cv. Kufri Chipsona-1 was the one found suitable for processing due to its high dry-matter content (22.07%), while, the other cultivars were found suitable for table purpose alone. Though cv. Kufri Chipsona-1 yielded higher (309.39 q/ha), its performance could not be predicted under various conditions owing to the data on regression coefficient (being less than one), and a significant deviation from regression. Cultivars Kufri Chipsona-1 and Kufri Chipsona-2 were found to be suitable for processing, with high tuber- and processing-grade yield, high dry-matter content, low amount of reducing sugars and phenols in the crop durations E_2 and E_3. Both these cultivars produced chips of acceptable colour in all three crop durations.

Key words: Potato, genotypes, autumn season, processing quality

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important food crops both in the developing and the developed countries. Potato processing is well advanced in developed countries, but now processing industry in the developing countries is also growing fast. In India, there is an increase in processing activity due to an increase in urban population, preference of the new generation for fast foods, rise in *per capita* income, and increase in the number of working women (preferring ready-to-cook foods) and an expanding tourism (Marwaha, 1997). Processing of potatoes solves the problem of storing large quantities of this commodity during periods of glut. A large number of companies, including multinationals, have stepped into the field of potato processing. The processing industry needs potatoes with high dry matter (over 20%), low amounts of reducing sugars (below 0.25% on fresh weight basis), and low amounts of phenols (Gaur *et al,* 1999).

In the state of Punjab, the main-season crop is grown under short-day conditions beginning in October, and is harvested in January/ February. During crop maturation, the average minimum temperature is 4.5°C, which results in relatively low dry-matter content and high levels of reducing sugars (Ezekiel *et al,* 1999). Days to harvest also affect processing quality in potato, viz., its dry matter content, sugar content, reducing sugars, phenolic compounds, and specific gravity (Marwaha and Sandhu, 2002). Therefore, identifying suitable varieties for late-autumn planting in the North-Western plains will not only ensure supply of fresh potatoes to the processing industry of North India for a longer period, but also minimize transportation charges, and save on the storage cost of tubers (Pandey *et al,* 2003).

MATERIAL AND METHODS

The experimental material comprised ten genetically diverse potato genotypes, viz., Kufri Badshah, Kufri Anand, Kufri Chandramukhi, Kufri Bahar, Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2, Kufri Ashoka, Kufri Jawahar and Russet Nor x 97-ES-33, obtained from Central Potato Research Institute (CPRI), Shimla. These were multiplied at Vegetable Research Farm of Department of Vegetable

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Science, PAU, Ludhiana. All the ten cultivars were evaluated in Randomized Block Design (RBD), with three replications. Each plot measured 3.2m x 1.2m, and consisted of 16 plants in each row. Seed-sized tubers were planted at row-to-row and plant-to-plant spacing of 60cm and 20cm, respectively. The crop was planted on 16th November 2005. Three experiments were laid out for different crop durations, viz., 80, 100 and 120 days. Various crop durations are symbolized below:

Environment-I (E_1) – Haulm cutting 80 days after planting

Environment-II (E_2)– Haulm cutting 100 days after planting

Environment-III (E_3)– Haulm cutting 120 days after planting

Each crop was harvested at 10-15 days at haulm cutting. The crop was raised following a package of practices for potato recommended by Punjab Agricultural University, Ludhiana. Processing characters like dry-matter content (%), total amount of sugars (Yemm and Willis, 1954), reducing sugars (mg/100g fresh weight) (Nelson, 1944), total amount of phenols (Swain and Hillis, 1959) and polyphenol oxidase chip colour (score), were all studied. Statistical analysis of variance was carried out for Randomized Block Design for each character separately, in each of the three environments (crop duration cycles). Phenotypic stability analysis of genotypes was assessed for their stability of performance over environments, using a model suggested by Eberhart and Russell (1966). The following model was used for describing performance of a variety over a series of environments:

$$Y_{ij} = \mu_i + \beta_i I_j + \delta_{ij}$$

where,

Y_{ij} = Variety Mean of the ith variety at the jth environment (where i=1, 2 …….g, j=1, 2 ……….n)

μi = Overall Mean of the ith genotype over all the environments

β_i = Regression coefficient of ith variety to the varying environments

I_j = Environmental index obtained as the Mean of all genotypes at the jth environment minus the Grand Mean, i.e.,

$$I_j = \frac{Y.}{g} \quad \frac{Y..}{gn}$$

where,

$$\Sigma_j I_j = 0$$

δ_{ij} = Deviation from regression of the ith variety in j^{th} environment

g = Number of genotypes

n = Number of environments

Stability parameters of individual genotypes were calculated as per Eberhart and Russell (1966).

RESULTS AND DISCUSSION

Cultivars Kufri Chipsona-1 (161.47 q/ha), Kufri Chipsona-2 (209.23 q/ha) and Kufri Ashoka (151.03 q/ha) had significantly higher processing-grade yield than the Mean value (124.49 q/ha) in E_1. However, in the case of E_2, cvs. Kufri Badshah (200.70 q/a), Kufri Bahar (169.30 q/ ha), Kufri Lauvkar (180.57 q/ha), Kufri Chipsona-1 (174.50 q/ha) and Kufri Chipsona-2 (189.57 q/ha) yielded significantly higher than the Mean (152.85 q/ha). In the third environment (E_3), cvs. Kufri Badshah (325.03 q/ha), Kufri Chipsona-1 (224.50 q/ha) and Kufri Jawahar (224.87 q/ha) gave significantly better yield than the Mean value (196.16 q/ha) (Table 1). Analysis of pooled data revealed that cvs Kufri Badshah and Kufri Chipsona-2 had significantly higher processing-grade yield (215.47 q/ha and 196.02 q/ha, respectively) than the pooled Mean (157.83 q/ha).

In the case of environment E_1, cvs. Kufri Chipsona-1 and Kufri Chipsona-2 showed significantly higher dry-matter content than the Mean value. However, in the case of E_2, cvs. Kufri Anand, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 showed significantly higher dry-matter content than the Mean. In E_3, cv. Kufri Chipsona-1 alone had significantly higher dry-matter content than the Mean value. Pooled data analysis indicated that cvs. Kufri Anand, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 had significantly higher Mean value than the pooled Mean. Kufri Chipsona-1 had the highest dry-matter (20.69%), followed by Kufri Chipsona-2 (19.93%), Russet Nor x 97-ES-33 (19.83%) and Kufri Anand (19.74%) (Table 2).

Cultivars Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 had a high Mean dry-matter content, with regression coefficient less than one (0.74, 0.22 and 0.90, respectively) and a non-significant deviation from regression, showing their suitability for cultivation under diverse environmental conditions. Studies of Patel *et al* (2003) and Pandey *et al* (2005) also support the above findings.

In environments E_1 and E_2, cvs. Kufri Chandramukhi, Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2 and

Table 1. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for processing-grade yield (q/ha) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	120.67	200.70	325.03	215.47	2.85	0.55
2.	Kufri Anand	90.30	147.47	212.70	150.16	1.69	50.02
3.	Kufri Chandramukhi	93.78	48.57	87.68	76.67	0.00	1203.67*
4.	Kufri Bahar	133.68	169.30	197.93	166.97	0.88	68.23
5.	Kufri Lauvkar	105.03	180.57	140.60	142.07	0.38	2482.04*
6.	Kufri Chipsona-1	161.47	174.50	224.50	186.82	0.90	93.30
7.	Kufri Chipsona-2	209.23	189.57	189.26	196.02	-0.26	90.91
8.	Kufri Ashoka	151.03	153.63	219.30	174.66	1.00	391.86*
9.	Kufri Jawahar	69.45	164.07	224.87	152.80	2.11	720.22*
10.	Russet Nor x 97-ES-33	110.27	100.20	139.73	116.73	0.45	310.28*
Mean		124.49	152.85	196.16	157.83		
CD (5%)		16.11	14.64	23.73	34.52	SE of bi = 0.45	
CV		5.95	5.40	8.76			

*Significant at 1%

Table 2. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for dry matter content (%) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	15.21	17.49	18.96	17.22	1.04	0.03
2.	Kufri Anand	18.58	20.29	20.36	19.74	1.04	0.09
3.	Kufri Chandramukhi	16.04	18.54	21.27	18.62	1.44	0.10
4.	Kufri Bahar	16.25	19.04	18.98	18.09	0.78	1.08*
5.	Kufri Lauvkar	16.81	17.02	18.72	1752	0.51	0.47
6.	Kufri Chipsona-1	19.37	20.63	22.07	20.69	0.74	0.04
7.	Kufri Chipsona-2	19.17	20.74	19.87	19.93	0.22	0.93
8.	Kufri Ashoka	14.44	17.10	21.29	17.61	1.87	0.85
9.	Kufri Jawahar	14.53	17.44	19.87	17.28	1.47	0.00
10.	Russet Nor x 97-ES-33	18.04	20.19	21.26	19.83	0.90	0.09
Mean		16.84	18.85	20.26	18.65		
CD (5%)		1.86	1.32	1.10	0.90	SE of bi = 0.24	
CV		5.78	4.14	3.41			

*Significant at 5%

Table 3. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for total amount of sugars (mg/100g fresh weight) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	975.67	816.33	571.67	787.89	1.59	9435.81*
2.	Kufri Anand	692.67	477.00	369.33	513.00	1.37	56.93
3.	Kufri Chandramukhi	494.67	371.33	326.00	397.33	0.72	21.95
4.	Kufri Bahar	736.67	467.00	356.67	520.11	1.62	16.19
5.	Kufri Lauvkar	487.67	334.00	293.00	371.56	0.85	208.03*
6.	Kufri Chipsona-1	347.67	288.67	268.67	301.67	0.34	10.07
7.	Kufri Chipsona-2	304.67	270.00	232.33	269.00	0.29	156.36*
8.	Kufri Ashoka	748.33	483.33	432.00	554.56	1.39	1268.33*
9.	Kufri Jawahar	668.33	405.67	375.67	483.22	1.31	2197.54*
10.	Russet Nor x 97-ES-33	511.00	416.67	390.67	439.44	0.52	70.49
Mean		596.73	433.00	361.60	463.78		
CD (5%)		16.57	12.10	12.39	54.43	SE of bi = 25.92	
CV		2.08	1.52	1.56			

*Significant at 1%

Russet Nor x 97-ES-33, had significantly less amount of total sugars than the Mean. Cultivars Kufri Anand, Kufri Chandramukhi, Kufri Lauvkar, Kufri Chipsona-1 and Kufri Chipsona-2 had significantly lower amount of total sugars than the Mean in E_3 (Table 3). In pooled analysis of data, four cultivars, viz., Kufri Chandramukhi, Kufri Lauvkar, Kufri Chipsona-1 and Kufri Chipsona-2, had significantly lower Mean of total level of sugars than did the pooled Mean. 'Kufri Chipsona-2' had the lowest mean of total level of sugars (269.00mg), followed by Kufri Chipsona-1 (301.67mg), Kufri Lauvkar (371.56mg) and Kufri Chandramukhi (397.33mg) per 100g on fresh-weight basis. Cultivars Kufri Chandramukhi, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 had lower Mean values for total amount of sugars as less than one regression coefficient (0.72, 0.34, 0.29 and 0.52, respectively), and non-significant deviation from regression, indicating that performance of these cultivars could not be predicted under unfavourable environments.

In this study, none of the cultivars showed regression coefficient equal to one or significant deviation from regression, indicating that no cultivar had average stability for this trait in all the three environments studied. The high amount of total sugars in environments E_1 and E_2 in our investigation could be attributed to low temperature (4.4°C), along with occurrence of frost during the vegetative phase and tuber development. Studies by Uppal *et al* (2003) also indicated that cvs. Kufri Chipsona-1and Kufri Chipsona-2 contained the lowest amount of total sugars (362 and 367mg/ 100g fresh weight, respectively).

Cultivars Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 had lesser amount of reducing sugars than the Mean in all the three environments (Table 4). Persual of pooled data also showed that cvs. Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 had significantly lower quantities of reducing sugars than the pooled Mean. 'Kufri Chipsona-1' had the lowest amount of reducing sugars (75.74mg/100g fresh weight), followed by 'Kufri Chipsona-2' (81.94mg/100g fresh weight), 'Russet Nor x 97-ES-33' (165.88mg/100g fresh weight) and 'Kufri Lauvkar' (202.90mg/100g fresh weight). Kumar *et al,* (2003) also reported reducing sugars to vary from season to season, and cool weather (1-5°C) led to an increase in sugar levels. This variation could be attributed to variation in crop durations under different environments. Cultivars Kufri Chipsona-1 and Kufri Lauvkar showed the content of reducing sugars within permissible limits, regression coefficient of less than one, and a non-significant deviation from regression, indicating suitability of these genotypes for cultivation under unfavorable environmental conditions. Gaur *et al* (1999) reported that potatoes grown in North-Western plains contained relatively low dry-matter and higher amounts of reducing sugars (which were attributed to the comparatively low temperature prevalent during crop maturation).

'Kufri Chipsona-1' had significantly less amounts of total phenols than the Mean value under environment E_1. However, in the case of E_2, cvs. Kufri Lauvkar and Kufri Chipsona-1 showed significantly lower amounts of total phenols than the Mean value. In the case of E_3, cvs. Kufri Anand, Kufri Lauvkar, Kufri Chipsona-2 and Kufri Ashoka

Table 4. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for levels of reducing sugars (mg/100g fresh weight) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	456.47	433.22	403.48	431.05	0.52	263.99
2.	Kufri Anand	340.54	234.52	238.95	271.34	1.29	240.20
3.	Kufri Chandramukhi	341.08	230.29	212.57	261.31	1.52	3.61
4.	Kufri Bahar	473.99	307.78	312.98	364.92	2.03	537.60*
5.	Kufri Lauvkar	211.36	202.72	194.62	202.90	0.17	17.22
6.	Kufri Chipsona-1	103.22	77.76	46.24	75.74	0.56	293.18
7.	Kufri Chipsona-2	102.08	94.92	48.81	81.94	0.45	820.55**
8.	Kufri Ashoka	320.86	282.79	308.91	304.19	0.27	451.78*
9.	Kufri Jawahar	402.15	298.87	267.36	322.79	1.53	61.12
10.	Russet Nor x 97-ES-33	253.82	124.50	119.33	165.88	1.65	147.58
Mean		300.56	228.74	215.35	248.21		
CD (5%)		30.60	24.99	21.08	24.99	SE of bi = 0.26	
CV		7.19	5.87	4.95			

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

showed significantly less amounts of total phenols than the Mean value (Table 5). From pooled analysis, cv. Kufri Lauvkar alone had a lower Mean of total phenols (37.56mg/ 100g fresh weight) than the pooled Mean. Though some of the cultivars showed a regression coefficient close to one (Kufri Anand, Kufri Ashoka and Kufri Jawahar), deviation from regression in their case was significant, thereby indicating poor stability of these cultivars for this trait. However, these values are higher than those reported by Marwaha (1999) in hybrids MP/90-94 (25.6mg), MP/91-G (27.1mg) and MP/90-83 (30.1mg) on per 100g fresh weight basis.

In the environment E_1, cv. Kufri Anand (0.06) alone had significantly less activity of polyphenol oxidase than the Mean value (0.08). In the case of E_2, cvs. Kufri Badshah and Kufri Chipsona-2 had significantly less value for polyphenol oxidase activity than the Mean value (0.08). However, none of the cultivars showed significantly less value of polyphenol oxidase than the Mean value in environment E_3 (Table 6). Analysis of pooled data indicated that cv. Kufri Chipsona-2 alone had on overall Mean (0.06) of polyphenol oxidase similar to the pooled Mean (0.06). However, Uppal (1999) reported that polyphenol oxidase activity was the highest in tubers of cv. Kufri Sutlej, and lowest in cv. Kufri Jawahar.

In the environment E_1, cvs. Kufri Chipsona-1 and Kufri Chipsona-2 produced chips of acceptable colour (each having a colour score of 2). In environment E_2, cvs. Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33 produced chips of acceptable colour (2.00, 2.00 and 2.67 score, respectively). However, cvs. Kufri Lauvkar, Kufri Chipsona-1, Kufri Chipsona-2 and Russet Nor x 97-ES-33

Table 5. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for total amount of phenols (mg/100g fresh weight) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	64.00	39.67	35.00	46.22	1.12	0.86
2.	Kufri Anand	59.33	44.33	27.67	43.78	1.07	62.77**
3.	Kufri Chandramukhi	58.33	38.00	37.33	44.56	0.85	7.65*
4.	Kufri Bahar	74.00	50.00	40.33	54.78	1.25	4.86
5.	Kufri Lauvkar	56.33	26.33	30.00	37.56	1.13	48.22**
6.	Kufri Chipsona-1	54.33	34.33	38.00	42.22	0.72	28.67**
7.	Kufri Chipsona-2	59.67	38.33	29.00	42.33	1.13	5.84*
8.	Kufri Ashoka	59.00	41.67	28.33	43.00	1.07	30.31**
9.	Kufri Jawahar	57.67	40.33	33.33	43.78	0.90	2.57
10.	Russet Nor x 97-ES-33	56.00	36.67	37.67	43.44	0.76	13.22**
Mean		59.86	38.96	33.66	44.16		
CD (5%)		4.00	3.00	2.67	6.72	SE of bi = 0.23	
CV		5.28	3.97	3.53			

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

Table 6. Mean ($\bar{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for polyphenol oxidase (IU) in potato during autumn season, 2005-06

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\bar{X}i$)	bi	S^2di
1.	Kufri Badshah	0.08	0.04	0.05	0.06	1.47	0.00
2.	Kufri Anand	0.06	0.05	0.07	0.06	-0.22	0.00
3.	Kufri Chandramukhi	0.07	0.06	0.05	0.06	0.96	0.00
4.	Kufri Bahar	0.09	0.06	0.06	0.07	1.13	0.00
5.	Kufri Lauvkar	0.09	0.08	0.05	0.07	1.43	0.00
6.	Kufri Chipsona-1	0.09	0.08	0.06	0.08	0.92	0.00
7.	Kufri Chipsona-2	0.08	0.04	0.05	0.06	1.32	0.00
8.	Kufri Ashoka	0.09	0.08	0.05	0.07	1.58	0.00
9.	Kufri Jawahar	0.08	0.09	0.05	0.07	1.39	0.00
10.	Russet Nor x 97-ES-33	0.08	0.07	0.07	0.07	0.02	0.00
Mean		0.08	0.06	0.05	0.06		
CD (5%)		0.02	0.02	0.01	0.01	SE of bi = 0.67	
CV		19.16	27.53	36.70			

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

Table 7. Mean ($\overline{X}i$), regression coefficient (bi) and deviation from regression (S^2di) for chip colour (score) in potato during the autumn season 2005-2006

Sl. No.	Genotype	E_1	E_2	E_3	Overall Mean ($\overline{X}i$)	bi	S^2di
1.	Kufri Badshah	6.33	5.67	5.00	5.67	1.25	0.00
2.	Kufri Anand	5.67	4.67	4.33	4.89	1.25	0.07
3.	Kufri Chandramukhi	5.33	4.67	3.67	4.56	1.56	0.02
4.	Kufri Bahar	5.33	5.67	4.67	5.22	0.62	0.30
5.	Kufri Lauvkar	4.67	3.67	3.00	3.78	1.56	0.02
6.	Kufri Chipsona-1	2.00	2.00	1.33	1.78	0.62	0.07
7.	Kufri Chipsona-2	2.00	2.00	1.33	1.78	0.62	0.07
8.	Kufri Ashoka	5.33	5.33	5.67	5.44	-0.31	0.02
9.	Kufri Jawahar	5.33	4.67	4.00	4.67	1.25	0.00
10.	Russet Nor x 97-ES-33	4.33	2.67	2.67	3.22	1.56	0.46*
Mean		4.30	4.10	3.57	4.10		
CD (5%)		1.81	0.92	1.07	0.48	SE of bi = 0.43	
CV		11.59	13.02	11.77			

*Significant at 5%

produced chips of acceptable colour (score ≤ 3.0) in environment E_3 (Table 7). Potatoes grown in a cool climate, particularly in areas where night-temperature drops below 10°C during the last month before harvest, are found not suitable for processing (Ezekiel *et al,* 1999). Pooled data analysis indicated that cvs. Kufri Chipsona-1 and Kufri Chipsona-2 produced chips of acceptable colour. 'Kufri Chipsona-1' and 'Kufri Chipsona-2' produced chips of excellent quality and light colour (score of 1.78 each). Pandey *et al* (2005) reported that in a late-planted crop at Modipuram, acceptable quality chips were produced only in cv. Kufri Chipsona-1. This cultivar produced light-coloured chips at all the stages of harvest and locations in North-Western and West-Central Indian plains (Pandey *et al,* 2005).

Among the three environments studied, 120 days' crop duration (E_3) yielded the highest total tuber-yield (q/ha) and processing-grade yield (q/ha), followed by E_2 (100 days' crop duration) and E_1 (80 days' crop duration) (Table 1). Besides total tuber-yield, most genotypes in group E_3 exhibited high dry-matter content and low levels of reducing sugars, compared to that in the other environments, and, these are desirable attributes for processing. Though in the environment E_1, cv. Kufri Ashoka yielded significantly higher (151.03 q/ha) than Mean, it had low dry-matter content. Therefore, this was unsuitable for processing purposes, but was suitable for table-purpose. In E_2, cv. Kufri Chipsona-1 and Kufri Chipsona-2 had high yield, high dry-matter content and low amounts of reducing sugars. Therefore, these cultivars are suitable for processing. In the environment E_3, cvs. Kufri Chipsona-1 and Kufri Chipsona-2 were found to have high tuber-yield, high dry-matter content, low levels of reducing sugars, and low amounts of total phenols. Also, both of these cultivars produced chips of acceptable colour in all the three environments. The potential of cvs. Kufri Chipsona-1 and Kufri Chipsona-2 is not fully exploited due to occurrence of frost at the vegetative stage of the crop. Kumar *et al* (2004) documented that 120 days' crop duration was most suitable for Kufri Chipsona-1 and Kufri Chipsona-2 in the spring season crop. From stability analysis data, it is concluded that cv. Kufri Chipsona-2 was stable for total tuber-yield in all the three environments. Therefore, cv. Kufri Chipsona-2 is recommended for cultivation for all the three crop durations to produce potatoes for the processing industry.

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Survey of nematode-destroying fungi from selected vegetable-growing areas in Kenya

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ABSTRACT

Plant-parasitic nematodes cause severe damage to a wide range of economic crops, causing upto 5% yield losses globally. In Kenya, vegetables are affected, among other pests, by parasitic nematodes, causing upto 80% loss in yield. Nematode control is very difficult and relies heavily on use of chemical nematicides. Use of these chemical nematicides leads to biological magnification, and elimination of natural enemies of other pathogens, thus creating a need for greater application of pesticides, increased production costs, and development of insecticide-resistance. These factors have led to a growing interest in search for alternate management strategies. The objective of this study was, therefore, to document nematode-destroying fungi in selected, major vegetable-growing areas in Kenya as a step towards developing a self-sustaining system for management of plant-parasitic nematodes. Soil samples were collected from five vegetable-production zones, viz., Kinare, Kabete, Athi-river, Machakos and Kibwezi, and transported to the laboratory for extraction of nematode-destroying fungi. The soil-sprinkle technique described by Jaffee *et al* (1996) was used for isolating the nematode-destroying fungi from soil, while, their identification was done using identification keys described by Soto Barrientos *et al* (2001). From this study, a total of 171 fungal isolates were identified as nematode-destroying. The highest population was recorded in Kabete, at 33.9% of the total, followed by Machakos, Kibwezi, Athi-river, with the least in Kinare, at 24.6, 22.2, 11.7 and 7.6% of the total population, in that order. *Arthrobotrys* was the most frequent genus, with mean occurrence of 7.3, followed by *Monacrosporium* with 6 and *Stylophage* with 5.2. *A. dactyloides* was significantly (*P=0.002*) affected by the agro-ecological zone, with the highest occurrence recorded in Kabete, and the least in Athi-river. Kibwezi recorded highest diversity index, with a mean of 1.017, while, Athi-river recorded the least, with a mean of 0.333. Kibwezi had the highest species richness, recording a mean of 3.4, while, the least mean of 1.6 was recorded in Athi-river. Mean species richness of 2.2 was recorded for both Kabete and Machakos, and 1.8 for Kinare. From the three genera recorded, *Arthrobotrys* was more effective at trapping nematodes compared to *Monocrosporium* and *Stylopage*. The genus *Arthrobotrys* had the highest number of trapped nematodes, with a total population of 57, followed by *Monacrosporium,* the least being *Stylopage*, with 45 and 36, respectively, in a period of 104 hours. From the study, it is evident that agricultural practices affect occurrence and diversity of nematode-destroying fungi, and, *Arthrobotrys* can be used as a bio-control agent for managing plant-parasitic nematodes.

Key words: *Artabotrys,* biological control, plant-parasitic nematodes

INTRODUCTION

Horticultural crops, both for local consumption and export, are important in Kenya. One-tenth of the vegetables in Kenya are grown for export. They are recognized for their health and nutritional benefits, and provide cash income and employment for close to two million people in Kenya (Dobson *et al*, 2004). Production of vegetables in Kenya, especially for an expanding domestic market, is even now limited by major pests and diseases (Dobson *et al*, 2004). Plant-parasitic nematodes have been identified as a major production constraint, affecting vegetable production, resulting in reduced yield quality and quantity (Nchore *et al*, 2010). They are responsible for upto 80%, on vegetable production (Kaskavalci, 2007). Vegetable production in Kenya is characterized by high chemical input for pest and soil fertility management (Mutsotso *et al,* 2005). These practices have been associated with increase in soil-borne diseases and decline in beneficial soil micro-organisms (Wachira *et al,* 2008). Specifically, vegetable damage by root-knot nematode has been reported in Kenya, with infected plants rendered unacceptable to international markets (Nchore *et al*, 2010). The root knot nematode increases wounding of the root system, thus providing points of ingress of the pathogen. The nematode may also modify the tissue in a way that it becomes more amendable to bacterial colonization (Hayward, 1991). Globally, it is

estimated that US $ 500 million is spent on root-knot nematode control strategy (Keren-Zur *et al,* 2000; Pinkerton *et al,* 2000), including use of nematicides, organic-manure amendment and use of resistant cultivars (Akhtar & Malik, 2000). Overall, though nematicides are effective in managing root-knot nematode and other plant-parasitic nematodes, they are expensive and become environmental pollutants when not applied at the right time, in the right manner and in the right dosage. This increases cost of production to the farmers, reducing their profit (Republic of Kenya, Taita District Development Strategies 2002-2006). Use of nematicides is also curtailed by their threat to groundwater, soil biodiversity, as well as long waiting-periods between use, harvesting and marketing of a crop (Bridge, 1996).

Alternatively, soil beneficial microorganisms can be used as an alternative, thereby helping reduce application of chemicals to the soil. This entails the use of natural enemies to control nematode pests. Beneficial micro-organisms are non-polluting and, thus, environmentally safe and acceptable. Usually, these are species-specific to the target pest, therefore with no chances of affecting non-target species (unlike chemicals, which are broad-spectrum in their action (Hein *et al,* 2007). Nematode-destroying fungi are one such group of beneficial microorganisms for use in control of plant-parasitic control of plant-parasitic nematodes. These micro-fungi are natural enemies of the nematodes. They naturally capture, kill and digest nematodes present in the soil (Rodrigues *et al,* 2001; Nordbring-Hertz *et al,* 2002). They comprise three main groups: the nematode trapping fungi, the endoparasitic fungi, and the egg-and cyst-parasitic fungi (Nordbring-Hertz *et al,* 2002; Masoomeh *et al,* 2004). After trapping the nematodes, the fungi penetrate their cuticle, invade their entire body-cavity and, then, digest them completely. This group of fungi has drawn much attention due to their potential as biological control agents for plant-parasitic nematodes (Jansson *et al,* 2000; Sanyal, 2000; Masoomeh *et al,* 2004). About 70% of the fungal genera and 160 species are associated with nematodes, but, only a few can be used as biological control agents for nematodes (Elshafie *et al,* 2006). This study was, therefore, aimed at documenting occurrence and diversity of nematode-destroying fungi and testing their efficacy on plant-parasitic nematodes, to harness their potential as bio-control agents against plant-parasitic nematodes.

MATERIAL AND METHODS

Soil samples were collected from five different vegetable-growing areas in Kenya, viz., Kinare, Kabete, Athi-river, Machakos and Kibwezi, in the order of altitude and temperature. Kinare was a high-altitude area and the coldest, with Kibwezi being the lowest and hottest. Vegetable gardens in each zone were dominated mainly by spinach, kale, tomato, cabbage and pepper, among other vegetables. From each of the study areas, five farms under intensive vegetable-production were selected randomly for this study. From each of the farms, five different vegetable gardens were sampled. From each vegetable garden in turn, five soil samples were collected and mixed together in a bucket to make a composite sample. One kilogram of soil was then re-sampled from the composite sample in the bucket, put into plastic bags, labelled and placed in a cool box. Soil sampling was done using a soil auger sterilized using ethanol after every sampling, to avoid cross-contamination. All the samples were later transported to the laboratory for isolating nematode-destroying fungi.

Isolation of nematodes-destroying fungi was done using the soil-sprinkle technique described by Jaffee *et al* (1996) where, tap water agar (TWA) was prepared by dissolving 20g agar in one litre of tap water. The medium was autoclaved and cooled before use after amending it with 0.1g per litre of streptomycin sulfate under a laminar air flow cabinet. One gram of soil sample was sprinkled on the medium in the Petri dish, and a suspension of *Meloidogyne* species consisting of approximately 1000 nematodes, was added to the Petri dishes as bait (Christina *et al,* 1999). The plates were then incubated at room temperature and observed daily from the third week, up to the sixth week, under a dissecting microscope. The examination was focussed on trapped nematodes, trapping organs and conidia of the nematode-destroying fungi (Wachira *et al,* 2008).

Taxonomic classification of the nematode-destroying fungi was done using the 'slide culture technique' where slides were observed under a microscope, while identification of the genus was done using identification keys described by Soto Barrientos *et al* (2001). After identification of nematode-destroying fungi, pure cultures of the three most-frequent fungal isolates were made for the experiment on efficacy. A 5mm mycelial block was inoculated into PDA in a Petri dish and allowed to grow for five days, before approximately 50 plant-parasitic nematodes were added. Efficacy of the fungal isolates was monitored for a period of 3-6 weeks. Trapped nematodes were counted for five days after 3 weeks of incubation. All the data in this study was analyzed by Analysis of Variance (Kindt & Coe, 2005)

RESULTS AND DISCUSSION

From this study, 171 fungal isolates were identified as nematode-destroying. They grouped into three genera and five taxa. The three genera were: *Arthrobotrys, Monacrosporium* and *Stylopage*. *Arthrobotry*s was a frequently-encountered genus. The genus *Arthrobotrys* was represented by *A. oligospora, A. dactyloides* and *A. longispora,* the genus *Monocrosporium* was represented by *M. cionopagium,* while the genus *Stylopage* was represented by *S. grandis. A. oligospora* had the highest frequency of occurrence, followed by *A. dactyloides, M. cionopagium, S. grandis;* the least frequent was *A. longispora* with occurrence of 46.20, 45.61, 5.85, 1.17 and 1.17%, decreasing in that order (Fig. 1).

Fungal isolates were recovered from all the vegetable-production zones. Excepting *A. dactyloides*, all the isolates were not significantly ($P > 0.05$) affected by the agro-ecological zone. Frequency of occurrence of *A. dactyloides* was significantly ($P=0.002$) affected by the agro-ecological zone. The highest occurrence of *A. dactyloides* was recorded in Kabete, while the least was recorded in Athi-river, with a total record of 40 and 4, respectively. The species was also recorded in Machakos, Kibwezi and Kinare, with occurrence of 19, 10 and 5, respectively, in decreasing order. Among the isolates, only *A. oligospora* and *A. dactyloides* were found to occur in all the agro-ecological zones. *M. cionopagium* occurred in all the zones except Kinare, while, *S. grandis* was present in both Kibwezi and Kinare. *A. longispora* was recorded in Kibwezi only (Table 1).

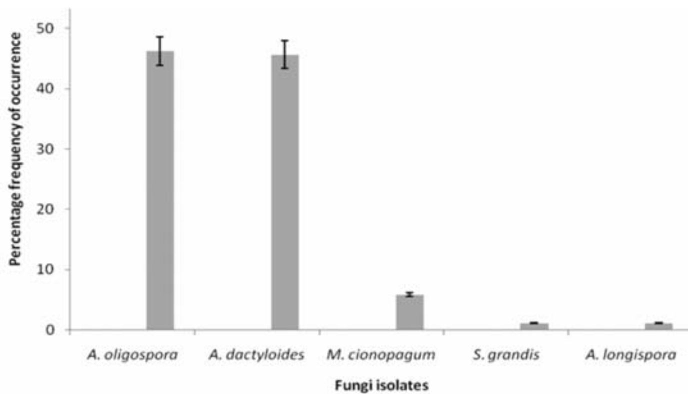

Fig. 1. Percentage occurrence of nematode-destroying fungi in some vegetable-producing areas of Kenya

Table 1. Occurrence of nematode-destroying fungi in major agro-ecological zones of Kenya

Zone	*A. dactyloides*	*A. oligospora*	*A. longispora*	*M. cionopagium*	*S. grandis*	Total
Kabete	20	17	0	1	0	58
Machakos	19	22	0	1	0	42
Kinare	5	7	0	0	1	13
Kibwezi	10	20	2	5	1	38
Athi-river	4	13	0	3	0	20
P value	0.002	0.395	0.062	0.165	0.062	

The highest number of nematode-destroying fungi was recorded in Kabete, followed by Machakos, Kiwezi, Athi-river and, finally Kinare, with total mean abundance of 11.6, 7.6, 7.4, 4.0, and 2.6 in that (decreasing) order. Kibwezi recorded the highest diversity index, with a mean of 0.930, followed by Machakos with 0.637, while Kabete recorded the least diversity index mean of 0.411. Mean richness and abundance varied between vegetable-production zones. The highest mean species richness was recorded in Kibwezi, while the least was recorded in Athi-river. All the agro-ecological zones differed significantly ($P = 9.587 \times 10^{-4}$) in terms of species abundance. Kabete had the highest species abundance with a mean of 11.6, and, the least was seen in Kinare with species mean abundance of 2.6 (Table 2).

More nematode-destroying fungi were detected with increase in the number of the soil samples under study. It is evident that all the possible isolates of nematode-destroying fungi were recorded in this study, from the samples collected. Collecting and processing additional samples may not have significantly increased the number of isolates (Fig. 2).

There was a significant ($P=0.003$) difference on efficacy between the three most-frequent nematode-destroying fungal species. *Arthrobotryrs oligospora* was the most efficient nematode-destroying fungus, with a mean of 7.3, followed by *Monacrosporium;* the least was *Stylopage,* with mean records of 5.9 and 5.1, respectively.

Nematode-destroying fungi were isolated from all the selected vegetable-production zones. These occurred at different frequencies and diversity. The study demonstrated

Table 2: Mean shannon, species richness and abundance of nematode-destroying fungi in different vegetable-growing areas in Kenya

Zone	N	Mean shannon	Mean richness	Mean abundance
Kibwezi	5	0.930	3.4	7.6
Machakos	5	0.637	2.2	7.4
Athi-river	5	0.483	1.6	4.0
Kinare	5	0.482	1.8	2.6
Kabete	5	0.411	2.2	11.6
P value				9.587×10^{-4}

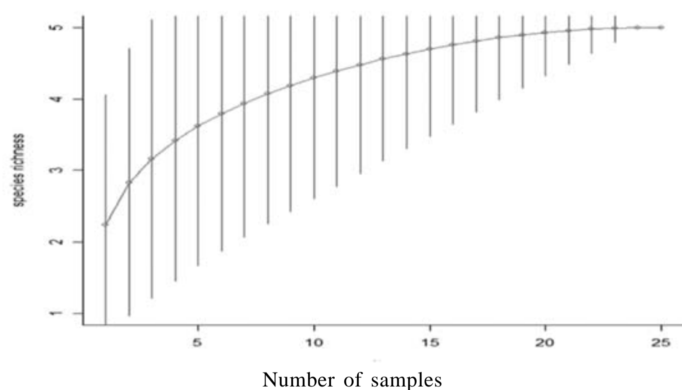

Fig. 2. A total-species cumulative curve for nematode-destroying fungi in some vegetable production zones of Kenya

the occurrence of diverse nematode-destroying fungi in nature, especially, in vegetable-production zones. These findings agree with previous reports on nematode-destroying fungi that indicate that these are wide-spread in all habitats, but, at different densities and diversities (Birgit *et al,* 2002; Wachira *et al,* 2008).

Arthrobotrys oligospora was the most abundant species of nematode-destroying fungi in the area under study. Other studies on nematode-destroying fungi report the same observation. It has never been clear why this is the most frequently encountered fungus. Wachira *et al* (2008) had suggested that farming practices like weeding could be the cause of such high occurrence. It has also been suggested that it is due to the ability of the fungus to exist both as a saprophyte and a plant-parasitic nematode feeder (Sobita and Anamika, 2011). Due to this high occurrence, this fungus has attracted several other interesting studies (Niu and Zhang, 2011).

It was expected that the highest divergence of fungi would be isolated from areas under low temperature (Kinare). However, this was not the case. Kinare had the least variety of fungi. This was attributed to the high use of chemical fertilizers and pesticides, since, all the vegetables were aimed for the market. In a study on long-term effects of manures and fertilizers on soil productivity and quality, it was reported that chemically fertilized soils had lower content of organic matter and fewer numbers of microfauna than manured soils (Edmeades, 2003). The highest number of nematode-destroying fungi was recovered from Kabete. Soils in this area had been collected from the farm at University of Nairobi to which animal manure had been frequently applied. This may explain the high number of nematode-destroying fungi here, since, these are associated with increase in beneficial micro-organisms in the soil (Wachira and Okoth, 2009). In our study, a high fungal population was found in areas where manure had been applied, and low fungal population in areas where chemical fertilizer was applied.

Temperature is an important factor in regulating microbial activity and shaping soil microbial communities. It determines moisture level in the soil, which is key to fungal spore germination and growth. High temperatures lead to low soil-moisture, which leads to low fungal-spore germination. A study by Haugen and Smith (1992) reported that at high temperatures, there was low germination of fungal spores, leading to low fungal population, while, under low temperatures there was high fungal germination, leading to a high fungal population. This was found to be in reverse in our study. Although Machakos and Kibwezi experience high temperatures, a high population of nematode-destroying fungi was seen here by us. This could be attributed to the irrigation activities undertaken at the farm which ensured moist conditions throughout the growth season. This soil enhanced moisture, coupled with high temperature, improved fungal spore germination (as, fungal spores germinate better under moist and warm conditions).

Efficacy test showed that the genus *Arthrobotrys* was most effective in trapping plant-parasitic nematodes. Previous studies on fungi of this genus have consistently showed that it is able to trap 90% of all the nematodes in Petri dishes and liquid cultures in 16-40 hours (Rajeswari and Sivakumar, 1999). Therefore, due to its high occurrence, this fungus can be used in management of plant-parasitic nematodes, and its potential for this ability should be investigated. The investigation should focus on finding suitable carrier/s for the fungus and its mode of application. This would reduce over-reliance on chemical nematicides and help develop a self-regulating system in the soil for control of soil borne pests.

CONCLUSION

Additional evidence has been provided by this study that nematode-destroying fungi naturally occurr in agricultural habitats. It is also evident that agricultural activities targeting high crop-production, like, application of chemical fertilizers and pesticides, directly affect soil biodiversity adversely. Results from this study can be used in further research for establishing the potential of nematode-destroying fungi in regulation of plant-parasitic nematode population.

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Economic analysis of post-harvest loss and marketing efficiency in guava (cv. *Allahabad safeda*) in Karnataka

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ABSTRACT

Post-harvest losses (PHL) in guava (cv. *Allahabad safeda*) were estimated at the field and retail levels in Karnataka, and impact of this loss on marketing efficiency was studied. Results indicated that the total PHL was 13.29% consisting of field-level loss (9.17%) and retail level loss (4.12%). The producer's share was 51.52% and PHL, when included as an item of cost, reduced the share to 45.80%. PHL also reduced marketing efficiency index from 1.06 to 0.88, thereby indicating the importance of PHL and scope for minimizing it to improve the efficiency of the marketing system in guava.

Key words: Guava, post-harvest losses, Allahabad safeda, economic analysis, marketing efficiency

INTRODUCTION

Guava (*Psidium guajava* L.) is an important nutritious fruit marketed in India and accounts for about 4% each of area and production among fruit crops grown in India. Like other fruits ((Srinivas *et al,* 1997; Jagtap and Katrodia, 1998; Wanjari *et al*, 2002; Gajanana *et al.,* 2011), guava is also subject to losses at various stages of handling after harvest. Information on economic aspects of marketing, associated costs and returns, and losses that occur at different stages of handling in guava in India is not available at present. Therefore, a study was undertaken to examine marketing arrangements and assess post-harvest losses in guava at different stages of handling in Karnataka, one of the major guava producing states of India.

MATERIAL AND METHODS

Karnataka is one of the major guava-producing states in the country producing 135,100 tonnes (5.4%) from an area of 7100 ha (3.23%). *Allahabad safeda* is the most popular variety of guava grown in Karnataka. Bengaluru (Rural & Urban) district produces the largest quantity of guava in the state, accounting for 19.7% area and 18.7% production in Karnataka (2011-12). Therefore, Bengaluru district was selected for the study at the first stage of sampling. At the second stage, three taluks, namely, Doddaballapur, Devanahalli and Bengaluru North, were selected and field-level loss was assessed from harvest at 39 sample-farmers' fields located in the three taluks. Retail-level loss was estimated from 31 retailers spread over the city of Bengaluru sourcing their material from K.R. market.

Estimating marketing efficiency: Efficiency of a marketing system is normally analyzed using the standard formula of Acharya and Agarwal (2001) which was later modified by Sreenivasa Murthy *et al* (2004) by including PHL as an item under the cost. The modified formula used in our study is given below:

$$ME = \frac{NP_F}{MC + MM + PHL}$$

where,

ME = Marketing efficiency index

NP_F = Farmer's net price

$NP_F = GP_F - \{C_F + (L_F \times GP_F)\}$ or

$NP_F = \{GP_F\} - \{C_F\} - \{L_F \times GP_F\}$

where,

NP_F represents the net price received by the farmer (Rs./kg)

GP_F represents the gross price received by the farmer (Rs./kg)

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C_F represents the cost incurred by the farmer in the course of marketing (Rs./kg)

L_F represents the physical loss of produce at field-level (kg)

MC = Marketing-cost to the intermediaries

$MC = C_F + C_R$

where,

C_F represents the cost to the farmer in marketing (Rs./kg)

C_R represents the cost to the retailer in marketing (Rs./kg)

MM = Marketing margin of the intermediary

$MM = MM_R$

where,

MM_R represents the marketing margin of the retailer

PHL = Post-harvest loss in the course of marketing

$PHL = \{L_F \text{ x } GP_F\} + \{L_R \text{ x } GP_R\}$

where,

L_F and GP_F are the same as indicated above

L_R represents the physical loss during retailing (kg)

GP_R represents the gross retail price (Rs./kg)

RESULTS AND DISCUSSION

Marketing practices in guava

Guava fields under harvest in Bengaluru district were visited. Marketing practices followed and losses incurred at the field-level were studied. The main marketing channels followed by the guava growers in Bengaluru district were: Self marketing in the auction at K.R. Market, Bengaluru, and Field sale of guava to contractors besides leasing out the orchard to the pre-harvest contractor (PHC).

- *Producer – Commission agent – Retailer – Consumer (Self marketing)*
- *Producer – Contractor – Commission agent – Retailer – Consumer (Field sale)*
- *Producer – PHC – CA – Retailer – Consumer (PHC)*

After harvest, ripe and green (mature) fruits were graded as large, medium or small. Fruits are then packed in bags of 20-22kg or 32-35kg (with a bamboo base) and brought to the market in tempos (vans) or mini-trucks. Sale in Bengaluru wholesale market, field-level sale and sale to pre-harvest contractors (PHC) were the main channels used by guava farmers in the area under study. In all, 56.67% of the farmers marketed 62.95% of the produce through the self-marketing channel. About 20% of the farmers sold 37.05% of their guava product at the field itself. Another 23.33% of the farmers leased out guava fields to the PHC.

Marketing cost and price realization

Farmers were found to incur an expenditure of Rs. 2.40/kg towards marketing of guava, which consisted of harvesting, grading and packing (15.19%), packing-material cost (1.26%), transportation (30.38%), unloading (2.53%) and commission (50.63%). Farmers realized a net price of Rs. 11.34/kg. The retailers realized a gross price of Rs. 22.01/kg and, after deducting the cost incurred, their margin worked out to Rs. 8.04/kg. In the process, the producer's share worked out to 51.52% (Table 1).

Post harvest loss (PHL) in guava

Losses during different stages of handling in the Self-marketing channel were assessed in 39 guava fields under harvest and from 31 retailers of guava in Bengaluru.

Total PHL was 13.29% which included field-level loss (9.17%) and retail-level loss (4.12%) (Table 2).

Field level loss

Field level loss in guava consisted of over-ripe fruits (2.93%), bird attack (0.24%), mealy bug (0.54%) and diseases like stylar-end rot (1.32%) and canker (1.29%). Further, scratches on surface fruit due to thrips, friction, etc. working out to 2.71% were also observed in our study. Over-ripe fruits accounted for 2.93% of field-level loss.

Table 1. Marketing cost, price realized and producer's share in guava

Sl. No.	Particulars	Amount or %
1	Marketing cost of producers	Rs. 2.4 /kg
	Harvesting, grading and packing	15.19 %
	Packing-material cost	1.26 %
	Transportation	30.38 %
	Unloading	2.53 %
	Commission	50.63 %
2	Net price	
	Producer	Rs.11.34 /kg
	Retailer	Rs. 8.04 /kg
3	Producer's share	51.52 %

Table 2. Post-harvest loss in guava at different levels of handling

Sl. No.	Stage/level	Loss (%)
1	Field level (after harvest and before marketing - grading, sorting for damages)	9.17
	Over-ripe fruits, discards	2.93
	Damage due to bird attack	0.24
	Damage due to blossom (Stylar) end rot	1.32
	Damage due to canker	1.29
	Damage due to mealy bug	0.54
	Others (scratches due to thrips, friction, etc.)	2.71
2	Retail market level (damage due to pressing & fruits crushed during transit & loading/ unloading)	4.12
3	Total PHL in guava	13.29

Table 3. Incidence of disease on guava fruits collected from various localities

Fruit status	Locality											
	1	2	3	4	5	6	7	8	9	10	11	12
Healthy (%)	50.00	56.67	43.33	63.33	43.33	36.67	56.67	60.00	46.67	40.00	53.33	56.67
Diseased (%)	50.00	43.33	56.67	36.67	56.67	63.33	43.33	40.00	53.33	60.00	46.67	43.33
Disease (%)												
Canker (*Pestaliopsis psidi*)	13.33	13.33	15.00	8.33	16.67	16.67	13.33	8.33	11.67	15.00	10.00	11.67
Stylar end rot (*Phomopsis psidi*)	28.33	23.33	30.00	20.00	30.00	31.67	21.67	25.00	28.33	33.33	30.00	23.33
Anthracnose (*Collectotrichum gloeosporioides*)	8.33	6.67	11.67	8.33	10.00	15.00	8.33	6.67	13.33	11.67	6.67	8.33

Table 4. Post-harvest storage losses in *Allahabad Safeda* guava fruits stored at RT & at 12°C

PLW (%)	Spoilage (%)							
	Days after Harvest				Days after harvest			
At RT	2	3	5	6	2	3	5	6
	2.51	3.53	6.35	8.16	0.00	0.00	7.29	17.28
At 12°C	3	7	10	14	3	7	10	14
	2.52	4.70	6.29	8.37	0.00	0.00	0.45	1.36

Table 5. Valuation of post-harvest loss in guava (*Allahabad Safeda*)

Sl. No.	Stage	PHL (%)	Value loss (Rs./kg)
1	Field level	9.17	1.26
2	Retail level	4.12	0.91
	Total	13.29	2.17

Hence, select harvest of fruits can reduce the loss due to over-ripe fruits. Further, losses occurring at different stages of handling guava due to stylar-end rot, anthracnose, canker, thrips' attack, etc. need to be addressed.

Retail-level loss

Loss at the retail-level was 4.12% and was due mainly to press-damage and fruits crushed in transit, unloading and loading. Farmers currently use gunny/plastic bags with a bamboo basket at the base. Instead, they could use plastic crates to reduce losses in transit.

Pathological investigation

Guava fruits collected from orchards in 12 different localities of Bengaluru district were assessed for infection with various diseases. Fruits were found to be seriously infected by diseases. Disease incidence percentage ranged from 36.67 (Locality 4) to 63.33 (Locality 6). Stylar end rot (*Phomopsis psidi*) was the major disease, causing maximum spoilage of fruits, and varied from 20 % (Locality 4) to 33.33 % (Locality 10). Canker (*Pestaliopsis psidi*) incidence varied from 8.33% (Locality 4) to 16.67% (Locality 5 & 6). Anthracnose (*Colletotrichum gloeosporioides*) incidence varied from 6.67% (Locality 2) to 15.00% (Locality 6). Appropriate, timely or effective pre-harvest disease management schedule was not practiced in these orchards (Table 3).

Post-harvest storage losses in *Allahabd safeda* guava fruits

Storage losses in *Allahabd safeda* guava were estimated as 3.53 % at 3 days storage at room temperature (24-32°C). This was mainly due to physiological loss in weight (PLW). Spoilage started after 5 days of storage (7.29%), and reached 17.28% by 6th day of storage.

By storing the fruits at low temperature (12°C), total losses at 10 days of storage were reduced to 6.74%. This was due to PLW 6.29% and 0.45% to spoilage loss. The total storage losses at 12°C increased to 9.73% when storage was prolonged to 14 days. Spoilage in storage at room temperature as well as at 12°C was found to be mainly due to blossom-end rot in *Allahabad safeda* guava variety (Table 4). It was observed that at 3 days of storage, guava fruits lost 3-4% weight and, after 5 days, spoilage set in. Therefore, care should be taken to dispose of the fruits within five days from harvesting. However, it is possible to delay spoilage by storing the guava fruits at 12°C.

Valuation of post-harvest loss, price spread and marketing efficiency

Post-harvest loss is calculated from the price prevalent at different levels of handling, and is presented in Table 5. Post-harvest loss accounts for 9.85% of the price to the consumer in a marketing channel (Table 6 & 7). As PHL escalates the cost of marketing, it has an impact on marketing efficiency. Price-spread was observed to be 54.2% which, minus the PHL, would be 48.48%. If PHL is to be included as an item under cost of marketing, efficiency of the marketing system would be reduced (Table 7). The producer's share in the consumer rupee is 51.52% indicating,

Guava harvest, sorting and packing in Bengaluru

Field-level loss in guava

Table 6. Price-spread in marketing of guava

Particulars	Price spread	
	Rs./kg	%
Net price received by the farmer	10.08	45.80
Marketing cost of the farmer	2.40	10.90
PHL at field level	1.26	5.72
Retailer's cost	0.23	1.04
PHL at retail level	0.91	4.13
Retailer's margin	7.13	32.39
Consumer price	22.01	100.00

Table 7. Efficiency in marketing guava and impact of post-harvest loss (PHL)

Sl. No.	Efficiency parameter	Efficiency parameter value
1	Producer's share (%)	51.52 (48.8)*
2	Marketing-cost (Rs./kg)	2.63 (4.80)*
4	Intermediary's margin (%)	36.53 (32.39)*
5	Post-harvest loss (PHL) (%)	9.85
3	Marketing-efficiency index	1.06 (0.88)**

*Producer's share, marketing-cost and margin after inclusion of PHL as an item of cost

** indicates marketing efficiency (ME) after inclusion of PHL as an item of marketing-cost

that, a scope exists for improving the marketing system. Therefore, it is inferred that inclusion of PHL in calculating marketing efficiency reduces the system's efficiency. This calls for efforts to reduce losses during post-harvest handling of guava, to help improve the efficiency of the marketing system.

Guava retailing in Bengaluru

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Evaluation of brinjal genotypes against bacterial wilt caused by *Ralstonia solanacearum*

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ABSTRACT

Forty brinjal genotypes were screened by artificial inoculation using *Ralstonia solanacearum* inoculum at a concentration of 1.0 X 10^8 cfu/ml ($O.D_{600}$ = 0.3). Genotypes Arka Nidhi, Haritha, Swetha, Surya, IIHR-3, IIHR-555, WCGR, R-2588, WL-2230, L-3261, L-3270, L-3272 and Arka Anand were found to be resistant to bacterial wilt, whereas, IIHR-7, L-3263, L-3268 and L-3269 were moderately resistant. Genotypes R-2584, R-2586, R- 2592, L-3260, L-3262, L-3264, L-3266 and L-3267 were moderately susceptible, and genotypes R-2580, R-2582, R-2587, R-2591, R- 2593 and R- 2595 were found to be susceptible. Lastly, genotypes R-2581, R- 2594, R-2589, R-2590, WL-2232, Pusa hybrid-6, Arka Shirish, R-2585 and R-2583 were found to be highly susceptible to bacterial wilt. Resistant and moderately resistant genotypes showed longer incubation period.

Key words: Brinjal, bacterial wilt, *Ralstonia solanacearum,* genotypes

INTRODUCTION

Brinjal (*Solanum melongena* L.) is one of the important vegetable crops of our own country and belongs to the family Solanaceae. It features on the menu of virtually every household in India, irrespective of food preference, income level or social status. Successful cultivation of the brinjal crop has been hindered by several insect pests and devastating diseases. Among the diseases, bacterial wilt caused by *Ralstonia solanacearum* (Yabucchi *et al*, 1995) is a major limiting factor. This has been the most ubiquitous and serious bacterial disease throughout tropical, sub-tropical and temperate regions of the world (Hayward, 1991).

In India, this disease is of a major concern and is serious in parts of Karnataka, Kerala, Orissa, Maharashtra, Madhya Pradesh and West Bengal (Rao *et al,* 1976). Yield losses ranging from 65 to 70% have been reported in brinjal (Das and Chattopadhyay, 1953). The disease is characterized by sudden wilting of the plant at flowering stage, by yellowing of foliage and stunted plant growth (Kelman, 1953; Rai *et al*, 1975) and an initial, brownish discoloration of vascular tissues occassionally accompanied by browning and rotting of tissues inside vascular bundles (Smith, 1920).

For management of bacterial wilt in the field, various control measures like crop rotation (cultural practice), use of antagonistic organisms (biological method) and application of chemicals (chemical control) are suggested. As the pathogen can survive or persist in the soil for several years, it is very difficult to control bacterial wilt by chemical applications, using antagonistic organisms or by cultural practices. Therefore, mitigation of the disease using appropriate farming practices needs further development and adaptation (Grimault and Prior, 1990). Therefore, search for resistant sources and incorporating those genes in commercial cultivars is a sound approach to the problem.

MATERIAL AND METHODS

The experimental material consisting of 40 genotypes was maintained in a homozygous state at the vegetable block, Post-Graduation Centre, UHS Campus, Bengaluru. Seeds of these genotypes were sown in protrays in the 1st week of August 2011. The experiment was laid out in Randomized Complete Block Design (RCBD), with three replications. A row consisting of 15 plants constituted a replication under each treatment. The 40 genotypes, including resistant (Arka Anand) and susceptible check (Pusa Hybrid-6) were

subjected to artificial inoculation which made on seedlings in portrays, a day prior to transplantation into the main field. A slight injury was made to the root with a sterile knife before inoculating while withholding irrigation for a day. Three ml volume of the inoculum at a concentration of 1.0 X 10^8 cfu/ml (O.D600 = 0.3) was poured into the root zone. Thereafter, the seedlings were transplanted into the main field. Ten days after inoculation, symptoms of wilting were seen. Observations were made as per the scale suggested by Zakir Hussain *et al* (2005). Observations on (i) days to 50% bacterial wilt, (ii) bacterial wilt at different stages of plant growth, and (iii) cumulative bacterial wilt incidence at 50 days after inoculation were recorded. Observations were recorded at intervals of 10 days, with the last observation made at 50 days after inoculation.

RESULTS AND DISCUSSION

Any breeding programme, including any that involves host-plant resistance to a pathogen, must begin with an extensive screening of germplasm. Success in finding resistance to bacterial wilt is directly related to availability of resistant genotypes in the germplasm. Development of varieties/ hybrids with suitable horticultural traits is a slow process, despite availability of sources of resistance. This is due to the unstable nature of resistance under different environmental conditions, which has necessitated the breeder to explore better sources of resistance in the cultivated brinjal for breeding bacterial wilt resistance.

The 40 genotypes were screened against Race-I, Biovar 3. Genotypes Arka Nidhi, Haritha, Shwetha, Surya, IIHR-3, IIHR-7, IIHR-555, WCGR, R-2588, R- 2592, WL-2230, L-3260, L-3261, L-3262, L-3263, L-3268, L-3269, L-3270, L-3272 and Arka Anand (Resistant check) showed no 50% wilt even at 50 DAI. However, most genotypes like Pusa Hybrid-6, L-3267, R-2581, R-2589, R- 2593 and R-2583 took the least number of days to show 50% wilt incidence. Genotypes R-2586, L-3266 and R-2584 took the maximum number of days to express 50% wilt. Least number of days taken to express 50% wilt in a genotype shows occurrence of a shorter incubation period, and, such genotypes were highly susceptible to *Ralstonia solanacearum* under field conditions; while, in some genotypes, no 50% wilt even at 50 DAI shows occurrence of a longer incubation period. Therefore, these genotypes are able to withstand attack from *Ralstonia solanacearum* under field conditions, without any great loss in economic yield. Results of the present study are in agreement with those of Zakir Hussain *et al* (2005) (Table 1).

Genotypes Pusa Hybrid-6 (at 0-10 and 11-20 DAI), followed by R- 2595 (at 0-10 DAI), R- 2593 (at 11-20 DAI) and R-2591 (at 11-20 DAI) recorded comparatively higher wilt incidence, indicating that it was the stage that was critical for genotypes becoming susceptible to bacterial wilt. Compare this to the genotypes Shwetha, Surya, IIHR-3, IIHR-7, IIHR-555, WL-2230, L-3261, L-3270 and WCGR, where none, or very low, wilt-incidence was recorded.

At 21-30, 31-40 and 41-50 DAI, most genotypes showed medium to low level of wilt. Most of the susceptible genotypes showed a susceptible reaction in their early stages of growth (0-10 and 11-20 DAI). Similarly, Hoque *et al* (1981) recorded higher incidence of wilt in tomato in the early stage of crop growth, i.e., the first symptom of wilt was observed by them on the 15th day from inoculation. Data on wilting collected by them at 43 days after inoculation varied from 13.3% to 100%.

Significant difference was observed for cumulative bacterial wilt incidence at 50 DAI among the eggplant genotypes studied. Highest incidence was recorded in WL-2232, followed by R-2590, Arka Shirish and Pusa Hybrid-6. Lowest incidence was recorded in the genotypes Surya, IIHR-3 and L-3270. In the present study, during screening of the genotypes, air temperature and relative humidity recorded were 19-28°C and 51-94%, respectively. These factors, together with impact from soil moisture and soil temperature, may have influenced resistance reaction of the genotypes.

Among the various genotypes used in this trial, only Arka Nidhi, Haritha, Shwetha, Surya, IIHR-3, IIHR-555, WCGR, R-2588, WL-2230,L-3261, L-3270, L-3272 and Arka Anand were resistant to bacterial wilt; IIHR-7, L-3263, L-3268 and L-3269 were found to be moderately resistant.

Vasse *et al* (2005) reported that resistance exhibited by various genotypes may be due to the secondary metabolism of polyphenols, and the higher concentration of steroidal glycoalkaloids present in resistant plants, thereby preventing bacterial movement into the vicinity of the plant system (by their action as a repellent). Further, Prior *et al* (1994) reported that inhibitor extracts, tyloses and gums in resistant plants act like filters, thereby preventing bacterial movement within a plant system.

Among the genotypes used in our experiment, Arka Nidhi, Haritha, Shwetha, Surya, IIHR-3, IIHR-555, WCGR, R-2588, WL-2230, L-3261, L-3270, L-3272 and Arka Anand graded as resistant to bacterial wilt, whereas, IIHR-7, L-

Table 1. Reaction of eggplant genotypes at different stages of plant growth to bacterial wilt pathogen (%) under field conditions

Sl. No.	Genotype	Days to 50% bacterial wilt	Bacterial wilt incidence (%)					Cumulative bacterial wilt incidence at 50 DAI (%)	Disease reaction
			0-10 DAI	11-20 DAI	21-30 DAI	31-40 DAI	41-50 DAI		
1	Arka Nidhi	-	5 (12.63)	2.5 (9.09)	5 (12.92)	2.5 (9.09)	0	15.00 (22.73)	Resistant
2	Haritha	-	2.5 (9.09)	0	0	12.5 (20.63)	1.66 (4.31)	16.67 (23.93)	Resistant
3	Shwetha	-	0	0	0	5 (12.92)	0	5.00 (12.92)	Resistant
4	Surya	-	0	0	0	0.83 (3.03)	1.66 (4.31)	2.50 (7.34)	Resistant
5	IIHR-3	-	0	0	0	0	2.5 (9.09)	2.50 (9.09)	Resistant
6	IIHR-7	-	0	0	0	15 (22.59)	10 (18.04)	25.00 (29.91)	Moderately resistant
7	Arka Shirish	18	36.04 (36.86)	25 (29.97)	13.63 (21.60)	0.83 (3.03)	12.5 (20.63)	88.00 (70.17)	Highly susceptible
8	IIHR-555	-	0	0	0	0	20 (26.44)	20.00 (26.44)	Resistant
9	WCGR	-	2.5 (9.09)	0	2.5 (9.09)	0	0	5.00 (12.92)	Resistant
10	R-2580	26	5 (10.45)	25 (29.91)	25 (29.91)	15 (22.59)	2.5 (7.34)	72.50 (58.89)	Susceptible
11	R-2581	12	35 (36.22)	37.5 (37.74)	7.5 (15.89)	0	2.5 (9.09)	82.50 (65.59)	Highly susceptible
12	R-2582	24	20 (26.53)	27.5 (31.60)	15 (22.73)	1.66 (4.31)	0	64.17 (53.23)	Susceptible
13	R-2585	18	27.5 (31.60)	35 (36.26)	20 (26.44)	2.5 (9.09)	0.83 (3.03)	85.83 (68.63)	Highly susceptible
14	R-2583	15	22.62 (28.36)	33.28 (35.20)	13.79 (21.73)	12.04 (19.19)	0	81.74 (63.94)	Highly susceptible
15	R-2584	36	0.93 (3.20)	27.59 (31.66)	19.48 (26.15)	5.52 (13.34)	0	53.52 (47.01)	Moderately susceptible
16	R-2586	50	2.5 (9.09)	15 (22.73)	10 (18.43)	17.33 (24.43)	5 (12.92)	49.83 (44.89)	Moderately susceptible
17	R-2587	19	7.38 (15.61)	42.62 (40.73)	14.06 (21.97)	0	0	64.06 (53.15)	Susceptible
18	R-2588	-	7.5 (15.23)	5 (12.63)	0	0.83 (3.03)	0	13.33 (20.75)	Resistant
19	R- 2592	-	3.57 (6.36)	7.04 (15.29)	21.47 (27.57)	10.23 (18.56)	0	42.33 (40.54)	Moderately susceptible
20	R-2589	14	35 (36.26)	27.5 (31.60)	15 (22.78)	2.5 (9.09)	1.66 (4.31)	81.67 (64.63)	Highly susceptible
21	R-2590	16	26.49 (30.93)	23.18 (28.76)	26.49 (30.95)	6.29 (14.40)	5.62 (13.36)	88.07 (69.88)	Highly susceptible
22	R-2591	19	5.29 (13.00)	47.02 (43.27)	8.77 (17.09)	0	14.73 (22.50)	75.82 (60.65)	Susceptible
23	R- 2593	14	12.5 (20.70)	50.34 (45.17)	9.46 (17.81)	6.08 (14.14)	0	78.38 (62.29)	Susceptible
24	R- 2594	30	12.5 (20.63)	25.34 (30.20)	15 (22.78)	15 (22.73)	13.5 (20.63)	81.33 (63.68)	Highly susceptible
25	L-3261	-	0	5 (12.63)	0	0	0	5.00 (12.63)	Resistant
26	R- 2595	20	38.25 (38.19)	15.75 (23.37)	11.08 (19.23)	1.5 (4.08)	1.85 (4.54)	68.44 (55.84)	Susceptible
27	WL-2230	-	0 7.5	(15.74)	5 (12.63)	5 (10.45)	0	17.50 (24.07)	Resistant
28	WL-2232	18	28.12 (32.05)	35.09 (36.28)	11.25 (19.54)	17.54 (24.72)	0	92.09 (73.78)	Highly susceptible
29	L-3260	-	2.9 (9.80)	17.53 (24.71)	0	14.9 (22.65)	11.25 (19.39)	46.59 (43.02)	Moderately susceptible
30	L-3262	-	5 (12.92)	17.5 (24.68)	10 (18.04)	10 (18.04)	5 (12.63)	47.50 (43.54)	Moderately susceptible
31	L-3269	-	2.54 (7.40)	15.4 (23.04)	7.63 (15.88)	0.86 (3.09)	5.03 (12.69)	31.46 (34.10)	Moderately resistant
32	L-3263	-	5 (12.63)	20 (26.55)	0.83 (3.03)	0	0.83 (3.03)	26.67 (31.07)	Moderately resistant
33	L-3264	26	22.5 (28.28)	25 (29.97)	5 (12.92)	1.66 (4.31)	0.83 (3.03)	55.00 (47.85)	Moderately susceptible

Table 1. Contd...

Sl. No.	Genotype	Days to 50% bacterial wilt	Bacterial wilt incidence (%) 0-10 DAI	11-20 DAI	21-30 DAI	31-40 DAI	41-50 DAI	Cumulative bacterial wilt incidence at 50 DAI (%)	Disease reaction
34	L-3266	42	13.38(21.42)	26.25 (30.80)	6.52 (14.72)	4.45 (11.84)	2.39 (8.89)	52.99 (46.70)	Moderately susceptible
35	L-3267	12	27.5 (31.60)	31 (33.81)	0	1.13 (3.54)	0	59.63 (50.55)	Moderately susceptible
36	L-3268	-	1.94 (4.64)	14.56 (22.38)	11.58 (19.82)	0	0	28.09 (31.89)	Moderately resistant
37	L-3270	-	0	2.5 (9.09)	0	0	0	2.50 (9.09)	Resistant
38	L-3272	-	2.5 (9.09)	5 (12.92)	0.83 (3.03)	0	2.5 (9.09)	10.83 (19.18)	Resistant
39	Arka Anand (Resistant Check)	-	5 (12.63)	2.5 (9.09)	0	0	0	7.50 (15.74)	Resistant
40	Pusa hybrid-6 (Susceptible check)	11	47 (43.26)	38.25 (38.18)	2.56 (7.46)	0	0	87.81 (69.69)	Highly susceptible
	Level of Significance		**	**	**	**	**	**	
	SEm±		1.14	0.93	1.22	0.93	0.56	2.03	
	CD @ 5%		3.21	2.64	3.45	2.64	1.58	5.72	
	CV (%)		12.21	7.35	16.59	18.65	15.66	8.47	

DAI – Days after inoculation; Figures in parentheses are angular transformed values

3263, L-3268 and L-3269 graded as moderately resistant to bacterial wilt. However, further research is needed to evaluate level of resistance of the genotypes under different agro-climatic zones of the country, to study the stability of resistance to various races of *Ralstonia solanacearum.*

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Evaluation of F_1 hybrids and their parents for growth, yield and quality in cherry tomato (*Solanum lycopersicum* var. *cerasiforme*)

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ABSTRACT

The present study was carried out to estimate the performance of F_1 hybrids and their parents for various yield and yield-attributing traits in cherry tomato, at Division of Vegetable Crops, Indian Institute of Horticultural Research (IIHR), Bengaluru, during the year 2010-11. Among the seven parents used, three parents, namely, IIHR-2866 (yielding 3.03kg/plant), IIHR-2864 (2.87kg/plant) and IIHR-2865 (2.73kg/plant) were found to be high-yielding. Among the 21 F_1 hybrids evaluated, three hybrids, namely, IIHR-2754 x IIHR-2860 (4.27kg/plant), followed by IIHR-2754 x IIHR-2865 (3.97kg/plant) and IIHR-2864 x IIHR-2865 (3.40kg/plant) recorded higher yield than the Check varieties, whereas, three hybrids, viz, IIHR-2754 x IIHR-2865 (54.38t/ha), succeeded by IIHR-2863 x IIHR-2866 (46.46t/ha) and IIHR-2858 x IIHR-2866 (44.79t/ha), recorded higher estimated yield per hectare than the Check varieties. Hybrid IIHR-2754 x IIHR-2860 was found promising for most of the traits studied. The best performing parents can be used for breeding further while, the hybrids can be exploited commercially.

Key words: Cherry tomato, high yield, hybrids, parents, breeding

INTRODUCTION

Cherry tomato (*Solanum lycopersicum* var. *Cerasiforme)* is a botanical variety of the cultivated tomato. It is thought to be the ancestor of all the cultivated tomatoes. It is marketed at a premium price compared to the regular tomatoes. Cherry tomatoes are widely cultivated in Central America and are distributed in California, Korea, Germany, Mexico and Florida. It is a warm-season crop, reasonably tolerant to heat and drought, and grows under a wide range of soil and climatic conditions (Anon, 2009a). Cherry tomato is grown for its edible fruits which are ideal for making processed products like sauce, soup, ketchup, puree, curry, paste, powder, *rasam* and sandwich. These also have good nutritional and antioxidant properties. The size of cherry tomatoes ranges from thumb-tip to the size of a golf ball, and, can range from being spherical to slightly oblong in shape (Anon, 2009b). Hybrid vigour in cherry tomato has not been exploited fully. Little attention has been paid by plant researchers on the performance for yield and yield-components in the hybrids of cherry tomato. Therefore, the present study was undertaken to evaluate the best-performing parents and their F_1 hybrids in cherry tomato.

MATERIAL AND METHODS

The present study was undertaken at Division of Vegetable Crops, ICAR-Indian Institute of Horticultural Research (IIHR), Hesarghatta, Bengaluru. The experimental field is located at an altitude of 890 meters above MSL, at $13^0 38'$ N latitude and 78^0E longitude. The parents and the hybrids were evaluated during July 2011 - May 2012. The experimental material consisted of seven parents, viz, IIHR-2754 (P_1), IIHR-2858 (P_2), IIHR-2860 (P_3), IIHR-2863 (P_4), IIHR-2864 (P_5), IIHR-2865 (P_6) and IIHR-2866 (P_7), three Check varieties, viz, IIHR-2871 (C_1), IIHR-2876 (C_2) and Arka Ashish (C_3), and 21 F_1 hybrids developed through half-diallele mating design, during *Kharif* 2011. Spacing between plants was 60cm, while, between rows it was 45cm.

All the twenty one hybrids, along with their corresponding parents, were evaluated in Randomized Block Design in three replications, during the summer of 2012. Observations on five randomly-selected plants were recorded for various yield-attributing traits to estimate performance of the parents and hybrids.

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RESULTS AND DISCUSSION

Per se performance of parental lines, check varieties and hybrids (Table 1) and the three best-performing parents, and hybrids, for various growth, yield and quality parameters are presented in Table 2.

Genotypes differed significantly in plant height which ranged from 98cm (P_2) to 140cm (P_6) among parents (Table 1), from 57.67cm (C_3) to 131.33cm (C_1) among Check varieties, and from 89cm (P_2 xP_4) to 165.67cm (P_1 x P_6) among hybrids (Table 1). Number of primary branches per plant ranged from 3 (P_2 and P_3) to 3.67 (P_1 and P_5) among parents, from 3 (C_2) to 4.33 (C_3) among check varieties, and from 3 (P_1 x P_7) to 3.67 (P_1 x P_2) among hybrids (Table 1). Number of secondary branches ranged from 8 (P_5) to 11 (P_1) among parents, from 6 (C_2) to 9 (C_1) among check varieties, and from 6 (P_5 x P_6) to 11.33 (P_1 x P_5) among hybrids (Table 1). A higher number of branches may have resulted in production of more number of leaves and greater size of the leaf. Total number of leaves on a plant could perhaps decide the efficiency of photosynthesis, thereby resulting in better growth and yield. These results are in confirmity with Deepa and Thakur (2008) and Arun *et al* (2004).

A significant difference was seen in the number of inflorescences per plant, ranging from 35 (P_3) to 48 (P_1) among parents, from 25 (C_3) to 35.33 (C_1) among Check varieties, and from 37 (P_3 x P_5) to 63.33 (P_2 x P_3) among hybrids (Table 1). Parents used in the experiment differed

Table 1. Mean performance of parents, F_1 hybrids and Check varieties for growth, yield and quality traits in cherry tomato

Sl. No.	Parent/ F1 hybrid / Check variety Parent	Plant height (cm)	No. of primary branches	No. of secondary branches	No. of inflore-scences	Average fruit weight (g)	No. of fruits/ kg	No. of fruits/ cluster	No. of fruits/ plant	Yield/ plant (kg)	Yield/ ha (t)	No. of locules/ fruit	Fruit firmness (kg/mm2)	Pericarp thickness (mm)
1	P1	130.67	3.67	11.00	48.00	10.36	96.67	10.33	498.67	2.20	21.46	2.33	4.40	2.20
2	P2	98.00	3.00	9.00	38.67	14.11	71.00	9.67	374.33	2.50	24.79	3.00	5.00	2.43
3	P3	115.67	3.00	9.33	35.00	14.66	68.33	9.33	326.33	2.20	27.92	2.33	4.20	3.87
4	P4	109.00	3.00	8.67	36.00	12.46	80.33	8.67	312.67	2.57	20.83	2.67	4.53	2.43
5	P5	131.00	3.67	8.00	38.33	31.05	32.33	7.00	269.33	2.87	33.33	2.33	7.20	4.80
6	P6	140.00	3.33	12.67	38.33	13.77	72.67	8.33	318.33	2.73	29.79	3.67	5.00	2.23
7	P7	127.67	3.33	9.67	38.00	13.41	74.67	8.33	316.00	3.03	30	2.33	4.57	4.03
F1 hybrid														
1	P_1 X P_2	117.33	3.67	9.33	44.33	12.83	78.00	9.33	416.67	3.20	38.96	2.67	4.60	2.40
2	P_1 X P_3	144.67	3.67	10.33	44.67	19.15	52.33	8.00	357.33	4.27	26.46	2.67	3.33	3.10
3	P_1 X P_4	154.00	3.67	8.67	56.33	16.68	60.00	7.33	414.67	2.70	32.92	2.00	8.20	3.13
4	P_1 X P_5	140.00	3.33	11.33	38.00	15.90	63.00	6.67	253.33	3.07	44.38	2.67	7.00	4.00
5	P_1 X P_6	165.67	3.33	9.33	42.33	16.59	60.33	8.33	352.00	3.97	40.63	2.67	6.00	3.20
6	P_1 X P_7	139.33	3.00	9.67	46.67	13.98	71.67	8.33	391.67	3.33	54.38	2.33	4.40	3.13
7	P_2 X P_3	115.67	3.00	9.33	63.33	15.41	65.00	9.00	570.00	3.27	32.5	2.00	5.00	3.17
8	P_2 X P_4	89.00	3.00	8.33	38.67	15.56	64.33	8.33	323.33	2.50	35.83	2.33	5.20	4.00
9	P_2 X P_5	149.33	3.33	7.67	40.33	20.02	50.00	6.33	256.00	3.37	39.17	2.00	7.20	6.00
10	P_2 X P_6	144.33	3.00	8.33	44.67	16.68	60.00	8.33	371.00	2.60	32.5	2.33	6.80	4.07
11	P_2 X P_7	149.00	3.00	8.00	42.67	18.10	55.33	8.33	357.33	3.03	44.79	2.33	7.17	4.20
12	P_3 X P_4	105.00	3.00	9.33	42.67	18.44	54.33	8.33	355.33	2.57	35.83	2.33	7.27	3.13
13	P_3 X P_5	141.67	3.67	7.67	37.00	23.68	42.33	6.00	222.00	3.03	42.71	2.33	9.53	5.00
14	P_3 X P_6	142.33	3.67	11.00	39.33	17.98	55.67	7.33	288.00	3.30	43.75	2.67	6.00	3.20
15	P_3 X P_7	152.00	3.00	8.67	38.00	15.32	65.33	7.00	266.67	2.93	36.25	2.33	7.80	4.10
16	P_4 X P_5	156.00	3.00	9.67	50.33	19.76	50.67	6.33	318.67	3.13	37.29	2.00	6.13	3.97
17	P_4 X P_6	148.67	3.00	7.67	45.00	16.68	60.00	8.00	360.00	3.20	36.88	2.67	4.80	3.20
18	P_4 X P_7	144.00	3.00	6.67	44.00	16.43	61.00	8.33	366.00	3.00	46.46	2.33	7.97	3.93
19	P_5 X P_6	127.67	3.00	6.00	40.33	15.24	65.67	6.67	268.33	3.40	38.33	3.00	5.97	4.07
20	P_5 X P7	131.67	3.00	7.33	42.33	18.10	55.33	7.67	325.33	3.07	36.04	2.67	6.20	4.20
21	P_6 X P_7	140.33	3.00	10.00	38.33	14.79	67.67	8.33	319.33	2.90	39.38	3.33	8.00	3.20
Check														
1	C1	131.33	3.67	9.00	35.33	17.68	56.67	8.00	282.66	2.10	23.12	2.00	5.80	3.00
2	C2	118.00	3.00	6.00	34.33	16.69	60.00	7.33	252.00	1.93	33.54	2.33	5.80	2.80
3	C3	57.67	4.33	6.33	25.00	91.41	11.00	4.67	117.67	3.10	21.46	3.33	8.20	7.40

Table 2. Three best-performing parents (Lines and Check varieties) and hybrids in cherry tomato for growth, yield and quality traits

Trait	Parent (Lines and Check variety)			F_1 Hybrid		
	I	II	III	I	II	III
Plant height (cm)	P_6 (140)	C_1(131.33)	P_5(131.00)	P_1 x P_6 (165.67)	P_4 x P_5 (156.00)	P_1 x P_4 (154.00)
No. of primary branches	C_3(4.33)	P_1,P_5 and C_1(3.67)	P_2, P_3 and p_4 (3.00)	P_1 xP_2 (3.67)	P_1 x P_5 (3.33)	P_1 X P_7 (3.00)
No. of secondary branches	P_6(12.67)	P_1 (11.00)	P_7 (9.67)	P_1 x P_5 (11.33)	P_3 x P_6 (11.00)	P_3 x P_6 (10.33)
No. of inflorescences	P_1 (48)	P_2(38.67)	P_5 and P_6(38.33)	P_2 x P_3 (63.33)	P_1 xP_4(56.33)	P_4 x P_5 (50.33)
Average fruit weight (g)	C_3 (91.41)	P_5 (31.05)	C_1 (17.68)	P_3 x P_5 (23.68)	P_2 x P_5 (20.02)	P_4 x P_5 (19.76)
No. of fruits/ kg	P_1 (96.67)	P_4 (80.33)	P_7(74.67)	P_1 x P_2 (78.00)	P_1 x P_7 (71.67)	P_5 x P_6 (65.67)
No. of fruits/ cluster	P_1 (10.33)	P_2 (9.67)	P_3 (9.33)	P_1 x P_2 (9.33)	P_2 x P_3 (9.00)	P_1 xP_6 (8.33)
No. of fruits/ plant	P_1 (498.67)	P_2 (374.33)	P_3 (326.33)	P_2 x P_3 (570)	P_1 x P_2 (416.67)	P_1 x P_4 (414.67)
Yield/ plant (kg)	C_3 (3.10)	P_7 (3.03)	P_5 (2.87)	P_1 x P_3 (4.27)	P_1 x P_6 (3.97)	P_5 x P_6 (3.40)
Yield/ ha (t)	C_2 (33.54)	P_5 (33.33)	P_7 (30.00)	P_1 x P_7 (54.38)	P_4 x P_7 (46.46)	P_2 x P_7 (44.79)
No. of locules/ fruit	P_6 (3.67)	C_3 (3.33)	P_2 (3.00)	P_6 x P_7 (3.33)	P_5 xP_6 (3.00)	P_1 xP_2 and P_1 x P_3 (2.67)
Fruit firmness (kg/mm2)	C_3 (8.20)	P_5 (7.20)	C_1 and C_2 (5.80)	P_3 x P_5 (9.53)	P_1 x P_4 (8.20)	P_6 x P_7 (8.00)
Pericarp thickness (mm)	C_3 (7.40)	P_5 (4.80)	P7 (4.03)	P_2 xP_5 (6.00)	P_3 x P_5 (5.00)	P_2 xP_7, P_5 x P_7 (4.20)

significantly among themselves for average fruit-weight which ranged from 10.33g (P_1) to 31.05g (P_5). Fruit weight ranged from 16.69g (C_2) to 91.41g (C_3) among Check varieties, and from 12.83g (P_1 x P_2) to 23.68 (P_3 x P_5) among hybrids (Table 1). Average fruit weight contributed directly towards fruit yield per plant. This is in agreement with the findings of Deepa and Thakur (2008) and Shivakumar (2000).

The genotypes under study differed significantly among themselves for number of fruits per kg which ranged from 32.33 (P_5) to 96.67 (P_1) among parents, from 11 (C_3) to 60 (C_2) among Check varieties, and from 42.33 (P_3 x P_5) to 70 (P_1 x P_2) among hybrids (Table 1). Number of fruits per cluster ranged from 7 (P_5) to 10.33 (P_1) among parents, from 4.67 (C_3) to 8 (C_1) among Check varieties, and from 6.33 (P_2 x P_5 and P_4 x P_5) to 9.33 (P_1 x P_2) among hybrids (Table 1). The genotypes differed significantly among themselves for number of fruits per plant which ranged from 269.33 (P_5) to 498.67 (P_1) among parents, from 117.67 (C_3) to 282.66 (C_1) among Check varieties, and from 222 (P_3 x P_5) to 570 (P_2 x P_3) among hybrids (Table 1). Increased fruit-set observed may be due to a higher rate of anther dehiscence and better pollen viability. Similar results were reported earlier by Shivanand (2008). Any deviation in results with the findings of others could be attributed to differences in genotypes under study, environmental conditions and stage of fruit harvest.

As for yield per plant, genotypes differed significantly, ranging from 2.20kg (P_1 and P_3) to 3.03kg (P_7) among parents, from 1.93kg (C_2) to 3.10kg (C_3) among Check varieties, and from 2.50kg (P_2 x P_4) to 4.27kg (P_1 x P_3) among hybrids (Table 1). Genotypes differed significantly among themselves for estimated yield which ranged from 20.83 tonnes per hectare (P_4) to 33.33 tonnes per hectare (P_5) among parents, from 21.46 tonnes per hectare (C_3) to 33.54 tonnes per hectare (C_1) among Check varieties, and from 26.46 tonnes per hectare (P_1 x P_3) to 54.38 tonnes per hectare (P_1 x P_7) among hybrids (Table 1). Hybrid P_1 x P_7 showed highest yield per plant and estimated yield per hectare. These results are in consonance with findings of Madalageri and Dharmatti (1991).

Genotypes differed significantly among themselves in number of locules per fruit which ranged from 2.33 (P_1, P_3, P_5 and P_7) to 3.67 (P_6) among parents, from 2(C_1) to 3.33(C_3) among Check varieties, and from 2.00 (P_1 x P_4, P_2 x P_3, P_2 x P_5 and P_4 x P_5) to 3.33 (P_6 x P_7) among hybrids (Table 1). Variation in fruit firmness depends upon stage of harvest, and at mature stage this ranged from 4.20 kg/mm^2 (P_3) to 7.20 kg/mm^2 (P_5) among parents, from 5.8kg/mm^2

(C_1 and C_2) to 8.20kg/mm^2 (C_3) among Check varieties, and from 3.33kg/mm^2 (P_1 x P_3) to 9.53 kg/mm^2 (P_3 x P_5) among hybrids (Table 1). Thus, hybrid P_3 x P_5 may be best suited for long-distance transport and for processing. Genotypes differed significantly among themselves for pericarp thickness (mm) which ranged from 2.20mm (P_1) to 4.80mm (P_5) among parents, from 2.80mm (C_2) to 7.40mm (C_3) among Check varieties, and from 2.40mm to (P_1 x P_2) to 6.00 (P_2 x P_5) among hybrids (Table 1). These results are similar to the findings of Thakur *et al* (2005), Hazarika and Phookan (2005) and Shivakumar (2000). Fruit firmness and pericarp thickness are important fruit-quality parameters. The three best overall performing parents (Lines and Check varieties) and hybrids are presented in Table 2 for different traits studied in cherry tomato.

In this study, parents IIHR-2866, IIHR-2864 and IIHR-2865 performed well for various traits under study. As such, these could be exploited further in various breeding programmes. Promising hybrids, IIHR-2754 x IIHR-2866 (P_1 x P_7) and IIHR-2754 x IIHR-2860 (P_1 x P_3), can be subjected further to selection for isolating desirable genotypes in cherry tomato.

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Plant traits in fig as indicators of resistance to shoot borer, *Dyscerus? fletcheri* Marshall (Coleoptera: Curculionidae)

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ABSTRACT

A comparative study was conducted on fig (*Ficus carica* L.) cultivars Deanna and Poona to test whether antixenosis due to plant traits was at least partially responsible for a differential susceptibility to the shoot boring curculionid weevil, *Dyscerus? fletcheri*. Field evaluation revealed significant difference in borer incidence in cvs. Poona (6.25%) and Deanna (75%). Further, traits of plant architecture such as number of primary/ secondary/ terminal shoots, plant vigour and density of terminal shoots were significantly higher in cv. Deanna, which was highly susceptible to shoot borer. However, latex-flow index was significantly higher in cv. Poona that was resistant to the borer. A step-wise multiple regression analysis revealed that the tested plant traits explained 60% of the total variation in stem borer infestation ($y=-0.96-0.02x_1+0.23x_2-0.03x_3+0.24x_4+1.28x_5-1.31x_6$, $R^2=0.60$) in the susceptible cultivar, Deanna. Role of these traits in preference/non-preference of *D. fletcheri* for a cultivar is discussed.

Key words: Fig, *Ficus carica* L., resistance, cultivars, stem borer, *Dyscerus? fletcheri*

INTRODUCTION

Cultivation of the common fig (*Ficus carica*) is picking up in India amid growing acceptance of the fruit with high curative and lacerative nutritional values. Commercial cultivation of the common (edible) fig is confined mostly to the western parts of Maharashtra, Gujarat, Uttar Pradesh (Lucknow & Saharanpur), Karnataka (Bellary, Chitradurga & Srirangapatna) and Tamil Nadu (Coimbatore). Of the 470 varieties listed, cvs.'Poona' and *'Deanna'* are popularly grown for fresh fruit. In India, fig trees are prone to attack by as many as 50 species of insect pests (Butani, 1979). Of these, the stem boring beetles (that include *Batocera rufomaculata*, *B. rubus*, *Aclees cribratus*, *Apriona cinerea*, *A. rugicollis*, *Olenecamptus bilobus* and *Rhytidodera* species) (Verghese *et al,* 2001, 2003) cause severe damage to plants. However, a new curculionid weevil, *Dyscerus? fletcheri* Marshall (Coleoptera: Curculionidae) has been found damaging fig plants heavily during the post-rainy season, by directly damaging the terminal fruit bearing shoots (Kamala Jayanthi *et al*, 2015, in press). Our preliminary studies showed differential susceptibility of fig cultivars to this stem borer, suggesting a need to identify marker traits involved in host-plant selection by the pest. However, no literature is available on the effect of plant architecture traits on incidence of shoot borer, *D. fletcheri,* in fig. Therefore, this study was carried out to determine whether these traits contributing to antixenosis in fig cultivars by *D. fletcheri.*

MATERIAL AND METHODS

In the present study, a differential susceptibility of two common fig varieties, Deanna and Poona, to the curculionid weevil *D. fletcheri,* was assessed through field evaluation at Indian Institute of Horticultural Research (IIHR), Bangalore (12°58'N; 77°35'E), India. Observations were recorded during September – December, 2010 to assess the incidence of *D. fletcheri* on two year old plants growing adjacent to each other. Each of the cultivars was planted in five rows, each row consisting of 16 plants. Plant architecture traits were recorded in both the varieties (n=80) before flowering (September – December). Traits like number of primary shoots, secondary shoots, terminal shoots, plant vigour, density of terminal shoots and latex-flow were measured to relate these traits to varietal preference and non-preference of the curculionid borer, *D. fletcheri* for a variety. Of these traits, the number of primary shoots, secondary shoots, terminal shoots and density of terminal

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shoots were grouped under canopy traits as these can be altered through canopy management, whereas, plant vigour and latex-flow index were grouped as inherent plant traits. Plant vigour was visually scored on a 1-5 scale where, 1= least vigorous and 5= most vigorous. Density of terminal shoots in each tree was also visually scored on a 1-5 scale where, 1= less dense with less compactness, and 5= highly dense with more compactness. Latex flow was measured on a 1-3 scale where, 1= low and 3= profuse. Latex-flow index was measured by uniformly piercing the base of the tender terminal shoot with a pin, and the amount of latex that oozed out was expressed in relative terms (as described above). Sampling for borer infestation was carried out on terminal, fruit-bearing shoots on each tree, based on fresh feeding-damage (external deposition of a fine powder at the base of the shoot), wilting and withering of tender shoots.

Data collected on plant traits, viz., number of primary shoots, secondary shoots, terminal shoots, plant vigour, density of terminal shoots and latex-flow index were analyzed using one way ANOVA to determine differences in the above-mentioned parameters as significant or non-significant, between the two cultivars as per Little and Hills (1978). Correlation, step-wise multiple regression and path-coefficient analyses between the plant parameters studied and stem-borer incidence were carried out. To get a further insight, a step-wise regression procedure (Ryan, 1997) was employed to select the most crucial plant traits influencing variability in borer incidence. This technique consists of essentially identifying, stage by stage, trait(s) significantly related to borer incidence (y). Further, as a measure of goodness-of-fit of the models developed, values pertaining to Co-efficient of Determination (R^2) (Agostid'no and Stephens, 1986) were calculated. Variance Inflation Factor (VIF) value was computed to test the multi-colinearity of variables.

RESULTS AND DISCUSSION

Severe borer infestation was noticed (75%) in cv. Deanna (n=80) and significantly ($P = 0.05$) lower infestation (6.5%) was observed in cv. Poona (n=80), during August-December. Within a tree too, significantly higher infestation was noticed on tender terminal-shoots (7.82%) in cv. Deanna (n=4286), and 0.32% in cv. Poona (n=1863) (t=8.17, df=79, P<0.01).

Among canopy traits, the number of primary shoots, secondary shoots, terminal tender-shoots, and density of terminal shoots ranged from 1-5, 0-28, 6-89 and 1-5 respectively in cv. Deanna and 2-4, 5-19, 4-48 and 1-4, respectively, in cv. Poona. Inherent plant traits, viz., plant vigour and latex-flow index ranged from 1-5 & 1-2, and 1-5 & 2-3, respectively, in cvs. Deanna and Poona, respectively (Table 1).

Data revealed significant variation in canopy and plant traits between cultivars Deanna and Poona. Mean number of primary shoots (3.80), secondary shoots (16.15), terminal tender-shoots (53.58), plant vigour (3.38) and density of terminal shoots (2.90) was significantly higher in cv. Deanna compared to cv. Poona (Table 1). Mean latex-flow index was significantly higher in cv. Poona (2.94) compared to cv. Deanna (1.06) (Table 1).

Influence of various plant traits on differential susceptibility of the two common fig varieties revealed that the number of primary shoots (r=0.28; P=0.01); number of secondary shoots (r=0.64; P=0.001), number of terminal tender-shoots (r=0.58; P=0.001), plant vigour (r=0.54; P=0.001), density of terminal shoots (r=0.67; P=0.001) had a significant, positive correlation with incidence of the shoot borer, *D. fletcheri*. However, latex-flow index had a significant, negative correlation with the incidence of *D. fletcheri* (r=-0.53; P=0.001) (Table 2).

Table 1. Plant traits in two fig cultivars

Variety	Canopy traits			Inherent traits of the plant				
	No. of primary shoots (+SE)	No. of secondary shoots (+SE)	No. of terminal tender shoots (+SE)	Density of shoots (+SE)	Plant vigour (+SE)	Latex-flow index (+SE)	Per cent infested trees(n=80)	Per cent infested shoots/ tree
Deanna	3.80 + 0.08	16.15 + 0.66	53.58 + 1.94	2.90 + 0.15	3.38 +0.14	1.06 + 0.03	75.00	7.82†
	(1.0 - 5.0)	(0.0 - 28.0)	(6.0 - 89.0)	(1.0 - 5.0)	(1.0 - 5.0)	(1.0 - 2.0)		
Poona	3.41 + 0.08	10.83 + 0.37	23.29 + 1.06	2.04 + 0.10	2.46 +0.11	2.94 + 0.03	6.25	0.32††
	(2.0 - 4.0)	(5.0 - 19.0)	(4.0 - 48.0)	(1.0 - 4.0)	(1.0 - 5.0)	(2.0 - 3.0)		
CD *(P=0.05)*	0.22	1.48	4.33	0.34	0.34	0.08		

Figures in parentheses show the range of values; †n = 4286; ††n = 1863

Multiple regression analysis indicated that plant traits could explain 60% of the total variation in stem-borer infestation. Considering the traits viz., number of secondary shoots, density of terminal shoots, and latex-flow index, being significant based on *r*/SE (a stringent criterion for identifying significant variables for regression analysis), variability in stem borer infestation on the two cultivars can be explained to an extent of 59% ($y=-1.56+0.18x_2+1.35x_5-1.04x_6$, $R^2=0.59$) (Table 3). Further, traits like number of primary shoots, secondary shoots, terminal tender-shoots, plant vigour, density of terminal shoots and latex-flow index as lone, independent factors explained 8, 41, 33, 29, 49, 28% of the total variation in stem borer incidence, respectively, in linear equations. Maximum variation in stem borer infestation was explained by canopy traits and density of terminal shoots (49%), followed by the number of secondary shoots (41%) (Table 4). However, step-wise multiple regression analysis showed that various combinations of host-plant traits could not explain variability in stem borer infestation beyond 60% (Tables 5-6). Nevertheless, canopy traits, viz., number of secondary shoots and density of terminal shoots, alone, could explain variability in stem borer infestation to an extent of 53% ($y=-4.83+0.25x_2+1.43x_5$, $P=0.01$; $R^2=0.53$), with lesser VIF value (2.13) indicating a low level of collinearity among variables (Table 5). Further, a combination of canopy traits, viz., number of terminal shoots and density of terminal shoots, could explain variability in stem borer infestation to an extent of 51% ($y=-3.97+0.05x_3+1.63x_5$, $R^2=0.51$). A combination of canopy traits (density of terminal shoots) and inherent plant traits (latex-flow index) could explain variability in stem borer

Table 2. Direct and indirect effects of plant traits in fig cultivars

Pathways of association	Direct effects	Indirect effects	'r'
1. Primary branches (No.)			0.28*
a. Direct effect	-0.05		
b. Indirect effect via			
Tertiary branches (No.)		-	
Secondary branches (No.)		-	
Plant vigour		-	
Density of branches		-	
Latex flow		-	
2. Secondary branches (No.)			0.64**
a. Direct effect	0.33		
b. Indirect effect via			
Primary branches (No.)		0.13	
Tertiary branches (No.)		0.24	
Plant vigour		0.16	
Density of branches		0.20	
Latex flow		-0.15	
3. Tertiary branches			0.58**
a. Direct effect	-0.16		
b. Indirect effect via			
Primary branches (No.)		-0.06	
Secondary branches (No.)		-0.12	
Plant vigour		-0.08	
Density of branches		-0.09	
Latex flow		0.12	
4. Plant vigour			0.54**
a. Direct effect	0.08		
b. Indirect effect via			
Primary branches (No.)		0.01	
Secondary branches (No.)		0.04	
Tertiary branches (No.)		0.05	
Density of branches		0.04	
Latex flow		-0.03	
5. Density of branches			0.67**
a. Direct effect	0.40		
b. Indirect effect via			
Primary branches (No.)		0.12	
Secondary branches (No.)		0.24	
Tertiray branches (No.)		0.22	
Plant vigour		0.26	
Latex flow		-0.13	
6. Latex flow			-0.53**
a. Direct effect	-0.33		
b. Indirect effect via			
Primary branches (No.)		0.08	
Secondary branches (No.)		0.15	
Tertiray branches (No.)		0.24	
Plant vigour		0.12	
Density of branches		-0.33	

*Significant at 1% level; **Significant at 0.1% level

Table 3. Linear regression models explaining the variability in shoot borer, *D. fletcheri*, infestation in fig using plant traits

Variables considered	Model	R^2	VIF
i) Significant variables based on *r** (x_1=no. of primary shoots; x_2=no. of secondary shoots; x_3= no. of terminal shoots.; x_4=plant vigour; x_5=density of terminal shoots; x_6=latex-flow index)	$y=-0.96-0.02x_1+0.23x_2-0.03x_3+0.24x_4+1.28x_5-1.31x_6$	0.60	2.47
ii) Only significant variables based on (*r*/SE)** (x_2=no. of secondary shoots; x_5=density of terminal shoots; x_6=latex-flow index)	$y=-1.56+0.18x_2+1.35x_5-1.04x_6$	0.59	2.42

r=correlation coefficient; **SE=Standard error

Table 4. Linear models to estimate variability in shoot borer, *D. fletcheri*, infestation in fig using various plant traits

Variables considered	Model	R^2	VIF
i) No. of primary shoots (x_1)	$y=-3.15+1.46x_1$	0.08	1.09
ii) No. of secondary shoots (x_2)	$y=-3.86+0.44x_2$	0.41	1.69
iii) No. of terminal shoots (x_3)	$y=-1.95+0.110x_3$	0.33	1.50
iv) Plant vigour (x_4)	$y=-2.87+1.71x_4$	0.29	1.40
v) Density of terminal shoots (x_5)	$y=-3.19+2.16x_5$	0.49	1.81
vi) Latex-flow index (x_6)	$y=6.25-2.06x_6$	0.28	1.38

Table 5. Various linear equations for estimating variability in shoot borer (*D. fletcheri*) infestation

Variables considered	Model	R^2	VIF
With the no. of primary shoots kept at a constant			
i) No. of primary shoots (x_1)+ no. of secondary shoots (x_2)	$y=-4.51+0.22x_1+0.43x_2$	0.41	1.70
ii) No. of primary shoots (x_1)+ no. of terminal shoots (x_3)	$y=-3.37+0.45x_1+0.10x_3$	0.34	1.52
iii) No. of primary shoots (x_1)+ plant vigour (x_4)	$y=-6.19+1.01x_1+1.61x_4$	0.32	1.47
iv) No. of primary shoots (x_1)+ density of terminal shoots (x_5)	$y=-4.58+0.44x_1+2.07x_5$	0.46	1.85
v) No. of primary shoots (x_1)+ latex-flow index (x_6)	$y=2.95+0.83x_1-1.91x_6$	0.30	1.43
With the no. of secondary shoots kept at a constant			
i) No. of secondary shoots (x_2)+ no. of terminal shoots (x_3)	$y=-3.89+0.33x_2+0.04x_3$	0.43	1.76
ii) No. of secondary shoots (x_2)+ plant vigour (x_4)	$y=-5.27+0.35x_2+0.94x_4$	0.47	1.89
iii) No. of secondary shoots (x_2)+ density of terminal shoots (x_5)	$y=-4.83+0.25x_2+1.43x_5$	0.53	2.13
iv) No. of secondary shoots (x_2)+ latex-flow index (x_6)	$y=-0.27+0.35x_2-1.16x_6$	0.48	1.92
With the no. of terminal shoots kept at a constant			
i) No. of terminal shoots (x_3)+ plant vigour (x_4)	$y=-3.88+0.08x_3+1.06x_4$	0.41	1.70
ii) No. of terminal shoots (x_3)+ density of terminal shoots (x_5)	$y=-3.97+0.05x_3+1.63x_5$	0.51	2.04
iii) No. of terminal shoots (x_3)+ latex-flow index (x_6)	$y=1.05+0.08x_3-0.91x_6$	0.36	1.56
With the plant vigour kept at a constant			
i) Plant vigour (x_4)+ density of terminal shoots (x_5)	$y=-3.85+0.52x_4+1.81x_5$	0.46	1.85
ii) Plant vigour (x_4)+ latex flow index (x_6)	$y=1.42+1.28x_4-1.51x_6$	0.42	1.72
With the density of terminal shoots kept at a constant			
i) Density of terminal shoots(x_5)+ latex-flow index (x_6)	$y=0.37+1.79x_5-1.32x_6$	0.55	2.22

Table 6. Step-wise linear models to estimate variability in shoot borer (*D. fletcheri*) infestation in fig

Variables considered	Model	R^2	VIF
i. No. of primary shoots (x_1) + no. of secondary shoots (x_2) + no. of terminal shoots (x_3)	$y=-4.25+0.12x_1+0.32x_2+0.04x_3$	0.43	1.75
ii. No. of primaries (x_1) + no. of secondary shoots (x_2) + no. of terminal shoots (x_3) + plant vigour (x_4)	$y=-5.69+0.18x_1+0.27x_2+0.026x_3+0.86x_4$	0.48	1.92
iii. No. of primary shoots (x_1) + no. of secondary shoots (x_2) + no. of terminal shoots (x_3) + plant vigour (x_4) + density of terminal shoots (x_5)	$y=-5.21+0.02x_1+0.19x_2+0.02x_3+0.31x_4+1.19x_5$	0.54	2.17
iv. No. of secondary shoots (x_2) + no. of terminal shoots (x_3) + plant vigour (x_4)	$y=-5.16+0.28x_2+0.03x_3+0.86x_4$	0.48	0.92
v. No. of secondary shoots (x_2) + no. of terminal shoots (x_3) + plant vigour (x_4) + density of terminal shoots (x_5)	$y=-5.14+0.19x_2+0.02x_3+0.03x_4+1.19x_5$	0.54	2.17
vi. No. of secondary shoots (x_2) + no. of terminal shoots (x_3) + plant vigour (x_4) + density of terminal shoots (x_5) + latex-flow index (x_6)	$y=-1.03+0.23x_2-0.03x_3+0.24x_4+1.28x_5-1.30x_6$	0.60	2.50
vii. No. of terminal shoots (x_3) + plant vigour (x_4) + density of terminal shoots (x_5)	$y=-4.33+0.05x_3+0.31x_4+1.45x_5$	0.51	2.04
viii. No. of terminal shoots (x_3) + plant vigour (x_4) + density of terminal shoots (x_5) + latex-flow index (x_6)	$y=-0.58+0.01x_3+0.26x_4+1.56x_5-1.15x_6$	0.55	2.22

infestation to an extent 55% ($y=0.37+1.79x_5-1.32x_6$; $R^2=0.55$) (Table 6).

Pathways through which the six plant traits studied operate, to produce their association with shoot borer infestation reveal direct and indirect contribution (Table 2). Path-coefficient analysis showed that direct effect of number of primary shoots on stem borer infestation was negative and was not too pronounced. Indirect effects through other traits also exhibited a similar trend. Direct effect of the number of secondary shoots on stem-borer infestation was positive and high in magnitude (0.33). The total correlation between number of secondary shoots and stem-borer infestation was highly positive and significant (0.64). Indirect effect of the number of secondary shoots via other plant traits, viz., number of primary shoots (0.13), number of terminal shoots (0.24), plant vigour (0.16) and density of terminal shoots (0.20) was positive and of a reasonable magnitude, contributing to the total correlation coefficient. However, indirect effect through latex-flow index was found to be negative (-0.15).

Number of terminal shoots exhibited moderate, negative, direct effect (-0.16) as well as indirect effects via the number of primary shoots (-0.06), number of secondary shoots (-0.12), plant vigour (-0.08) and density of terminal shoots (-0.09). However, it exhibited a positive, indirect effect through latex-flow index (0.12). Similarly, plant vigour also showed moderate, positive, direct effect (0.08) besides indirect effects via the number of primary shoots (0.01), number of secondary shoots (0.04), number of terminal shoots (0.04) and density of terminal shoots (0.05). However, it exhibited a negative, indirect effect through latex-flow index (-0.03).

Density of terminal shoots exhibited a very high magnitude of positive, direct effect with reference to stem borer infestation (0.40). Indirect effects via the number of primary shoots (0.12), number of secondary shoots (0.24), number of terminal shoots (0.22) and plant vigour (0.26) were positive and high in magnitude. Total correlation coefficient (0.71) was also found to be highly significant. However, with latex-flow index, it exhibited a negative, indirect effect (-0.13) for stem borer incidence. Therefore, by managing canopy traits such as the number of secondary shoots, terminal shoots and density of terminal shoots, stem borer infestation can be reduced.

Latex-flow index showed a negative, direct effect of high magnitude (-0.33), but showed positive, indirect effects through the number of primary shoots (0.08), number of secondary shoots (0.15), number of terminal shoots (0.24), density of terminal shoots (0.11) and plant vigour (0.12). Therefore, inherent plant characters, viz., plant vigour and latex-flow index, can be used as marker traits to induce resistance against stem borer in the common fig cultivars.

Plant genotypes possess trait-variations that can alter insect preference/non-preference (also referred to as antixenosis), i.e., insects are attracted to, or repelled by, a plant due to a variety of plant characteristics (Karban *et al,* 1997; Ernest, 1989) such as plant shape, size, surface texture, presence of trichomes and toughness of the tissue, tough vascular bundles, etc. Antixenosis refers to potential plant-traits, either morphological or allelochemical, impairing or altering insect behaviour towards the host (preference) in a way as to reduce chances of infestation by insects, for oviposition, food or shelter.

Preliminary comparative study conducted during 2010-11 showed significant differences in susceptibility of fig genotypes, viz., Deanna and Poona, to shoot borer, *D. fletcheri* (Table 1). These variations can be attributed to several canopy traits and inherent plant-trait variations, as explained in this study (Tables 1-6). The present study clearly revealed highly significant differences in per cent stem-borer infestation among two common fig cultivars, Deanna and Poona. Further, canopy traits, viz., number of secondary shoots, number of tertiary shoots, plant vigour and density of terminal shoots had a significant, positive relationship with stem borer infestation, and, latex-flow index had a significant, negative relationship with stem-borer incidence. High concentration of fresh latex in *Ficus* spp. (Moraceae) was reported to range between 15-30% (Mooibroek and Cornish, 2000). It is generally accepted that the primary function of latex is to provide stickiness to entrap whole insects (Dussourd 1993, 1995) or mire their mouthparts (Dussourd & Eisner 1987); the latex is mobilized and transported to the site of damage immediately upon onset of damage. However, the mechanism of these effects (even for stickiness) is not well-documented. In the present study, the high latex-flow index in cv. *Poona* can be seen as primarily effective on early-instar grubs of *D. fletcheri*, as reported by Zalucki *et al* (2001 a and b) in the case of milkweed caterpillars. They reported that mortality in specialist caterpillars armed with tiny mandibles feeding on milkweeds is the highest in earlier instars, and, especially high at the first bite after hatching (as, latex is mobilized and transported to the site of damage immediately upon the damage, and can travel over 70cm to the point of damaged, as reported in *Cryptostegia grandiflora*) (Buttery and Boatman, 1976). However, larger herbivores that feed on whole-plants can be expected to be much less affected, because, accumulation of latex at the site of damage in this case will be ineffective. Therefore, in the present study, the high latex-flow index observed in cv. Poona may have hampered establishment of early-instar grubs of *D. fletecheri*.

Canopy traits, viz., number of primary shoots, secondary shoots, terminal shoots and the density of terminal shoots, were found to be higher in the susceptible cv. Deanna, where, heavy incidence of stem borer was noticed. Usually, host-preference of herbivourous insects is attributed to their behavioral response to visual, tactile or chemical cues received from the plants when pests encounter (Bernays and Chapman, 1994; Briese and Walker, 2002). This provides the insects with positive and negative signals which enable them to identiy a right host (Bernays, 1989). Earlier studies reported that plant traits such as odour, colour, morphological and anatomical characteristics were also important factors influencing insect host-choice (Bernays

and Chapman, 1994). In the present study, the susceptible variety Deanna was found to be highly vigorous, with higher number of secondary shoots and, terminal shoots, leading to a dense canopy structure. This may have attracted the stem borer to cv. Deanna, compared to cv. Poona which was less vigorous, having a less dense canopy architecture. Connections between general host-vigour and herbivore preference have been found, especially in gall-inducing insects (Craig *et al,* 1989; Horner and Abrahamson, 1992; Fritz *et al,* 2003; Price and Hunter, 2005). Plant vigour hypothesis by Price (1991) states that females prefer to oviposit on fast-growing plants because of the plant's better nutritional quality or higher general vigour. Further, it is also reported that some plant genotypes endowed with a higher level of defense chemicals, are more resistant to insects than plants with lower concentrations of the same (Diego *et al,* 2011). We too observed in the present study that cv. Poona with a higher latex-flow index recorded a lower incidence of stem borer. Inherent plant traits, viz., high latex-flow index and plant vigour in cv. Poona may be responsible for the non-preference of stem borer to this genotype. These can be used as marker traits in breeding programs for developing stem-borer resistant varieties. It is clear from the present study that canopy traits that influence stem borer infestation. The density of terminal shoots, and the number of secondary shoots can be managed to develop a less dense canopy in the susceptible variety, Deanna. As reported in earlier studies, herbivorous insect do not attack plants indiscriminately but prefer to feed/ oviposit on specific plant species, or, genotypes of a single species (Jaenike, 1990; Hjalten *et al,* 2007; Crawford *et al,* 2007; Tommi *et al,* 2011). Further, in addition to genetic effects (i.e., species and genotype effects) on host-plant quality, other factors such as shading, soil fertility, etc. may influence suitability of a host (Mutikainen *et al,* 2000; Lower *et al,* 2003; Osier and Lindroth, 2006). Semiochemical cues in successful host-plant location and colonization can be expected to play a role in primary host attraction, and probably provide a basis for future olfaction-based studies.

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Short communication

A guide to *in silico* identification of miRNAs and their targets

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ABSTRACT

MicroRNAs (miRNA) are non-coding RNA molecules that play a critical role in gene regulation including translational repression in animals and mRNA cleavage in plants. MicroRNAs control various cellular, metabolic and physiological processes in living organisms. In this paper, we provide an overview on the significance of miRNA, nomenclature, their biogenesis and the pipelines for prediction of miRNA and their targets. These tools are important for identification of conserved miRNAs in crops where miRNAs have not been previously discovered. The newly-identified miRNAs and their targets play an important role in understanding regulation of growth, development and gene silencing in various life forms.

Key words: miRNA, bioinformatics, miRNA targets, structure, software tools

MicroRNAs (miRNAs) are non-coding RNAs (19-22 nt) molecules that are derived from one arm of the precursor miRNA sequences. These are produced from the non-coding portion of DNA and are generally transcribed as independent units. In plants, miRNAs bind to protein-coding regions of mRNAs and cause mRNA degradation (Llave *et al*, 2002) and translational repression at the 'seed region' (i.e., 2-8 nts at 5' end of a mature miRNA). In plants, miRNAs are processed from transcripts that can fold into a stable hairpin (Llave *et al*, 2002). Several miRNA sequences have been found to be highly conserved in different species, and, pre-miRNAs have a unique secondary structure, which helps identify them through *in silico* approaches.

Nomenclature

Predicted miRNAs are named as per MiRBase guidelines (Griffiths–jones, 2006). Name of the microRNA consists of the prefix 'mir', followed by a dash. For example, osa-miR444 is a miRNA where 'osa' indicates the name of the species, *Oriza sativa,* 'miR' indicates mature sequences, and '444' indicates the order of its discovery. Sometimes, both miR444a and miR444b are present. Here '444a' indicates that it was discovered before miR444b. Sometimes, microRNAs are denoted as miR-444-5p or miR-444-3p, which indicates the origin of microRNAs from the 3' and 5' end, respectively.

Biogenesis of microRNAs

MicroRNA genes are found in the intergenic regions of DNA sequences. The process of miRNA biogenesis starts from the nucleus, and is completed in the cytoplasm. In the nucleus, sequences that contain mature miRNA sequences are transcribed by RNA polymerase II (polu II) into a primary RNA. The primary miRNA is then processed in the nucleus by endonuclease into a precursor miRNA sequence, containing 60-100nts long stem loop structure. The pre-miRNA is then cleaved into a miRNA:miRNA* duplex by a Dicer-like enzyme (DCL-1) in the nucleus, and, these sequences are exported from nucleus to the cytoplasm. In the cytoplasm, one of these strands of precursor miRNA produces the mature miRNA, which is approximately 22nts. This gets associated with the RNA-induced silencing complex (RISC) to interact with its mRNA targets.

Source of sequences for miRNA prediction

MicroiRNA can be predicted from different sequences, viz., expressed sequence tags (ESTs) (Reddy *et al*, 2012), genomic survey sequences (GSS), new generation sequences (NGS) (Kanupriya *et al*, 2013), or unigenes. These sequences can be generated or extracted from any public repository database and used for the prediction of miRNA. Known miRNA sequences are available in miRBase database (http://www.mirbase.org).

Prediction of conserved miRNAs

BLASTX/ BLASTN tools help identify query sequences that contain miRNA homologs. Precursor miRNA sequences are extracted from sequences containing miRNA homologs by taking into consideration 50 nucleotides upstream and 50 nucleotides downstream from the mature miRNA position.

Secondary structure and mature microRNA prediction

Secondary structure of these pre-miRNA sequences is then predicted and the minimum free energy is computed. Identification of mature miRNA depends on the following parameters (Reddy *et al*, 2012):

1. RNA sequences should fold into a complete stemloop hairpin
2. Length of mature miRNAs should be between 19 and 21 nts
3. Predicted miRNAs should have ≦ 2 nt mismatches
4. Minimum free-energy of the secondary structure should be ≧ 18 kcal mole^{-1}
5. A+U content should be in the range of 30-70%

Target prediction

MiRNA target genes control biological, metabolic and physiological processes in plants and, hence, identification of their targets is important. They help understand the role and functional importance of miRNAs. It has been shown that one miRNA can target more than one regulatory gene. Functional characterization of a miRNA target is essential for providing a biological insight into each miRNA-mediated pathway (Reddy *et al*, 2012). In plants, miRNAs are important in regulating plant growth and development**.** A flow-chart depicting various steps in the prediction of miRNA and their targets is presented in Fig. 1.

Functional annotation of miRNA targets

Identification of biological information of the coding portion of a sequence is an important aspect. MicroRNAs play an important role in regulating gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation, in both plants and animals. The role of individual miRNAs in an organism, namely biochemical, biological, metabolic, gene expression, and physiological function, can be predicted using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) tools.

Tools for miRNA and target prediction

Various tools, both online and offline, are available for predicting miRNA, their secondary structures and targets (Table 1); miRAuto (Lee *et al*, 2013) is a comprehensible tool for miRNA prediction from small RNA sequencing data in plant species. miRAuto software analyzes the expression

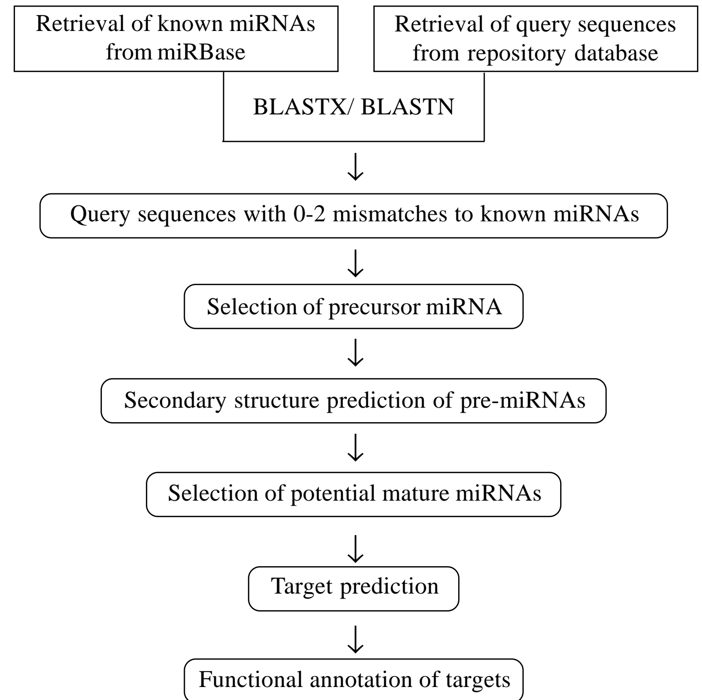

Fig. 1. Flowchart for the prediction of miRNA and their targets

Table 1. Tools for miRNA analysis

Tool	Website
Tools for miRNA and Target prediction.	
miRAuto	http://nature.snu.ac.kr/software/miRAuto.htm
MaturePred	http://nclab.hit.edu.cn/maturepred/
miRPara	http://www.whiov.ac.cn/bioinformatics/mirpara
miRDeep	*www.australianprostatecentre.org/research/software/ mirdeep-star*
MicroPC	http://www.biotec.or.th/isl/micropc
C-mii	http://www.biotec.or.th/isl/c-mii/documentation.Php
miRTour	http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/
psRNATarget	http://plantgrn.noble.org/psRNATarget/
TAPIR	http://bioinformatics.psb.ugent.be/webtools/tapir
Tools for structure prediction	
RNAfold	*subtiliswiki.net/wiki/index.php/RNAfold_WebServer*
UNAfold	http://www.bioinfo.rpi.edu/applications/hybrid/ download.php
MFold	http://www.bioinfo.rpi.edu/applications/mfold
Tools for functional annotation	
GO	*www.geneontology.org*
KEGG	*www.genome.jp/kegg*

of 5' -end position of compared RNAs in reference sequences, to candidate miRNAs, for the possibility of presence of miRNA fragments. MaturePred tool, based on machine learning method, is used for accurately predicting plant miRNAs. Using this tool, we can extract the position, structure and energy related information from real/ pseudo miRNA:miRNA* duplex; miRPara (Wu *et al*, 2011) is based on SVM, and predicts mature miRNA coding regions from genome-scale sequences. In this tool, sequences are classified from miRBase into animal, plant and overall categories, and it uses a support vector machine to train the three models based on an initial set of 77 parameters related to physical properties of the pre-miRNA and its miRNAs; miRDeep is a non-comparative computational method developed for identification of miRNAs from a pool of sequenced RNA transcripts, obtained by deep-sequencing experiments (An *et al*, 2013). This method at first aligns the transcript reads to genomic locations, and selects genomic sequences from locations that can form hairpin secondary structures.

MicroPC (μPC) (Mhuantong *et al*, 2009) is an online tool for predicting and comparing plant miRNAs and their targets. It offers three, main interactive pages for comparing, searching and predicting plant miRNAs. Target-align was proposed for plant miRNA target identification, and developed as both web and command line versions. C-mii (Numark *et al*, 2012) is a stand-alone software package, with graphical user interface for identifying, manipulating and analyzing plant miRNAs and targets. C-mii tool performs sequence-similarity search, secondary-structure folding, automatic stem-loop identification and manipulation, and, functional and gene ontology (GO) annotation. It can be used for plant miRNA and target prediction only; miRTour (Milev *et al*, 2011), based on comparative approach, is used for both miRNA and target prediction. All the steps of miRNA and target prediction like homolog search, miRNA precursor, target prediction and annotation, are performed by the same set of input sequences. psRNATarget (Dai *et al*, 2011) is a plant small RNA target analysis server, which consists of two important functions: (i) Reverse complementary matching between small RNA and target transcript using a proven scoring schema, and (ii) Target-site accessibility evaluation by calculating unpaired energy (UPE) required to 'open' secondary structure around small RNA's target site on mRNA. The psRNA Target incorporates recent discoveries in plant miRNA target recognition. TAPIR (Bonnet *et al*, 2010) is a web server designed for the prediction of plant microRNA targets. The server offers a possibility of searching for plant miRNA targets, using a fast and a precise algorithm.

Tools for miRNA structure prediction

RNAfold (Zuker and Stiegler, 1981) is a tool which reads RNA sequences, calculates their minimum free energy and structure, and, returns the structure in bracket notation and its free energy. UNAFold software (Markham *et al*, 2008) is a collection of several programs that simulate folding, hybridization, and melting pathways for one or two single-stranded nucleic acid sequences. Secondary structure prediction for single-stranded RNA or DNA combines free energy minimization, partition function calculations and stochastic sampling. It is an offline tool. Mfold is a web server for prediction of secondary structure of single-stranded nucleic acids.

MicroRNAs regulate gene expression in a variety of ways such as translational repression, mRNA cleavage and deadenylation in plants. A number of computational tools based on comparative and non-comparative algorithms are available for identification of mature miRNA and their targets. In this study, an algorithm for prediction and analysis of miRNAs through bioinformatics tools has been presented.

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Short communication

Studies on fruit and yield traits in indigenous coloured varieties of mango (*Mangifera indica* L.) in South Gujarat, India

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ABSTRACT

An investigation on fruit descriptors and yield in twelve mango varieties was conducted under South Gujarat conditions. Maximum fruit length was recorded in cv. Totapuri (16.23cm). Vanraj showed the highest values for fruit width (11.67cm), fruit circumference (37.37cm), fruit weight (729g), fruit volume (575.59cm^3) and fruit pulp (78.93%). Maximum TSS (21.20%), acidity (0.42%) and fruit firmness (7.00 rating) was observed in cvs. Deshi-1, Deshi-3 and Makaram, respectively. 'Totapuri' had maximum total shelf-life (21.33 days), number of fruits per tree (383.00) and fruit yield (236.80kg/tree). The varieties had green to yellow ground-colour of peel. All the varieties had red-blush peel colour, excepting cvs. Dadamio, Makaram and Swarnarekha which were purplish-red. Similarly, pulp colour ranged from light yellow to light orange. Based on overall performance, cvs. Alphonso, Deshi-1, Deshi-2, Kesar, Khandesi Borasio, Totapuri and Vanraj proved to be superior to the other varieties.

Key words: Colour, indigenous, varieties, mango

Mango (*Mangifera indica* L.) belongs to the family Anacardiacea, and is native to the Indo-Myanmar region (Mukherjee, 1953). In India, there exist hundreds of mango cultivars (Chadha and Pal, 1986), and Gujarat figures in the mango belt of the country. In particular, the southern part of Gujarat is well-suited for mango cultivation and is home to several indigenous coloured varieties, owing to a favourable tropical climate. Today, there is a good demand in international markets for varieties with attractive peel colour. Although numerous studies have been conducted on mango in the region, there is dearth of information on coloured mango varieties. These are distinct from each other in terms of gradation, intensity of colour and other attributes (Pleguezuelo *et al*, 2012). In view of the popularity and importance of coloured mango varieties globally, the aim of the present study was to assess physico-chemical and other characteristics of coloured mango fruits, especially, locally grown varieties in South Gujarat. This study will help identify suitable parents and potential mango varieties for further evaluation, conservation and utilization in crop improvement programmes. In the long run, this could prove important to guage consumer preference and emerging market-expectations.

The present study was carried out at Germplasm Evaluation Block, Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, NAU, Navsari, during the fruiting season in year 2012. Varieties selected for this study were: Alphonso, Batli, Dadamio, Deshi-1, Deshi-2, Deshi-3, Kesar, Khandesi Borasio, Makaram, Swarnarekha, Totapuri and Vanraj. Age of the trees used in this experiment was 20-30 years. Plants were maintained under uniform conditions as per the recommended package of practices of Navsari Agricultural University. Fully mature mango fruits were harvested and collected randomly (as and when the fruits matured on the tree). After uniform ripening at room temperature, 15 fruits per variety were used in the study. Fruit description, viz., fruit length, fruit width, fruit circumference, fruit weight and fruit volume were recorded as per standard methods at ready-to-eat, ripe stage. Fruit pulp percentage was calculated as per Peter *et al* (2007). Total shelf-life was noted under room temperature for both pre- and post-ripening period in fruits starting with the day of harvest. Physiological loss in fruit weight was determined at 3-day intervals using standard formulae and was expressed in percentage (AOAC, 1994). Fruit firmness was rated as per DUS, with rating of low firmness (3), medium (5) and high firmness (7) (DUS, 2008). Fibre attachment to stone was observed and different ratings were given (DUS, 2008). A panel of five judges scored each variety, and the average score was taken as the final rating for the variety. Number of fruits per tree was recorded at

harvest. Fruit yield in term of kg per tree was obtained by multiplying average fruit-weight with number of fruits per tree. Total soluble solids (TSS) were determined with a digital hand-refractometer (HI 96801) at three different points on the fruit, i.e., shoulder, middle and distal end of the fruit, after thorough mixing. The values were expressed as percentage (Ranganna, 1986). Titratable acidity was estimated as per Ranganna (1986). Fruit parameters, viz., peel and pulp colour, pulp fibre, lenticel density and nature, depth of sinus, fruit shape, fruit apex and depth of fruit-stalk cavity, were determined by five judges who used DUS guidelines (DUS, 2008). The experiment was laid out in Randomised Block Design (RBD), with three replications, with three trees per replication. Data on various parameters were analyzed using Analysis of Variance (ANOVA) employing Statistical Package for Agricultural Workers (STAT OP Sheoran). Differences among individual means were tested using Least Significant Difference (LSD) test at $P< 0.05$ level.

Result showed that physico-chemical characteristics of the fruit were highly significant ($P< 0.05$) for differences among varieties (Table 1). Maximum fruit length was observed in cv. Totapuri (16.23cm), while this was minimum in Deshi-3 (9.43cm). 'Vanraj' recorded maximum fruit-width (11.67cm), while, cv. Makaram recorded the least (7.00cm). Fruit circumference was highest in 'Vanraj' (37.37cm), and lowest in Makaram (23.20cm). Several workers have reported mango cultivars to differ in fruit length and width, according to their genetic make-up (Jilani *et al*, 2010).

Highest fruit-weight was recorded in cv. Vanraj (729.03g). In contrast, 'Makaram' had lowest fruit-weight (235.73g). The remaining varieties had fruits ranging in weight from 300 to 363g. Maximum fruit volume was noted in 'Vanraj' (739.33cm^3), while, the minimum was recorded in 'Makaram' (240.00cm^3). Sarkar *et al* (2001) also reported variation in fruit-weight among different mango cultivars, which could be due to genetic or physiological factors (Uddin *et al,* 2006).

A distinct variation was observed in pulp content in different varieties (Table 1). Maximum pulp percentage was obtained in 'Vanraj' (78.93). This is in accordance with Kulkarni and Rameshwar (1981) among varieties evaluated by them. Similarly, pulp colour ranged from light-yellow to light-orange. These findings fall in the range reported by several researchers in mango (Sarkar *et al,* 2001; Jilani *et al,* 2010).

Fruit-firmness, as indicated in Table 2, rated maximum in 'Makaram' (7.00) and minimum (3.00) in 'Alphonso'. TSS content and acidity are also considered as a measure of fruit quality (Shafique *et al*, 2006). TSS recorded maximum in cv. Deshi-1 (21.20%), and minimum in cv. Totapuri (15.63%). Highest acidity was recorded in 'Totapuri' (0.42%), and least in 'Deshi-1' (0.24%) and Kesar (0.25%). Variation in chemical constituents among varieties too has been reported by researchers earlier (Syed, 2009).

Data on ripening behaviour in various mango varieties showed highly significant differences (Table 2). Maximum

Table 1. Fruit and yield descriptors in mango

Variety	Pulp colour	Fruit shape	Fruit length (cm)	Fruit width (cm)	Fruit circumference (cm)	Fruit weight (g)	Fruit volume (cm^3)	Fruit pulp (%)	TSS (%)	Acidity (%)	Number of fruits per tree	Yield (kg/tree)
Alphonso	Medium yellow	Ovate oblique	10.53	8.30	26.77	331.34	351.00	77.18	19.67	0.27	307.67	113.22
Batli	Light yellow	Ovate oblong	13.43	7.83	24.60	363.10	378.00	69.12	18.50	0.36	187.67	112.99
Dadamio	Light yellow	Ovate	10.30	8.70	26.73	358.23	372.67	66.29	17.63	0.38	210.33	124.52
Deshi-1	Medium yellow	Ovate	10.93	8.77	24.27	315.90	335.67	76.15	21.20	0.24	329.33	106.07
Deshi-2	Medium yellow	Ovate	10.50	8.47	23.70	301.23	312.00	71.43	20.40	0.28	292.33	95.61
Deshi-3	Light orange	Ovate	9.43	7.83	24.17	236.27	241.00	64.46	17.50	0.42	108.33	44.78
Kesar	Medium yellow	Oblong	12.13	7.97	24.80	319.67	326.33	72.23	18.80	0.25	273.33	97.80
Khandesi Borasio	Light orange	Ovate oblong	9.80	7.60	24.23	302.83	340.00	76.30	20.70	0.35	311.67	113.67
Makaram	Medium yellow	Oblong	12.60	7.00	23.20	235.73	240.00	60.14	16.70	0.37	119.33	45.95
Swarnarekha	Light orange	Ovate oblong	12.90	9.20	24.77	424.27	458.33	75.36	17.67	0.30	262.67	163.40
Totapuri	Medium yellow	Oblong with pointed tip	16.23	9.10	24.53	618.77	630.67	67.91	15.63	0.42	383.00	330.27
Vanraj	Medium yellow	Ovate oblique	15.07	11.67	37.37	729.03	739.33	78.93	17.23	0.33	172.67	399.39
CV	-	-	4.51	3.94	4.14	41.21	34.66	3.49	1.61	11.73	6.06	3.86
± SEM	-	-	0.32	0.19	0.62	13.96	11.74	1.18	17.00	0.20	3.06	2.03
CD (P=0.05)	-	-	0.93	0.57	1.82	6.39	5.15	2.87	0.01	0.07	8.15	5.27

Table 2. Ripening and shelf-life in mango fruits after harvest

Variety	Time taken to ripening (days)	Post-ripening life (days)	Total post-harvest life (days)	Fruit firmness (rating)
Alphonso	7.67	8.67	16.33	3.00
Batli	6.67	7.67	14.33	4.33
Dadamio	6.00	6.33	12.33	5.00
Deshi-1	5.67	8.00	13.67	3.00
Deshi-2	6.67	7.67	14.33	3.67
Deshi-3	4.67	6.33	11.00	6.33
Kesar	7.33	7.67	15.00	3.00
Khandesi Borasio	5.33	6.00	11.33	3.00
Makaram	5.67	9.00	14.67	7.00
Swarnarekha	7.33	7.67	15.00	5.00
Totapuri	8.67	12.67	21.33	3.67
Vanraj	4.67	6.67	11.33	3.67
CV	11.74	11.38	7.16	2.14
± SEM	0.43	0.51	0.56	0.35
CD. (0.05)	1.27	1.52	1.67	1.06

Table 3. Physiological weight loss (%) in mango fruits at various intervals after harvest

Variety	3 DAH	6 DAH	9 DAH	12 DAH	15 DAH	18 DAH	21 DAH
Alphonso	5.87	10.17	14.83	17.03	19.14	-	-
Batli	5. 26	11.58	16.52	18.40	20.94	-	-
Dadamio	7.96	14.05	16.82	19.36	23.48	-	-
Deshi-1	6.07	11.35	15.15	19.50	21.17	-	-
Deshi-2	7.34	11.72	16.48	19.19	21.06	-	-
Deshi-3	7.03	15.63	19.06	23.86	25.63	-	-
Kesar	6.28	11.06	15.15	18.08	19.94	-	-
Khandesi Borasio	6.94	15.20	18.23	21.85	23.74	-	-
Makaram	5.63	11.67	15.03	17.72	19.57	-	-
Swarnarekha	6.00	11.38	15.33	17.27	20.13	-	-
Totapuri	5.66	9.66	13.27	16.07	18.05	18.63	20.05
Vanraj	6.47	12.69	18.26	21.72	23.86	-	-
CV	0.53	2.45	0.67	1.35	2.69	-	-
± SEM	0.02	0.17	0.06	0.15	0.33	-	-
C.D. (0.05)	0.06	0.5	0.19	0.44	0.98	-	-

DAH: Days after harvest; (-), not determined, as, 91.67% of varieties lost their post-harvest life, with exception of 'Totapuri'

number of days taken to ripen after harvest was observed in 'Totapuri' (8.67), while this was minimum in 'Vanraj' and 'Deshi-3' (4.67). Similarly, 'Totapuri' recorded longest post-ripening life (12.67 days), the shortest was observed in 'Dadamio' and 'Deshi-3' (6.33 days). Total post-harvest life significantly higher in 'Totapuri' (21.33 days), and lowest in 'Deshi-3' (11.00 days). These finding are in accordance with Herianus *et al* (2003). Variation in post-harvest life in mango varieties could be due to their unique genetic make-up.

The physiological weight-loss in fruits differed significantly with variety (Table 3). At three days after harvest (DAH), least physiological weight-loss was noticed in 'Batli' (5.26%), while maximum weight-loss was recorded in 'Dadamio' (7.96 %). However, 'Totapuri' recorded minimum physiological weight-loss. 'Deshi-3' showed maximum physiological weight-loss at all intervals of observation, with an exception at 3 DAH (7.03%). Reduction in weight is attributed to physiological loss in weight due to respiration, transpiration of water through the peel tissue and due to other biological changes occurring in the fruit (Rathore *et al,* 2007), depending upon the genetic constitution of variety (Rymbai *et al,* 2014).

Good appearance of mango fruit has the highest phenotypic acceptability in consumers (Uddin *et al,* 2006). Among various varieties, green ground colour of mango peel was observed in cvs. Dadamio, Makaram and Swarnarekha, yellow colour in cvs. Alphonso, Batli, Deshi-1, Deshi-2, Deshi-3, Kesar and Totapuri, while, only 'Khandesi Borasio' showed greenish-yellow colour. All the varieties had red-blush peel colour, except cvs. Dadamio, Makaram and Swarnarekha, which showed purplish-red colour (Table 4). Pulp fibre was scarce in cvs. Alphonso, Deshi-1, Deshi-2, Kesar, Khandesi Borasio and Totapuri medium in cvs. Batli, Dadamio, Swarnarekha, and abundant in cvs. Deshi-3 and Makaram. Lenticel density ranged from sparse in cvs. Alphonso and Swarnarekha, to dense in cvs. Dadamio, Deshi-1, Deshi-2, Kesar, Khandesi Borasio and Totapuri. Cultivars Batli, Deshi-3 and Makaram had medium lenticels-density. Varieties Deshi-1, Deshi-2, Khandesi Borasio and Swarnarekha are the only ones with prominent lenticels. Sinus was absent in 'Deshi-3', very shallow in cvs. Batli and Dadamio, and shallow in all other varieties. Fruit in cv. Alphonso was ovate-oblique, Kesar and Makaram cvs. had oblong fruit, Batli, Khandesi Borasio and Swarnarekha had ovate-oblong fruits, Totapuri fruit was oblong with a pointed tip, and the rest were ovate. Fruit apex of all the varieties was obtuse, except in 'Dadamio' and 'Totapuri' where it was round and acute, respectively. Depth of fruit stalk cavity was shallow in cvs. Alphonso, Deshi-1, Deshi-2 and Swarnarekha, but the cavity was absent in all other varieties. Variation in external appearance among varieties may be attributed to genetic make-up, as, each genotype is unique.

Differences in fruit yield among varieties were highly significant (Table 1). Number of fruits per tree varied from as low as 108.33 in 'Deshi-3', to as high as 383.00 in 'Totapuri'. Eight of the 12 varieties studied had more than

Table 4. Secondary descriptors in mango fruits

Variety	Peel colour		Pulp fibre	Lenticel			Fruit apex	Depth of fruit-stalk cavity
	Ground	Blush		Density	Nature	Sinus		
Alphonso	Yellow	Red	Scarce	Sparse	Less prominent	Shallow	Obtuse	Shallow
Batli	Yellow	Red	Medium	Medium	Less prominent	Very shallow	Obtuse	Absent
Dadamio	Green	Purplish-red	Medium	Dense	Less prominent	Very shallow	Round	Absent
Deshi-1	Yellow	Red	Scarce	Dense	Prominent	Shallow	Obtuse	Shallow
Deshi-2	Yellow	Red	Scarce	Dense	Prominent	Shallow	Obtuse	Shallow
Deshi-3	Yellow	Red	Abundant	Medium	Less prominent	Absent	Obtuse	Absent
Kesar	Yellow	Red	Scarce	Dense	Less prominent	Shallow	Obtuse	Absent
Khandesi Borasio	Greenish-yellow	Red	Scarce	Dense	Prominent	Shallow	Obtuse	Absent
Makaram	Green	Purplish-red	Abundant	Medium	Less prominent	Shallow	Obtuse	Absent
Swarnarekha	Green	Purplish-red	Medium	Sparse	Prominent	Shallow	Obtuse	Shallow
Totapuri	Yellow	Red	Scarce	Dense	Less prominent	Shallow	Acute	Absent
Vanraj	Greenish Red	Red	Medium	Medium	Less prominent	Shallow	Obtuse	Shallow

200 fruits per tree. Similarly, highest fruit-yield (kg per tree) was recorded in 'Totapuri' (236.80kg/tree), while, 'Deshi-3' had the lowest yield (25.56 kg/tree). This is in line with findings of Sarkar *et al* (2001). Exceptional results obtained in 'Totapuri' may be attributed to unique genetic features of an individual variety.

The present investigation concludes that of the 12 mango varieties studied, fruits of Alphonso, Deshi-1, Deshi-2, Kesar, Khandesi Borasio, Totapuri and Vanraj were superior in various fruit parameters, as well as yield. Of these, cvs. Deshi-1 and Deshi-2 are promising, local genotypes. These varieties can be studied in-depth for further evaluation and use in mango breeding programmes, to help assess consumer preference and emerging market-expectations.

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Short communication

Effect of *in situ* rainwater harvesting and mulching on growth, yield and fruit quality in mango var. Arka Neelachal Kesri in Eastern India

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ABSTRACT

A field study was conducted at Central Horticultural Experiment Station (ICAR-IIHR), Bhubaneswar, India, during 2007-2013 in a new mango orchard of the variety 'Arka Neelachal Kesri' at 5m x 5m spacing, to conserve rain-water and to enhance soil moisture availability during dry periods for augmenting plant growth and fruit production. Among the four *in situ* rain-water harvesting techniques (cup-and-plate, half-moon, full-moon, and trench) evaluated in combination with three types of mulch (no mulch, inorganic mulch, and organic mulch), the cup-and-plate system resulted in maximum annual increment in vegetative growth and fruit yield (4.67kg/plant), while, organic (paddy straw) and inorganic (black polythene, 100µ thickness) mulches improved vegetative growth, fruit yield and TSS in fruit significantly over no mulch.

Key words: Mango (*Mangifera indica* L.), Arka Neelachal Kesri, *in situ* rain-water harvesting, mulching

Though considered drought-hardy, mango (*Mangifera indica* L.) requires watering for orchard establishment and good fruiting, even in heavy rainfall zones like coastal Odisha, where soil moisture deficit occurs during February-May. *In situ* rain-water harvest by building trenches, bunds, circular basins, etc. can increase soil water content by reducing surface runoff (Panigrahi *et al*, 2008). Mulching conserves soil moisture and controls weeds (Lal *et al*, 2003). Therefore, the present study was undertaken to assess the effect of *in situ* rain-water harvesting structures and mulching on performance of the mango variety 'Arka Neelachal Kesri' under rain-fed conditions.

The experiment was conducted at ICAR-IIHR-Central Horticultural Experiment Station, Bhubaneswar, Odisha, during 2007-2013. The soil at the experimental site is red lateritic, with poor organic matter content (0.2%) and meagre water holding capacity. The orchard of 'Arka Neelachal Kesri' mango was developed *in situ,* on its own rootstock, by sowing seeds at 5m x 5m spacing with onset of monsoon in 2007, and top-grafting the seedlings so-raised a year later. The experiment was laid out in split-plot design, with 12 treatment combinations consisting of four *in situ* rain-water harvesting structures, viz., half-moon or semi-circular basin, full-moon or circular basin, cup-and-plate, and trench system as the main plot, and three levels of mulching (no mulch, organic mulch and inorganic mulch) as sub-plot treatments (Table 1) with five replications. The trees were maintained under rain-fed conditions from the inception of the experiment.

Initial growth parameters, i.e., plant height, canopy diameter, scion girth and primary girth, were recorded during November-December, 2009. Thereafter, annual increment in growth was noted for three consecutive years, from 2010 to 2012. Fruits were harvested at full maturity and observations were recorded on fruit yield and quality

Table 1. Treatment details with specification of *in situ* rain-water harvesting structures and measures of mulching

Treatment	Specification
Four *in situ* rain water harvesting structures as main plot treatments:	
Half-moon	Semi-circular basin at 1m distance from main trunk
Full-moon	Circular basin at 1m distance from main trunk
Cup-and-Plate	Circular pit of 0.5m width and 0.5m depth around the tree at 1m distance from main trunk
Trench	Trench of 2m length, 0.5m width and 0.5m depth at 1m distance from main trunk
Three levels of mulch as sub-plot treatments:	
No mulch	Without cover
Inorganic mulch	UV-stabilized black polythene (100µ thickness) around the tree at 1m radius
Organic mulch	10cm thick layer of paddy-straw around the tree at 1m radius

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Table 2. Effect of *in situ* rain-water harvesting and mulching on annual increase in vegetative growth in mango 'Arka Neelachal Kesri'

Treatment	Annual increase in vegetative growth															
	Plant height (cm)				Canopy diameter (cm)				Trunk girth (cm)				Primary girth (cm)			
	2010	2011	2012	Pooled	2010	2011	2012	Pooled	2010	2011	2012	Pooled	2010	2011	2012	Pooled
In situ rain-water harvesting structures:																
Half-moon	44.92	40.63	47.88	44.48	52.98	58.8	51.73	54.50	6.97	7.2	6.01	6.73	3.66	5.01	4.14	4.27
Full-moon	46.88	40.67	49.53	45.69	52.39	60.59	53.21	55.40	7.33	7.59	6.67	7.20	3.91	5.15	4.42	4.49
Cup-and-Plate	50.28	54.09	64.46	56.28	60.41	79.22	71.73	70.45	7.81	10.17	9.31	9.10	4.69	7.72	6.86	6.42
Trench	53.13	46.46	56.35	51.98	53.31	68.67	62.37	61.45	7.68	8.89	8.06	8.21	4.21	6.36	5.69	5.42
SE(m)±	2.38	2.29	2.13	1.05	5.06	2.03	2.59	1.55	0.29	0.37	0.36	0.18	0.35	0.38	0.32	0.16
CD *(P=0.5)*	NS	7.13	6.34	3.26	NS	6.33	8.06	4.83	NS	1.14	1.12	0.58	NS	1.18	1.00	0.51
Mulch:																
No mulch	46.12	40.31	50.19	45.54	50.56	58.75	51.84	53.72	6.76	7.63	6.55	6.98	3.93	5.24	4.43	4.53
Inorganic mulch	50.45	47.19	56.13	51.26	56.2	69.22	62.97	62.8	7.84	8.92	8.01	8.26	4.16	6.43	5.63	5.41
Organic mulch	49.83	48.89	57.35	52.02	57.57	72.48	64.47	64.84	7.75	8.83	7.98	8.19	4.26	6.51	5.77	5.51
SE(m) ±	2.45	1.99	1.8	1.81	3.09	2.44	3.27	1.36	0.36	0.36	0.26	0.14	0.31	0.36	0.24	0.11
CD *(P=0.5)*	NS	5.76	5.21	3.04	NS	7.05	9.47	3.94	NS	1.04	0.74	0.39	NS	1.04	0.69	0.32

Table 3. Effect of *in situ* rain-water harvesting and mulching on fruit yield and quality in mango 'Arka Neelachal Kesri'

Treatment	Fruit yield									Fruit quality				
	No. of fruits/tree			Average fruit weight (g)			Total weight of fruits (kg/tree)			Pulp (%)	Peel (%)	Stone (%)	TSS (°B)	Acidity (%)
	2012	2013	Pooled	2012	2013	Pooled	2012	2013	Pooled					
In situ rain-water harvesting structures														
Half-moon	11.33	15.91	13.62	165.72	151.97	158.85	1.87	2.41	2.14	68.32	13.59	18.10	20.01	0.25
Full-moon	13.44	18.73	16.09	156.78	169.27	163.03	2.09	3.10	2.59	68.16	14.80	17.05	19.91	0.26
Cup-and-Plate	23.27	32.55	27.91	164.97	167.98	166.48	3.87	5.46	4.67	67.53	14.11	18.36	19.71	0.27
Trench	18.22	27.18	22.7	167.01	157.70	162.35	2.94	4.28	3.61	69.20	13.92	16.89	18.80	0.28
SE(m)±	1.46	2.03	1.42	4.69	5.95	3.25	0.23	0.36	0.22	0.78	0.48	0.45	0.35	0.2
CD *(P=0.5)*	4.54	6.31	4.42	NS	NS	NS	0.71	1.11	0.69	NS	NS	NS	NS	NS
Mulching														
No mulch	12.65	17.55	15.10	158.10	158.87	158.48	1.99	2.79	2.39	68.30	14.48	17.23	18.74	0.29
Inorganic mulch	18.79	25.88	22.33	165.85	162.81	164.33	3.06	4.17	3.61	69.03	13.40	17.57	19.87	0.26
Organic mulch	18.27	27.35	22.81	166.92	163.52	165.22	3.03	4.47	3.75	67.57	14.43	18.00	20.22	0.25
SE(m)±	1.69	2.21	1.44	4.58	5.8	4.04	0.28	0.35	0.24	0.83	0.53	0.42	0.28	0.2
CD *(P=0.5)*	4.90	6.40	4.16	NS	NS	NS	0.80	1.01	0.69	NS	NS	NS	0.81	NS

parameters (pulp, peel and stone details, total soluble solids and titratable acidity) when fruiting started in the year 2012. Fruit and its fractions, namely, peel and stone, were weighed and their contents calculated as percentage. TSS was determined using a hand-held digital refractometer. Acidity was estimated by titrating fresh fruit-juice with 0.1N NaOH, using phenolphthalein as an indicator, and was expressed as per cent citric acid equivalents. Data generated on various parameters were tabulated and statistically analyzed.

Annual increase in vegetative growth for three consecutive years, along with pooled data, is presented in Table 2. Cup-and-plate system of *in situ* rain-water harvesting resulted in significant increase in plant height, canopy diameter, scion girth and primary girth. This treatment also gave the highest fruit yield (27.91 fruits weighing 4.67kg/tree) (Table 3). However, no significant differences were observed with use of various *in situ* rain-water harvesting structures for average fruit weight and fruit quality (Table 3). Better growth and yield observed in the cup-and-plate system, may be due to improved rain-water harvest using this structure, and consequent increased soil-water available to the plants for longer duration than with the other structures.

Mulching had significant influence on vegetative growth (Table 2), yield and TSS (Table 3). Maximum annual increase in plant height, canopy diameter and primary girth were recorded in the organic mulch, followed by inorganic mulch. Enhanced plant growth observed could be due to availability of sufficient moisture and enhanced lateral growth of roots in the upper layers of soil which, in turn, may have resulted in better nutrient uptake, as reported in citrus (Panigrahi *et al*, 2008). Beneficial effects of black polythene

and straw mulch on plant growth have also been reported in guava by Das *et al* (2010).

Use of organic mulch resulted in the highest yield, which was at par with yield recorded in the inorganic much treatment. Increase in the yield under these mulches was due to a significant increase in number of fruits, over no mulch. Average fruit weight under both organic and inorganic mulch was also high, although statistically at par with no mulch. Higher yield under mulching due to better conservation and improved availability of soil moisture, suppression of weed growth and decrease in soil temperature (which, in turn, resulted in better fruit retention and reduced fruit-drop) have been reported by Shirgure *et al* (2005) in acid lime, by Ghosh and Tarai (2007) in *ber,* and by Sharma and Kathiravan (2009) in plum.

TSS in the fruit was significantly influenced by application of organic and inorganic mulch, but not so for the other fruit-quality parameters. Improvement in TSS by use of mulch may be due to soil moisture conservation which, ultimately, may have caused mobilization of soluble carbohydrates to the fruit (Nath and Sharma, 1994). Improvement in fruit quality with application of mulch was also observed by Ghosh and Tarai (2007) in *ber*.

Cup-and-plate system of *in situ* rain-water harvesting and mulching either with paddy-straw or black polythene (100μ thickness) could, therefore, be useful for providing better growth, fruit yield and quality in rainfed mango in the humid tropics of Eastern India.

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Short communication

Effect of hormonal treatment and mulching on fruit drop and quality in mango

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ABSTRACT

An experiment was laid out to assess the effect of hormonal treatment and mulching on fruit drop and quality in cvs. Mallika, Amrapali and Dashehari of mango at the experimental farm Bhota of IBES Neri, Hamirpur, during the years 2010-2012. Eight treatments, viz., T_1 & T_2: 2, 4-D (20 and 40ppm), T_3 & T_4: NAA (25 and 50ppm), T_5: 2, 4-D (20ppm) + Black polythene mulch, T_6: NAA (25ppm) + Black polythene mulch, T_7: Black polythene mulch, and T_8: Control, were applied during the last week of April at the pea stage of fruit development in the years 2011 and 2012. Observations were recorded on marked panicles at monthly intervals until harvest. All the hormonal treatments, mulching and combination thereof, showed significant reduction in fruit drop in all the three cultivars under study. Fruit retention at harvest in cvs. Amrapali, and Mallika and Dashehari was maximum (5.95, 9.5 and 8.3%, respectively) with T_5 (2, 4-D 20ppm + Black polythene mulch) which was statistically at par with T_1 (2, 4-D 20ppm), T_7 (Black polythene mulch) and T_2 (2, 4-D 40ppm). Effect of treatments on TSS content was non-significant. Highest TSS content (14.5°B) was noted in cv. Dashehari which was significantly higher than in Mallika (11.7°B) or Amrapali (11.4°B). Titratable acidity was significantly low in all the treatments than that in untreated plants. Highest acidity (0.53%) was recorded in Control. 'Dashehari' recorded the highest (0.63%) acidity, followed by Mallika (0.49%) and Amrapali (0.46%).

Key words: Mango, NAA, 2,4-D, mulch, fruit-drop, fruit quality

Mango is one of the most important tropical fruits worldwide in terms of production and consumer-acceptance (FAO, 2010). Mango (*Mangifera indica* L.) belongs to the order Sapindales and the family Anacardiaceae, and is cultivated primarily under tropical and subtropical climate. Foot hills of Himachal Pradesh present semi-arid type of a climate but, generally, the whole area around is characterized by a sub-tropical climate. Mango is one of the leading fruit crops grown in the low-hill and valley areas of Himachal Pradesh, with 28927 MT production from 39568 ha under mango cultivation (Anon., 2012). Despite adequate annual rainfall in the region, drought-like situation is fairly common due to a skewed distribution of rainfall. Owing to these sub-optimal growth conditions, establishing new plantations and attaining normal vegetative and reproductive growth is an uphill task. Fruit growth and fruit maturity in mango grown in these areas coincides with a period of heavy water-stress often resulting in low fruit-set, high fruit-drop, low fruit-size and poor fruit-quality.

Natural fruit-drop in mango is very high, especially during the initial four weeks of fruit-set. Chadha and Singh (1964) reported fruit-drop of 98, 95 and 99% in cvs. Langra, Dashehari and Fazli, respectively, during the 'on year'. Incidence of fruit-drop is more severe during the 'on year' in biennial-bearing cultivars. Various factors are associated with fruit-drop, such as, lack of cross-pollination, deficient nutrition, self-incompatibility, formation of abscission layer, hormonal imbalance, position of the fruit, and prevalence of pests and diseases (Chadha, 1993). Various workers have reported that just 0.1% of perfect flowers reach maturity in mango. Extent of the fruit-drop varies among cultivars (Chadha and Singh, 1964). Higher fruit-drop is generally associated with low auxin concentration (Singh *et al,* 2005), gibberellins & cytokinins (Ram, 1983). The period of heavy fruit drop in mango corresponds with high concentration of growth inhibitors (Murti and Upreti, 1995).

Among the control measures, mulching, proper fertilization and hormonal treatments have been found promising by a number of workers. Swake *et al* (1990) reported an increase of 2% in the yield over Control using polythene mulch in mango. Singh and Singh (1976) reported that NAA at 10ppm and 2, 4-D at 10 or 15ppm gave the

highest retention of fruits. Therefore, the present study intended to assess the effect of plant hormonal treatments, in combination with mulching, on reducing fruit-drop in mango.

An experiment was laid out to assess the effect of hormonal treatments and mulching on fruit drop and quality of mango cultivars Mallika, Amrapali and Dashehari at the experimental farm Bhota of IBES Neri, Hamirpur during the years 2010 -2012. The experimental site lies in Hamirpur district representing the sub-mountain region of Himachal Pradesh. Average mean maximum and minimum temperatures here are 31.3^{0}C and 12.4^{0}C, respectively, and relative humidity is 60.9%. Eight treatments, viz., T_1 & T_2: 2, 4-D (20 and 40ppm), T_3&T_4: NAA (25 and 50ppm), T_5: 2, 4-D (20ppm) + Black polythene mulch, T_6: NAA (25ppm) + Black polythene mulch, T_7: Black polythene mulch, and T_8: Control, were applied during the last week of April at the pea-size stage of fruits. Each treatment was replicated on three mango trees. Randomized Block Design was set up for applying treatments and for data analysis. Each treatment was replicated thrice. To record observations on the effect of treatments on fruit-drop, four panicles from all around the tree were marked on each plant. Data on initial fruit-set per panicle was recorded in these marked panicles before commencing the experiment. Subsequently, fruit-retention on the marked panicles was recorded at monthly intervals until harvest. Fruit samples, comprising ten fruits per tree, were used for determining physico-chemical characteristics like fruit-length, diameter, fruit-weight, TSS and titrable acidity.

Perusal of data (Table 1) revealed that the highest fruit-retention (22.2%) at 30 days after fruit set was found with T_4 (50ppm NAA) in cv. Amrapali, followed by that in the Control (21.6%), 2,4-D 40ppm (21.2%), and NAA 25ppm (20.6%). In cv. Mallika, highest fruit-retention (29.5%) at the same stage was recorded with T_1 (2,4-D 20ppm), followed by NAA 20ppm, and Control. Treatment T_1 (2,4-D 20ppm) had the highest fruit-retention (23.4%) 30 days after fruit-set in cv. Dashehari, which was at par with NAA 25ppm (22.5%) and Black polythene mulch (22.4%). At 60 days after fruit-set, maximum fruit-retention (13.3%) was noted with T_5 (2,4-D 20ppm + Black polythene mulch), which was statistically at par with NAA 50ppm (12.8%) and the Control (12.4%). Maximum fruit-retention in cv. Mallika at this stage was recorded with T_7 (Black polythene mulch), which was statistically at par with T_2 (2,4-D 40ppm), T_4 (50ppm NAA) and T_5 (2,4-D 20ppm + Black polythene mulch). In cv. Dashehari, T_5 (2,4-D 20ppm + Black polythene mulch) recorded the highest fruit-retention (17.5%), whereas, the lowest retention (8.1%) was found

Table 1. Effect of hormonal treatments and mulching on fruit-retention in three cultivars of mango

Treatment	Fruit-retention (%) days after fruit-set														
	Amrapali					Mallika					Dashehari				
	30	60	90	120	At harvest	30	60	90	120	At harvest	30	60	90	120	At harvest
2,4-D (20ppm)	19.6	10.5	8.2	5.7	5.73	29.5	14.8	9.4	6.7	6.51	23.4	8.1	6.1	5.2	4.41
	(4.42)	(3.21)	(2.83)	(2.38)	(2.37)	(5.43)	(3.81)	(3.03)	(2.56)	(2.54)	(4.81)	(2.82)	(2.45)	(2.27)	(2.10)
2,4-D (40ppm)	21.2	11.6	8.2	5.7	5.28	26.8	16.4	11.3	7.6	6.77	21.3	14.7	9.5	6.3	4.31
	(4.60)	(3.38)	(2.83)	(2.38)	(2.28)	(5.15)	(4.02)	(3.31)	(2.73)	(2.59)	(4.60)	(3.81)	(3.06)	(2.49)	(2.05)
NAA (25ppm)	20.6	12.3	9.2	5.6	4.05	27.3	14.9	9.5	6.8	5.19	22.5	13.7	9.5	7.3	4.27
	(4.53)	(3.48)	(3.01)	(2.35)	(2.01)	(5.20)	(3.85)	(3.06)	(2.57)	(2.25)	(4.72)	(3.68)	(3.06)	(2.68)	(2.04)
NAA (50ppm)	22.2	12.8	7.9	4.9	4.54	25.6	16.1	10.2	8.3	5.09	20.8	11.77	8.6	6.4	4.54
	(4.70)	(3.56)	(2.80)	(2.21)	(2.11)	(5.03)	(4.00)	(3.17)	(2.86)	(2.23)	(4.55)	(3.41)	(2.90)	(2.50)	(2.13)
2,4-D (20ppm) +	19.6	13.3	9.8	7.4	5.95	26.2	15.8	11.7	9.5	7.15	21.3	17.5	11.6	8.3	5.25
Black polythene mulch	(4.42)	(3.61)	(3.11)	(2.70)	(2.43)	(5.10)	(3.97)	(3.40)	(3.06)	(2.65)	(4.60)	(4.15)	(3.38)	(2.86)	(2.27)
NAA (25ppm) +	20.4	11.9	8.3	5.6	5.23	23.9	13.3	8.2	5.7	4.68	18.9	11.6	9.3	8.1	4.89
Black polythene mulch alone	(4.51)	(3.43)	(2.87)	(2.35)	(2.26)	(4.86)	(3.64)	(2.84)	(2.36)	(2.14)	(4.32)	(3.39)	(3.02)	(2.83)	(2.20)
Black polythene	19.3	10.2	8.6	5.9	5.35	26.3	17.3	11.6	7.1	4.27	22.4	15.8	11.3	8.9	4.58
mulch	(4.39)	(3.18)	(2.91)	(2.40)	(2.30)	(5.10)	(4.12)	(3.38)	(2.65)	(2.04)	(4.70)	(3.95)	(3.35)	(2.95)	(2.12)
Control	21.6	12.4	9.2	6.5	3.28	27.2	16.3	11.4	8.4	4.15	20.8	14.5	9.5	7.4	4.12
	(4.62)	(3.50)	(3.01)	(2.5)	(1.80)	(5.18)	(4.01)	(3.35)	(2.86)	(2.02)	(4.55)	(3.78)	(3.06)	(2.70)	(2.01)
CD 0.05	1.69	1.38	NS	1.41	1.27	3.25	2.95	1.71	1.28	1.07	3.13	4.90	4.21	2.39	0.43

*Figures in parentheses are square-root transformed values

Table 2. Effect of hormonal treatments and mulching on fruit quality in three cultivars of mango

Treatment	Fruit weight (g)				Fruit length (cm)				Fruit diameter (cm)				TSS (^{0}B)				Acidity (%)			
	Amra-pali	Mallika	Dashe-hari	Mean	Amra-pali	Mallika	Dashe-hari	Mean	Amra-pali	Mallika	Dashe-hari	Mean	Amra-pali	Mallika	Dashe-hari	Mean	Amra-pali	Mallika	Dashe hari	Mean
2,4-D (20ppm)	135.21	329.25	240.34	234.20	8.41	12.95	10.43	10.59	5.98	7.11	5.32	6.14	11.4	11.5	13.8	12.2	0.44	0.37	0.52	0.44
2,4-D (40ppm)	124.53	328.46	238.35	230.45	8.39	12.99	10.59	10.66	5.87	7.22	5.36	6.15	11.6	11.9	13.7	12.4	0.39	0.40	0.55	0.45
NAA (25ppm)	130.63	323.78	234.13	229.51	8.31	12.74	10.45	10.50	5.84	7.06	5.69	6.20	11.3	12.6	13.9	12.6	0.42	0.53	0.44	0.46
NAA (50ppm)	121.27	324.52	241.25	229.01	8.41	12.75	10.41	10.52	5.81	7.15	5.55	6.17	11.8	12.8	13.3	12.6	0.35	0.57	0.56	0.49
2, 4-D (20ppm) + Black polythene mulch	139.46	339.45	248.75	242.55	8.45	13.19	10.66	10.76	5.69	7.36	5.54	6.20	12.1	11.7	14.8	12.8	0.34	0.34	0.48	0.38
NAA (25ppm) + Black polythene mulch	137.58	337.75	245.48	240.77	8.40	13.32	10.74	10.82	6.19	7.55	5.77	6.50	11.6	12.3	14.6	12.8	0.37	0.46	0.47	0.43
Black polythene mulch alone	126.16	323.45	233.96	227.85	8.25	13.15	10.71	10.70	6.01	7.12	5.66	6.26	11.5	11.8	14.3	12.5	0.40	0.44	0.50	0.44
Control	123.17	321.28	206.74	217.06	8.22	12.81	10.01	10.34	5.35	6.95	5.29	5.86	11.4	11.7	14.5	12.5	0.46	0.49	0.63	0.53
$CD_{0.05}$				11.23				0.16				0.17				NS				0.09
Treatments				67.35				1.35				0.86				1.26				0.11
Variety TxV				93.58				1.68				1.04				1.94				0.21

with 2,4-D 20 ppm. Treatment T_5 (2,4-D 20ppm + Black polythene mulch) recorded highest fruit-retention in cv. Amrapali (9.8%), followed by Mallika (11.7%) and Dashehari (11.6%) at 90 days after fruit-set, and was statistically at par with T_4 (NAA 50ppm) and T_7 (Black polythene mulch). A similar trend was observed at 120 days after fruit-set where T_5 (2,4-D 20ppm + Black polythene mulch) had highest fruit-retention in all the three cultivars under study. Enhancement in (flowering 35 to 50%), fruit retention and minimum fruit-drop with enhanced yield in trees mulched with black polythene was also reported by Singh *et al* (2009) in cvs. Langra and Chausa of mango.

A perusal of data on fruit-retention at harvest revealed that maximum (5.95%) retention of fruits in cv. Amrapali was observed with T_5 (2,4-D 20ppm + Black polythene mulch), which was statistically at par with T_1 (2,4-D 20ppm), T_7 (Black polythene mulch) and T_2 (2,4-D 40ppm). In cvs. Mallika and Dashehari too, the same treatment, i.e., T_5 (2,4-D 20ppm + Black polythene mulch) resulted in the highest fruit-retention of 9.5% and 8.3%, respectively. Chattaha and Anjum (1999) also found 2,4-D @ 40ppm to be the most effective treatment in controlling fruit-drop in cv. Samar Behisht Chausa of mango, as compared to NAA or 2,4,5-T. During our investigation at harvest, it was noticed that all the hormonal treatments, mulching and combinations thereof, had significant effect on reduction in fruit-drop in all the three cultivars under study. Results obtained in the present study are in conformity with Ahmed *et al* (2012) who reported that treating plants with NAA, 2,4-D and 2,4,5-T significantly influenced the number of fruits retained at pea, marble, and harvest stages of fruit growth, compared to than in Control. Kulkarni (1983) also reported that application of 2,4-D @ 25ppm to half-grown fruits of mango cv. Alphonso reduced fruit-drop. 2,4-D reduced the fruit-drop by antagonizing adverse effects of growth inhibitors like ABA and ethylene.

All the treatments tested enhanced fruit-weight over the untreated Control (Table 2). Maximum fruit-weight (242.55g) was recorded with T_5 (2,4-D 20ppm + Black polythene mulch), which was statistically at par with the treatments NAA (25ppm) + Black polythene mulch, and 2,4-D 20ppm. Among the three cultivars, highest fruit-weight (321.28g) was recorded in cv. Mallika, followed by Dashehari (206.74g) and Amrapali (123.17g). Treatment T_5 (2,4-D 20ppm + Black polythene mulch) was found to give maximum fruit-length (10.82cm) and fruit-diameter (6.50cm), which was statistically at par with T_6 (NAA 25ppm + Black polythene mulch) and T_7 (Black polythene mulch alone). Lowest value for fruit-length (10.34cm) and fruit-diameter (5.29cm) was recorded in the untreated Control. Among the cultivars, Amrapali had the maximum (12.81cm) fruit-length, and Mallika had the largest (6.95cm) fruit-diameter. Effect of various treatments on Total Soluble Solids (TSS) content was non-significant. Highest TSS content (14.5°B) was noted in cv. Dashehari, which was significantly higher than that in Mallika (11.7°B) or Amrapali (11.4°B). Titratable acidity was significantly low in all the treatments, than in Control (untreated) plants. Highest acidity (0.53%) was recorded in the Control. 'Dashehari' recorded the highest (0.63%) acidity, followed by 'Mallika' (0.49%) and 'Amrapali' (0.46%).

Results obtained in the present experiment showed that T_5 (2,4-D 20ppm + Black polythene mulch) produced the best results in terms of enhanced fruit-retention and improved fruit-size and quality. Ahmed *et al* (2012) also reported similar results in cv. Dashehari, where, application of 2,4-D @ 15ppm enhanced fruit-size (in terms of fruit-weight) by 8.7% over the Control. 2, 4-D (35ppm) recorded significantly higher TSS (19.5°B), and, TSS to titratable acidity ratio over the Control. This confirms the role of application of exogenous auxins in reducing fruit-drop in mango.

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Short communication

Bio-fortification with iron and manganese for enhanced bunch yield in 'Robusta' banana through direct nutrient-feeding

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ABSTRACT

Enhancement of bunch weight together with bio-fortification with Fe and Mn was attempted in 'Robusta' banana by enriching with 0-1.25g bunch^{-1} each of $FeSO_4$ (heptahydrate) and $MnSO_4$ (monohydrate). Bunch yield and content of Fe and Mn in the pulp substantially increased by direct nutrient feeding of bunches with 7.5g each of urea and SOP besides 0.75g each of $FeSO_4$ and $MnSO_4$. The improved technique holds promise for combating anemia in humans by bio-fortification of banana with Fe besides supplemental Mn in diet.

Key words: Bunch size, direct nutrient feeding, 'Robusta' banana, *Musa* sp., Bio-fortification, Fe and Mn content of pulp

Manipulation of bunch development in banana to ensure uniformity in fruit size and high yield was achieved in different varieties of banana by Kotur *et al* (2012) using direct nutrient-feeding to the de-navelled distal end of rachis after fruit set. Using ^{15}N-labelled urea, it was demonstrated earlier (Kotur and Keshava Murthy, 2008) that over 42% of N blended in cow-dung slurry enriched with urea and sulphate of potash (SOP) could be mobilized into the bunch, with concomitant inflow of other nutrients present in the enriched cow-dung. Improvement in Fe and Mn content from 53 and 4.8µg g^{-1}, respectively, in the whole banana fruit (pulp + peel) under 'Control', to 115 and 14.9µg g^{-1}, respectively, was obtained in direct nutrient-feeding with a blend of 7.5g each urea and SOP in cow-dung in 'Robusta' banana (Kotur and Keshava Murthy, 2010). Therefore, further enhancement in bunch weight, and fruit bio-fortification with these micronutrients important for human nutrition (Nair and Iyengar, 2009; INSA, 2011) was attempted by enriching the blend with $FeSO_4$ (heptahydrate) and $MnSO_4$ (monohydrate). 'Control' bunches retained the male flower until harvest, while, other treatments involved direct nutrient-feeding of the de-navelled distal end of rachis (after shed of 15-18 spathes) with 500g fresh cow-dung enriched with 7.5g each of urea and SOP dissolved in 100ml of water. Uniform bunches carrying 10 hands (with average number of fingers at 132 ± 6.8) were selected for receiving the treatments. The blend was further enriched with $FeSO_4$ and $MnSO_4$ in the range of 0-1.25g each (Table 1) used in 3 replications. After harvest, fruit and bunch weight was recorded. Pulp from fruits ripening at ambient conditions was sampled, sliced, held in on oven at 70°C to dryness, and powdered. The powder was digested in 9:4 nitric:perchloric acid mixture. Iron and manganese in the digest were determined using an atomic absorption spectrophotometer. Data was analyzed taking the experiment design as a completely randomized unit.

Table 1. Effect of de-navelling and direct feeding of Fe and Mn blended with urea, SOP and cow-dung in 'Robusta' banana bunch

Treatment	Fruit weight (kg/bunch)	Bunch weight (kg)	Fe content (µg g^{-1})	Mn content (µg g^{-1})
Control	13.934	14.685	25.8	1.1
Cow dung + Urea + SOP	19.551	20.621	31.8	2.4
Cow dung + Urea + SOP + 0.25g each of $FeSO_4$ and $MnSO_4$	20.702	21.833	35.2	2.8
Cow dung + Urea + SOP + 0.50g each of $FeSO_4$ and $MnSO_4$	22.329	23.496	48.5	3.0
Cow dung + Urea + SOP + 0.75g each of $FeSO_4$ and $MnSO_4$	24.466	25.806	59.9	3.8
Cow dung + Urea + SOP + 1.00g each of $FeSO_4$ and $MnSO_4$	19.413	20.465	58.9	4.3
Cow dung + Urea + SOP + 1.25g each of $FeSO_4$ and $MnSO_4$	17.246	18.194	43.5	3.2
SEm (±)	0.4459	0.4730	1.74	0.21
CD *(P=0.05)*	1.3013	1.3803	5.08	0.35

Direct nutrient feeding using cow-dung enriched with urea and SOP increased fruit and bunch weight by 40% to 76% over the 'control' owing to enrichment with $FeSO_4$ and $MnSO_4$ (Table 1). This accounted for 25% enhancement over application of cow-dung enriched with only urea and SOP. Significant decline in fruit and bunch weight occurred at 1.00 and 1.25g each of $FeSO_4$ and $MnSO_4$, but, fruit and bunch weight was similar to that obtained in just urea + SOP blended with cow-dung. With regard to Fe and Mn content of pulp, significant increase was observed in enriched cow-dung with up to 1.0g each of $FeSO_4$ and $MnSO_4$ per bunch, declining significantly at 1.25g each of the two nutrients. However, these values were smaller than reported earlier (Kotur and Keshava Murthy, 2010), as, only the pulp was studied which has much lower nutrient content relative to the fruit peel. These results indicate that it is possible to increase bunch yield further as also the content of Fe and Mn in pulp substantially, by direct nutrient-feeding of 'Robusta' bunches with 7.5g each urea and SOP along with 0.75 each of $FeSO_4$ and $MnSO_4$. There is also a scope for adding nutrients other than N, K, S, Fe and Mn to the blend of cow-dung to optimize the use of direct nutrient-feeding of banana bunch. This can help maximize bunch yield and improve nutrient status in the pulp, to boost the food-value of banana fruit. However, neutraceutical implication in terms of bio-availability of Fe and Mn to humans remains to be seen through suitable clinical trials. This improved technique holds promise for combating anemia among humans besides supplementing Mn in their diet. Nair and Iyengar (2009) opined that food-based approaches for increasing iron and other haematopoietic nutrient content are important for correction of iron deficiency anemia in humans.

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Short communication

Effect of pinching and growth retardants on growth and flowering in African marigold cv. Pusa Narangi Gainda

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ABSTRACT

A study on the effect of pinching and application of growth retardants on growth and flowering in African marigold cv. 'Pusa Narangi Gainda' was carried out in the experimental field of Division of Floriculture and Landscaping, Indian Agricultural Research Institute, New Delhi. Treatments comprised pinching, CCC applied at 1000ppm, 1500ppm or 2000ppm, MH at 500ppm, 1500ppm or 2000ppm; B-9 at 500ppm, 750ppm or 1000ppm, and a Control (no pinching). CCC at 2000ppm recorded minimum plant height (46.0cm), maximum plant-spread (56.0cm) and maximum number of branches (19.0), whereas, maximum plant height (67.0cm), minimum plant-spread (29.66cm) and minimum number of branches (5.33) were recorded in Control (non-pinching). As for flowering and yield, application of CCC at 2000ppm recorded maximum flowering-duration (25.33 days), number of flowers per plant (40), single-flower weight (119.46g), flower yield per plant (408.10g), flower yield per unit area (17.83t/ha) and seed yield per plant (17.80 g), Maximum flower diameter (7.93cm) was recorded with application of CCC 2000ppm, whereas, minimum was recorded with pinching (6.2cm). Spray of growth retardants enhanced flower yield compared to that in Control (no pinching). Maximum shelf-life of flower was recorded with CCC 2000ppm (3.66 days), whereas, minimum was recorded with pinching and non-pinching (2.33 days). Thus, application of CCC at 2000ppm is superior to other treatments tested for increasing flower yield in marigold.

Key words: Marigold, pinching, non-pinching, growth retardants

Marigold is one of the most popular flowering annuals cultivated in India. It is one of the commonly grown flowers, and is used extensively in religious and social functions in different forms. It has gained popularity among gardeners and flower dealers on account of ease of cultivation. In the recent past, the enterprise has become highly remunerative to traditional floriculture in India on account of various commercial uses of this flower. Marigold is often referred to as the versatile crop with golden harvest. Flower yield is mainly dependent on the number of flower-bearing, branches which can be manipulated by arresting vertical growth of the plant and by encouraging side shoots to develop, with apical-bud pinching. Such side shoots have a better chance of bearing flowers and, in turn, lead to higher flower yield. Similarly, application of growth retardants in horticultural crops has a marked broad-range of effects, both morphological and physiological. Effect of growth retardants varies with plant species, variety, concentration, method of application, frequency of application and various other factors influencing uptake and translocation of nutrients. In view of its importance in commercial flower production, the present investigation was initiated with an objective to develop suitable agro-techniques for enhanced flower production in African marigold cv. Pusa Narangi Gainda.

An experiment was conducted at the research farm of Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi. Eleven treatments were imposed viz., Chloremequat chloride (CCC) at 1000ppm (T1), 1500ppm (T2), 2000 ppm (T3); Malic hydrazide (MH) at 500ppm (T4), 1000ppm (T5), 1500ppm (T6); Alar (B-nine) at 500ppm (T7), 750ppm (T8), 1000ppm (T9); Pinching (T10), and non-pinching [Control] (T11). The experiment was laid out in Randomized Block Design, with three replications. Seedlings were transplanted at a spacing of 45cm x 45cm. Meristematic bud was pinched three weeks after transplant. Freshly-prepared growth retardants were sprayed at different concentrations. The first spray was

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Table 1. Effect of pinching and growth retardants on growth and flowering in marigold

Treatment	Plant height (cm)	Plant spread (cm)	No. of branches per plant	Days to first flowering	Flowering duration	Number of flowers per plant	Flower diameter (cm)	Single flower weight (g)	Flower yield per plant (g)	Flower yield per unit area (t/ha)	Seed yield per plant (g)	Shelf-life of flower (days)
T1-CCC 1000ppm	46.83	50.00	16.67	63.66	22.61	33.33	7.56	101.27	361.66	16.50	15.61	2.00
T2-CCC 1500ppm	48.00	50.66	17.00	64.33	24.33	34.33	7.60	104.60	376.10	16.96	16.86	3.00
T3-CCC 2000ppm	46.00	56.00	19.00	66.66	25.33	40.00	7.93	119.46	408.10	17.83	17.80	3.66
T4-MH 500ppm	48.00	35.66	14.66	63.66	21.66	26.66	6.63	101.83	350.00	16.53	16.18	3.00
T5-MH 1000ppm	48.00	44.00	15.66	66.66	22.66	27.33	7.00	107.23	341.03	14.53	16.86	3.33
T6-MH 1500ppm	49.00	47.33	17.66	60.00	23.33	30.33	7.46	101.73	345.70	15.03	17.03	2.66
T7-B-nine 500ppm	48.00	37.33	10.66	61.66	22.00	31.33	7.10	94.87	385.46	16.56	17.73	3.00
T8-B-nine 750ppm	54.33	39.83	14.00	56.00	22.33	30.00	7.40	98.13	378.90	14.96	17.48	3.00
T9-B-nine 1000ppm	56.33	44.66	16.33	62.00	22.66	28.33	7.60	104.73	382.43	15.16	16.78	3.00
T10-Pinching	52.33	34.00	9.33	60.00	23.00	24.00	6.20	93.90	388.30	13.13	13.01	2.33
T11-Non Pinching (Control)	67.00	29.66	5.33	50.33	21.00	17.00	6.23	90.55	243.23	10.03	7.33	2.33
CD (P=0.05)	16.15	6.02	2.96	4.22	2.99	5.31	0.42	10.68	38.91	1.98	0.76	0.75

applied three weeks after transplanting, while the second spray was scheduled at five weeks after transplanting. Five plants were randomly selected in the net plot area and tagged with labels in each treatment to record observation on growth and yield. Crop management practices like nutrient irrigation weed management and plant protection measures were included as per requirement of the crop. Data on various parameters were recorded and subjected to statistical analysis.

Data presented in Table 1 reveal that pinching and application of different growth retardants at various levels influenced growth, flowering and yield significantly in marigold. The treatments were effective in suppressing plant height compared to Control. CCC at 2000ppm recorded minimum plant height (46.0cm), maximum plant-spread (56.0cm) and maximum number of branches (19.0), whereas, maximum plant height (67.0cm), minimum plant-spread (29.66cm) and minimum number of branches (5.33) were recorded in the Control (non-pinching). These findings are in accordance with Jay *et al* (1991), Girwani *et al* (1990), Narayana Gowda and Jayanthi (1991), and Dutta and Ramadas (1997) in chrysanthemum. This response may be due to inhibition of GA synthesis and breakdown of apical dominance, thereby resulting in auxin balance and enhanced differentiation of branching caused by CCC, as proposed by Ninnemann *et al* (1964). Early flowering (50.33 days) was recorded in non-pinching (Control). Late flowering was recorded with CCC 2000ppm and MH 1000ppm (66.66 days). These results are in congruence with Narayana Gowda and Jayanthi (1991) and Parmar and Singh (1989) in chrysanthemum. Delay in flowering may have been due to inhibition of GA synthesis.

As for flowering and yield parameters, application of CCC at 2000ppm recorded maximum flowering-duration (25.33 days), number of flowers per plant (40), single-flower weight (119.46g), flower yield per plant (408.10g), flower yield per unit area (17.83t/ha) and seed yield per plant (17.80g), whereas, minimum flowering-duration (21 days), flower number per plant (17.0), flower weight (19.55g), flower yield per plant (243.23g), flower yield per unit area (10.03 t/ha) and seed yield (7.33g) were recorded in Control (non-pinching). Maximum flower diameter (7.93cm) was recorded with CCC at 2000ppm. Results of the present study are in agreement with Leena *et al* (1992) in gladiolus, Dutta and Ramadas (1997) and Takuldar and Paswan (1994) in chrysanthemum, and, Syamal *et al* (1990) in marigold and China aster. This may probably be due to suppression of apical dominance, resulting in increased number of flowers

per plant and, ultimately, increased flower yield per hectare. Our results clearly showed that spray of growth retardants enhanced flower yield compared to that in Control (non-pinching). Maximum shelf life of flower was recorded in CCC 2000 ppm (3.66 days) .Similar results were obtained by Raju Dantuluri, (2000) who reported improved shelf life of flowers in Asiatic hybrid Lily cv. Corrida with CCC treatment. The minimum shelf life of flowers was recorded in pinching and non pinching (2.33 days).

Thus, the present investigation revealed that application of CCC at 2000ppm was superior among the treatments tested for increasing flower yield in marigold.

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Short communication

Antioxidant composition of guava (*Psidium guajava* L.) beverage blended with black-carrot juice

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ABSTRACT

An investigation was undertaken to study guava beverage blended with black-carrot juice, during 2011-2012. Enzyme-assisted processing of guava significantly improved the juice yield, total soluble solids, titratable acidity pH, ascorbic acid and sugars by using pectinase enzyme. The blending of guava beverage with black carrot juice significantly improved the functional properties of the guava RTS. Anthocyanin and ascorbic contents of blended guava RTS with black-carrot juice decreased with advancement of storage condition and period.

Key words: Guava, enzyme-assisted processing, functional quality, black carrot

Guava (*Psidium guajava* L.) is known as the apple of the tropics and is grown throughout the tropical and subtropical regions of India. It is a rich source of Vitamin C and minerals, viz., calcium, phosphorus and iron, necessary for human health. The guava fruit, and its juice, is popularly consumed for its great taste, nutritional benefits and nutrient content. Clarified guava juice is more acceptable and is used in the manufacture of clear guava-nectar or jelly, guava powder, or mixed-fruit juice blend. However, extraction of juice from guava is difficult and protracted owing to the gritty texture of its pulp and its pectineous nature. Enzyme-assisted processing with pectinolytic enzymes is an effective approach for degrading the pectinaceous material, to yield a free-flowing juice. Several researchers have reported recently that pectinase and cellulase enzyme treatments can significantly enhance recovery of phenolics and improve its functional properties (Collin *et al,* 1998). Black carrot has been in the focus as a source of natural food colorants (Collins *et al,* 1998), and high content of its anthocyanin pigments is being used for blending it with guava juice. Considering the enormous potential of guava as a source of phenolics, the objective of the current study was to study the effect of enzyme-assisted processing and blending of guava RTS with black-carrot juice, and to evaluate its functionality for imparting color appeal and antioxidant activity.

The present study was carried out for preparation of clarified guava juice using pectianase enzyme, and to utilize it for preparation of guava RTS (Ready-To-Serve juice) blended with black-carrot juice. Fully mature, ripe guava fruits (cv. L-49) free from blemishes and mechanical injury were procured from the local market. The location of the study was Department of Horticulture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. Fruits of guava were washed thoroughly in tap water and cut into thin slices using a stainless-steel knife, and subjected to hot-breaking at 80°C for 20 min, for softening the fruit pieces. These were subsequently passed through a laboratory-scale pulper for extracting a homogeneous pulp without seeds. Pulp samples were weighed out in 500ml glass bottles and the enzyme preparation (pectinase EC 3.2.1.1 from *Aspergillus niger*, 1 U/mg from *Aspergillus sp.*) was added at four concentrations: 0.5, 1, 1.5 and 2% E/S. Control (straight-pressed) pulp samples were incubated without the enzyme under the same conditions. For each concentration, 500ml of the pulp was taken in three replicates for analysis. The bottles were capped and incubated at 50°C in a thermostatically controlled water-bath for 1 hour. The pulp was then pressed using a hydraulic press with a nylon filter-bag to extract the juice. The filtrate was centrifuged at 5000rpm for 10 min to obtain the clarified juice (whose yield was determined by weighing the juice extracted, subsequently heat-processing it at 90°C for 1 min, and packing it in clean, sterilized glass-bottles finally upturned

and sealed). The clarified juice was then used for preparation of guava RTS by adding sugar and water. The RTS was standardized by conducting preliminary trials with the juice; TSS, acidity and more combinations were used for preparing the RTS beverage. RTS was blended with black-carrot juice at 5%, 10%, 15% or 20% concentrations. The blended RTS was heated at 90°C for 1 min, and packed in clean and sterilized bottles, upturned, sealed and stored under ambient conditions or at 7°C for use in analysis. Anthocyanin content was determined by pH differential method (Spectrophotometeric method) described by (Wrolstad *et al,* 2005). Each experimental unit was replicated thrice. Data were subjected to Analysis of Variance, using Completely Randomized Design.

Physico-chemical composition of guava fruit and pulp

Data presented in Table 1 reveal fresh guava fruit as recording 10% TSS, 4.1 pH, 0.54% titrable acidity, 200.5 mg/100g ascorbic acid, 10.6% total sugars, 6.23% reducing sugars, and 4.37% non-reducing sugars; whereas, guava pulp recorded 9% TSS, 4.1pH, 0.64% titrable acidity, 198.7mg/100 ml ascorbic acid, 4.21% reducing sugars, 1.37% non-reducing sugar, and 5.58% total sugars. Results on total acidity, total soluble solids, pH, ascorbic acid and sugar content in guava are in agreement with earlier findings of Kumar and Honda (1994), Chatterjee *et al* (1992), Tondon and Kalra (1984), Tiwari (2000), and Gowda (1995).

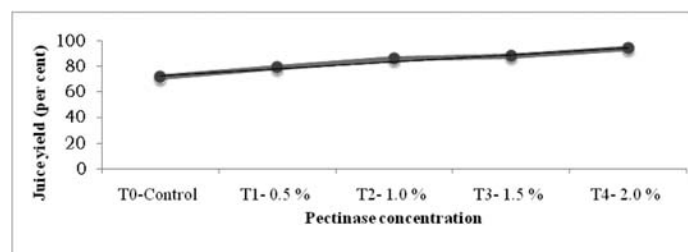

Fig. 1. Effect of pectinase concentration on juice yield in guava

Table 1. Physico-chemical composition of guava fruit

Traits	Fresh fruit	Fruit Pulp
TSS (%)	10.0	9.0
pH	4.10	4.1
Titrable acidity (%)	0.54	0.64
Ascorbic acid (mg/100g)	200.5	198.7
Reducing sugars (%)	6.23	4.21
Non-reducing sugars (%)	4.37	1.37
Total sugars (%)	10.60	5.58

Effect of enzyme-assisted processing on juice yield in guava

Data on effect of pectinase enzyme in varying concentrations (0.5%, 1.0%, 1.5% or 2.0%) for liquefication of guava juice, and enzymatically obtained clarified juice, compared to the juice in Control (without enzyme) as per cent juice-yield, are presented in Fig. 1. Significant increase in juice-yield was seen in enzyme-assisted processing. Juice yield in the Control sample T_0 was 71.3%, while, with increasing concentration of pectinase enzyme, the juice-yield increased to 94% in treatment T_4, followed by that in T_3 (88%), T_2 (85.80%) and T_1 (79%). Overall, 22.7% increase in juice-yield was seen as a result of degradation pectinase catalyzed in the plant cell-wall matrix. These results confirm the findings of Tiwari (2000) in guava.

Effect of enzyme-assisted processing on physic-chemical composition of guava juice

Data presented in Table 2 reveal that maximum TSS percentage (12.5), pH (4.66), titrable acidity (0.72), ascorbic acid content (25.15 mg/100g) and sugar content were recorded in treatment T_4, over the Control. Physico-chemical composition of the juice, viz., total soluble solids, pH, titrable acidity, total sugars, reducing sugars, non-reducing sugars and ascorbic acid content increased with pectinase concentration over the Control (untreated).

Physio-chemical composition of guava RTS blended with black-carrot juice

Data presented in Table 3 reveal that TSS of blended guava RTS ranged from 11.10% to 12.03%. Lowest value (11.10%) of TSS was recorded in treatment (T_4)

Table 2. Effect of enzyme-assisted processing on physico-chemical composition of guava juice

Treatment	TSS (%)	pH	Titrable acidity(%)	Ascorbic acid (mg/100g)	Sugars (%)		
					Reducing	Non-reducing	Total
T_1 (0.5%)	9.40	4.06	0.65	20.02	4.23	1.37	5.60
T_2 (1.0%)	11.00	4.36	0.67	22.36	4.23	1.38	5.61
T_3 (1.5%)	12.00	4.50	0.70	24.94	4.25	1.40	5.66
T_4 (2.0%)	12.50	4.66	0.72	25.15	4.26	1.42	5.68
T_0 (Control)	9.00	3.80	0.64	19.86	4.22	1.36	5.58
SE m+	0.05	0.12	0.004	0.33	0.005	0.003	0.012
CD *(p=0.05)*	0.16	0.37	0.015	1.05	0.018	0.012	0.038

Table 3. Physico-chemical composition of guava RTS blended with black-carrot juice

Treatment	TSS (%)	pH	Titrable acidity (%)	Ascorbic acid (mg/100ml)	Anthocyanin (mg/l)	Sugars (%)		
						Reducing	Non-reducing	Total
T_1 (5%)	11.86	5.51	0.31	9.52	20.91	2.56	8.23	10.79
T_2 (10%)	11.73	5.31	0.28	9.31	24.44	2.55	8.19	10.74
T_3 (15%)	11.60	5.11	0.26	9.01	26.45	2.53	8.18	10.71
T_4 (20%)	11.10	4.90	0.25	8.81	29.83	2.52	8.15	10.66
T_0 (Control)	12.03	5.73	0.32	9.76	-	2.57	8.31	10.88
SE m+	0.005	0.005	0.005	0.005	0.003	0.005	0.006	0.005
CD *(p=0.05)*	0.018	0.018	0.018	0.018	0.011	0.016	0.019	0.018

Table 4. Sensory evaluation of guava RTS blended with black-carrot juice (Scale 0-9)

Treatment	Colour	Consistency	Flavour	Aroma	Overall acceptability (Scale 0-9)
T_1 (5%)	8.25	8.41	7.41	7.41	7.86
T_2 (10%)	7.37	7.44	7.32	7.00	7.28
T_3 (15%)	7.30	7.30	6.85	7.11	7.06
T_4 (20%)	7.20	7.21	7.20	6.93	7.18
T_0 (Control)	6.07	6.29	7.06	7.31	6.66
SE m+	0.003	0.005	0.005	0.015	0.005
CD *(p=0.05)*	0.014	0.017	0.016	0.048	0.18

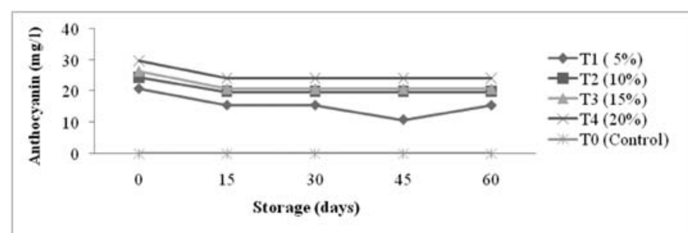

Fig. 2. Effect of storage conditions on anthocyanin content guava RTS blended with black-carrot juice at ambient temperature

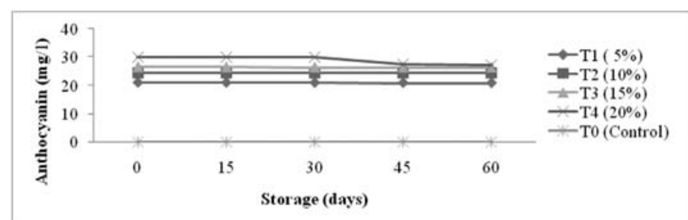

Fig. 3. Effect of storage conditions on anthocyanin content in guava RTS blended with black-carrot juice at 7°C

guava RTS blended with 20% black-carrot juice, compared to the T_0 Control (12.03%). Highest titrable acidity was recorded in the Control (0.32%) guava RTS, while the lowest was recorded in treatment (T_4) guava blended RTS with 20% black carrot juice (0.25). Highest ascorbic acid content was recorded in the Control treatment T_0 (9.76mg/100 ml), whereas, the lowest ascorbic acid content was recorded in guava RTS blended with 20% black-carrot juice (8.81mg/ 100 ml). A similar trend was observed in total sugar content, reducing sugars and non-reducing sugars in guava RTS blended with black-carrot juice.

The highest total anthocyanin content was recorded in treatment (T_4) guava RTS blended with 20% black-carrot juice, followed by treatment (T_3) that blended with 15% black-carrot juice, (T_2) 10% black-carrot juice and (T_1) 5% black-carrot juice, in that order. These results are in agreement with Bhuvaneshwari and Doreyappa Gowda (2006), and Garymain *et al* (2001).

Sensory analysis of guava RTS blended with black-carrot juice

Blending guava RTS with black-carrot juice improved organoleptic quality remarkably in guava juice blended with 5 or 10% black-carrot juice (Table 4). The highest colour score (8.25), consistency score (8.41), flavour score (7.41), aroma score (7.41) and overall acceptability score (7.86) was recorded in treatment (T_1) fallowed by in treatment T_2, while lowest colour (6.07), consistency (6.29), flavor (7.06), aroma (7.31) and overall acceptability (6.66) score was recorded in treatment (T_0). The product developed had a preponderant flavor of the original fruit used, lack of the earthy, raw flavor or added phytochemical content. The products show a good potential for anthocyanin-rich, healthy drinks for the food industry looking for natural alternatives to synthetic drinks. Results obtained by us are in agreement with Bhuvaneshwari and Doreyappa Gowda (2006).

Storage of guava RTS blended with black-carrot juice

Total anthocyanin content

Data on anthocyanins in guava RTS blended with black-carrot juice at room temperature and at 7°C are presented in Figs. 2 and 3. Anthocyanin content of guava RTS blended with black-carrot juice was found to decrease with advancing storage period, irrespective of the storage conditions. Initially, the highest value (29.85 ml/ l) for anthocyanin was recorded in treatment (T_4) 20% black-carrot juice blended with guava RTS. No anthocyanin content was found treatment (T_0) in the Control guava RTS.

Similar trend was observed in 15, 30 or 45 days stored guava RTS blended with black-carrot juice, at room temperature. At 60 days, the highest value (24.20ml/ l) for anthocyanin content was recorded in treatment (T_4) 20% guava RTS blended with black-carrot juice at 7°C. Total anthocyanin content during storage at different temperatures decreased in comparison to non-stored juice. These results are in agreement with Alighourchi and Barzegar (2009).

Results indicated that use of enzymes for liquefaction prior to pressing can improve the quality of guava juice remarkably, culminating in enhanced juice-yield. Blend of guava RTS with black-carrot juice enhanced anthocyanin content in the juice, which is directly related to health-promoting capacity; it also contained high anthocyanin content, with a stable and attractive strawberry-red colour. Blending of guava with black-carrot juice in the preparation of RTS beverage resulted in a product with good organoleptic (colour) properties and can be used as a health-drink.

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Short communication

Influence of nitrogen and phosphorus on flowering in African marigold (*Tagetes erecta* L.) var. Cracker Jack

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ABSTRACT

An investigation was conducted during the year 2000 to study the effect of nitrogen and phosphorus on flowering in African marigold. Results revealed that among the four levels of nitrogen tested, highest level of nitrogen (N_3) led to minimum number of days for the first flower-bud to become visible (31.66 days), days to flower-break (38.55 days), days to full-flowering (50.66 days). Plants receiving N_2 recorded significantly high number of flower heads per plant (28.42) and yield (11.11 t/ha). Among the three levels of phosphorus tested, days taken to appearance of the first flower-bud, flower-break and full-flowering were significantly earlier in a treatment with no phosphorus (P_0). However, number of flower heads per plant was significantly higher in P_2 (28.3). As for interaction effect, a combination of the highest level of nitrogen with no phosphorus (N_3P_0) recorded early flowering. Number of flower heads per plant was higher in N_3P_2 (31.83). Highest flower-yield (11.65 t/ha) was recorded in N_3P_2. Thus, it is concluded that nitrogen application advances flowering, while, phosphorus application delays flowering.

Key words: Marigold, nitrogen, phosphorus, flowering

Marigold is one of the most important commercially exploited flower crops of India. Among crop production technologies, balanced N and P fertilization are essential for better plant-spread and flower yield per unit area. Nitrogen and phosphorus are required in adequate quantity to attain ideal growth and to promote flowering (Pandey and Mishra, 2005). Adequate supply of N results in vigorous plant growth, consequently superior yield of flowers of better quality. Phosphorus is needed for normal growth and development of the plant due to its vital role in chlorophyll synthesis and physiological / metabolic processes of the plant. Nutrient supply needs to be adjusted to specific requirement by the plant during various stages of its growth, to attain maximum yield (Mengal, 1969). Nitrogen is well known for its influence on plant growth, flower production and quality of bloom in marigold (Noggle and Fritz, 1979).

In the absence of precise recommendations for some areas, growers impose manurial schedules of their own accord, resulting in improper nutrition to the crop. This upsets nutrient balance in the plant and is a major factor for low yield in many flower crops, posing a serious problem in flower production. Therefore, an attempt was made to improve flowering in marigold by applying various levels of nitrogen and phosphorus fertilizers to the plant.

The experiment was conducted at Horticultural Garden, Sri Venkateswara Agricultural College, Tirupati, during year 2000. Soil in the experimental plot was red sandy-loam with good drainage and a low water-holding capacity. Soil samples were collected before applying the manure from a depth of 20cm in the experimental area from some randomly selected spots. The composite sample was analyzed for chemical characteristics and content of nitrogen, phosphorus and potassium. Chemical analysis of the soil indicated that it was low in nitrogen and available phosphorus, high in available potash, and was alkaline in nature (Table 1). Raised nursery beds of 3m and 0.5m size

Table 1. Chemical analysis of soil in Horticultural Garden at S.V. Agricultural College, Tirupati

Particulars	Quantity
pH	7.4
EC	0.39 m. mhos per cm
Organic carbon	Low (below 0.5)
Available Nitrogen	201 kg/ha
Available P_2O_5	9.2 kg/ha
Available K_2O	130 kg/ha

were prepared well in advance for seed sowing. Seeds were treated with 0.3% Captan prior to sowing on 20-07-2000. Two hundred kilograms of farm yard manure was applied as a basal dose and mixed well into the soil at the last ploughing. N and P were applied in the form of urea (46.4%) and superphosphate (16.0% P_2O_5), respectively. The entire quantity of phosphorus and potash, and 50% of total nitrogen was applied as the basal dose. The remaining 50% of the nitrogen was applied as top-dressing three weeks after transplantation to the main field. Thirty-day-old, healthy seedlings of uniform growth were used for transplanting, and, planted at 40cm x 40cm spacing. All the other field operations were performed as per the recommended package of practices.

Treatments comprised four N levels [0 (N_0), 100 (N_1), 150 (N_2) and 200 (N_3) kg N per ha] and three P levels [0 (P_0), 100 (P_1) and 200 (P_2) kg P_2O_5 per ha]. The experiment was laid out in Factorial Randomized Block Design, with three replications. Data on days taken to appearance of the first flower-bud, days to flower-break, days to full-flowering, number of flower heads per plant, and yield (t/ha) were recorded. Fischers' (1963) method of analysis of variance was followed for analysis, and data was interpretation. F and t tests were applied and the results were tabulated.

Days taken to visibility of first flower-bud

Results revealed significant variation among the four levels of nitrogen for days taken to visibility of the first flower-bud (Table 2). Number of days taken got progressively and significantly reduced with increasing levels of nitrogen. Plants receiving the highest dose of nitrogen (N_3) took the least number of days (31.66) for appearance of the first flower-bud, whereas, plants treated with the lowest level of nitrogen (N_0) took more number of days (34.66). However, levels of nitrogen were seen to be independent of each other, and were significantly superior over the treatment without nitrogen.

As the level of phosphorus increased, time taken for appearance of the first flower-bud also increased. However, treatments P_0 and P_1 were of the same order and took nearly similar number of days (32.33, 32.58), but were significantly different from P_2 treatment.

Interaction between nitrogen and phosphorus with regard to appearance of the first flower-bud was significant. Minimum number of days (30.66) were required for this trait under the treatment of the highest level of nitrogen with no phosphorus (N_3P_0), closely followed by N_2P_1, N_3P_1 and N_2P_0, which were all of the same order.

Table 2. Influence of nitrogen, phosphorus and their interactions, on flower characters in marigold var. Cracker Jack

Treatment	Days to visibility of first flower-bud	Days to flower-break	Days to full flowering	Number of flower heads /plant	Yield t/ha
Nitrogen					
N_0	34.66	41.88	53.66	23.04	9.23
N_1	33.66	40.33	52.33	26.20	9.98
N_2	32.44	39.33	51.66	28.42	11.11
N_3	31.66	38.55	50.66	26.02	10.38
S.Em	0.19	0.31	0.49	0.11	0.35
CD *(P=0.05)*	0.58	0.93	1.44	0.34	0.10
Phosphorus					
P_0	32.33	39.58	51.41	24.22	9.60
P_1	32.58	39.99	51.83	25.23	10.12
P_2	34.41	40.49	52.99	28.30	10.80
S.Em	0.17	0.27	0.42	0.09	0.03
C.D *(P=0.05)*	0.5	0.80	1.25	0.29	0.09
Nitrogen x Phosphorus					
N_0P_0	34.00	42.33	53.00	21.43	8.75
N_0P_1	34.33	42.00	54.00	22.46	9.23
N_0P_2	35.66	41.33	54.00	25.23	9.71
N_1P_0	33.00	40.00	51.66	25.33	9.51
N_1P_1	33.33	40.33	52.00	26.06	9.90
N_1P_2	34.66	40.66	53.33	27.20	10.52
N_2P_0	31.66	39.00	51.00	27.96	10.83
N_2P_1	31.33	38.66	51.33	28.33	11.18
N_2P_2	34.33	40.33	52.66	28.96	11.31
N_3P_0	30.66	37.00	50.00	22.16	9.31
N_3P_1	31.33	39.00	50.00	24.06	10.18
N_3P_2	33.00	39.66	52.00	31.83	11.65
S.Em	0.34	0.54	0.85	0.19	0.061
C.D *(P=0.05)*	1.00	1.61	2.50	0.58	0.181

Days to flower-break

Data presented in Table 2 show that Control plants (N_0) took significantly higher number of days (41.88) than nitrogen treatments for flower-break. With increase in level of nitrogen, time taken for flower-break decreased significantly. However, treatments N_3 and N_2 were at par, but, significantly different from N_1. A contrary influence of phosphorus level was observed on this trait. As the level of phosphorus increased, time taken for flower-break too increased. Treatments P_0, P_1 and P_1, P_2 were of the same order, but P_0 and P_2 were statistically different. Plants receiving the highest level of nitrogen with no phosphorus (N_3P_0) showed the earliest flower-break (37.00 days). This was significantly superior to all other treatments. Control plants (N_0P_0) receiving neither nutrient flowered late (42.33 days), closely followed by N_0P_1 (42.00 days). Difference between the early-flowering plants and the late flowering plants was found to be 5 days.

Days to full flowering

A perusal of data (Table 2) indicates that among the four nitrogen levels tested, the highest level resulted in the shortest duration (50.66 days) for full flowering, closely followed by next highest N level (51.66 days), which was on par. A similar trend was observed in N_2 and N_3. Highest level (N_3) and lowest level (N_0) of nitrogen differed significantly in their effect. Various levels of phosphorus too exhibited a significant effect. As the level of phosphorus increased, the duration of full flowering reduced significantly. Treatments P_0, and P_1 and P_2 were of the same order, but P_0 was statistically different from others. Highest level of applied nitrogen with no phosphorus (N_3P_0) significantly reduced the number of days to full flowering (50.00 days), while this was highest in the treatment with the highest level of phosphorus with no nitrogen, and, both were independent.

Number of flower-heads per plant

Information in Table 2 shows that increase in number of flower heads did not corroborate with increase in levels of nitrogen. Highest number of flower heads (28.42) was recorded in plants receiving an intermediate level of nitrogen (N_2). The highest dose of nitrogen (N_3) resulted in fewer flower heads (26.02). The two were independent of each other. Number of flowers was lowest (24.22) under no phosphorus treatment, (P_0), and maximum (28.30) under the highest level of phosphorus (P_2). Production of flowers was significantly influenced by various combination treatments of nitrogen and phosphorus. N_3P_2 was superior to all other treatments.

Table 3. Nitrogen and phosphorus content (percentage) of the plant in marigold var. Cracker Jack as influenced by nitrogen, phosphorus and interaction thereof.

Treatment	Nitrogen content (%)	Phosphorus content (%)
Nitrogen		
N_0	1.17	0.22
N_1	2.40	0.25
N_2	2.85	0.26
N_3	3.25	0.28
S.Em	0.04	0.006
C.D *(P=0.05)*	0.13	0.017
Phosphorus		
P_0	2.36	0.24
P_1	2.40	0.25
P_2	2.49	0.26
S.Em	0.04	0.005
C.D *(P=0.05)*	0.11	0.015
Nitrogen x Phosphorus		
N_0P_0	1.06	0.21
N_0P_1	1.17	0.23
N_0P_2	1.30	0.22
N_1P_0	2.16	0.24
N_1P_1	2.53	0.25
N_1P_2	2.53	0.26
N_2P_0	2.72	0.25
N_2P_1	2.83	0.27
N_2P_2	3.00	0.27
N_3P_0	3.20	0.28
N_3P_1	3.25	0.29
N_3P_2	3.31	0.28
S.Em	0.08	0.01
C.D *(P=0.05)*	0.23	0.03

Flower yield

Highest flower-yield (11.11 t/ha) was recorded in plants receiving an intermediate level of nitrogen (N_2), while, the highest dose of nitrogen (N_3) produced 10.38 t/ha yield. Treatment P_2 recorded significantly high flower yield (10.80 t/ha). This was significantly superior to that in the other P treatments. N_3P_2 was superior to all other treatments with reference to flower yield (11.65 t/ha).

Effect of nitrogen on flowering

Table 2 indicates that flowering was earlier in plants receiving nitrogen, compared to those receiving no nitrogen. However, the difference between highest level of nitrogen and no- nitrogen was just 3 days and, from a practical point of view, this is not appreciable. Number of days taken to appearance of the first flower-bud decreased progressively with increase in nitrogen level. Number of days taken to 50% flowering was observed to be reduced with increasing level of nitrogen (0-90 kg N/ha) in marigold on sandy-loamy soil by Anuradha *et al* (1988, 1990). Chadha *et al* (1999) obtained earliest bud-initiation in plants treated with 30 kg N/ha. Thus, result of the present experiment is in line with the above findings, however, our results differ from the findings of Arora and Khanna (1986) who reported delayed commencement of flowering in marigold with application of graded doses of nitrogen (0-40g/m^2). No probable explanation for this was given. Views are divergent on the effect of nitrogen on flowering. Increased vegetative growth may help production of greater amount of photosynthates, leading to flowering stimulus, thus inducing early flowering. Increased nitrogen levels stimulating early-flowering may sound contradictory to the general belief that plants normally remain vegetative, thus delaying flowering, due to high nitrogen; But, this does not seem to be true in all the cases. Butters (1971) and Vijaykumar and Shanmugavelu (1978) reported early flowering in chrysanthemum with application of increased levels of nitrogen.

Effect of phosphorus on flowering

Plants receiving phosphorus took more number of days for appearance of the first flower-bud, flower bud break and full-flowering, while, Control plants receiving no phosphorus came to flowering earlier. In other words, application of phosphorus delayed flowering. However, no statistical difference was seen between Control plants and plants receiving 100kg P_2O_5 / ha (P_1) with regard to date of appearance of the first flower-bud, flower-break and full-flowering. Except for appearance of the first flower-bud, the two levels of phosphorus tested were found to be at par with each other. These observations indicate that application of phosphorus does not favour early flowering in marigold. However, these results are not in line with findings reported by others. For instance, Anuradha *et al* (1990) and Dahiya *et al* (1998) reported that the number of days required for 50% flowering reduced with application of phosphorus in marigold. Reasons for early flowering due to phosphorus application, however, were not elucidated by them.

Interaction between nitrogen and phosphorus for flower induction

Flowering in marigold responded significantly to treatment combinations of nitrogen and phosphorus. Plants treated with the highest dose of nitrogen with no phosphorus (N_3P_0) were the earliest in the appearance of first flower-bud (30.66 days), closely followed by N_3P_1, N_2P_1 and N_2P_0 which were of equal order statistically, but differed from the other treatments. Nitrogen is known to promote vegetative growth and advances the reproductive phase in plants. This may have occurred in the present case too. Phosphorus with no nitrogen (N_0P_2) delayed appearance of the flower-bud (35.66 days), and was of same order as N_1P_2. Days to flower bud-break were the least (37 days) under N_3P_0, which differed significantly from the others. For full flowering, fewer days were recorded in N_3P_0 and N_3P_1 (50 days), while this value was highest (54 days) in phosphorus applied with no nitrogen (N_0P_1 & N_0P_2). Our data indicate that flowering is influenced greatly with treatment combinations having nitrogen; flowering was delayed in the absence of nitrogen, whatever the rate of phosphorus applied.

Increase in the content of nitrogen and phosphorus, singly or in combination, helped the plant in terms of better growth and production, as ,these two nutrients play a very important role in the plant. Yield is the net result of several contributing traits like number of flowers per plant, weight of the flower and the nutrient content in the plant and exhibited a positive correlation (Table 3).

Thus, it is concluded that nitrogen application advances flowering, while, phosphorus application delays flowering in marigold.

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Short communication

Effect of integrated nutrient management on vegetative growth and yield in mango cv. Himsagar

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ABSTRACT

An experiment was conducted to study the effect of various combinations of integrated nutrient management schedules on vegetative growth and yield in mango cv. Himsagar at Regional Research Station, Gayeshpur, B.C.K.V., Nadia, West Bengal, during the years 2009-2011. Maximum total increment in plant height (108.00 cm), plant spread in E-W direction (123.00 cm) and N-S direction (105.00 cm), and tree volume (85.95 m^3) was recorded in 500:250:250g NPK/tree/year + 50kg FYM + 250g *Azospirillium* (T_6) compared to that in other treatments. This treatment (T_6) also significantly increased total number of fruits (234.12 fruits / tree), average fruit weight (263.10g) and yield (58.56kg /tree).

Key words: Mango, Himsagar, biofertilizer, INM

Mango (*Mangifera indica* L.), the ‘King of Fruits,’ is an evergreen fruit crop of the tropical and sub-tropical regions with a great economic potential, for, it fulfils the requirement for nutritional, medicinal, commercial, industrial and religious needs (Bihari *et al*, 2012). In India, it is a part and parcel of life, being connected with all phases of life from birth to death (Bose *et al*, 2001). Among fruit crops, it occupies the first place in area in India, occupying 2.29 mha with a production of 151.88 lakh tonnes, constituting 45% of the total world mango production. Production has been increasing since independence, contributing 20.3% of the total fruit produced in India, after banana (39.8%). Uttar Pradesh tops in total production (23.9%), followed by Andhra Pradesh (22.1%). West Bengal, falling also under the major mango-growing belt, contributed about 4.1% of total mango production in India (Indian Horticulture Database, 2011). West Bengal too is a major mango-producing state in India in terms of area and production, and new mango plantations need to be raised every year to supply an increased demand for this fruit. However, indiscriminate application of inorganic fertilizers leads to changes in physical, chemical and biological properties of the soil, besides reducing its fertility and leading to decline in its organic content (Singh *et al*, 2001). Also, use of inorganic carbon fertilizers is detrimental to human health and environment (Arisha and Bardisi, 1999). Estrada (2002) reported that agricultural lands get impoverished with application of high doses of fertilizer which, in turn, pollute the ecosystem significantly. Besides, information on effects of integrated nutrient management on vegetative growth and yield in mango cv. Himsagar in the alluvial tract of West Bengal is lacking. Therefore, the present experiment purported to develop an integrated nutrient management package for mango consisting of organic manure (FYM), inorganic fertilizers and biofertilizers for improving growth and yield in ‘Himsagar’.

The present investigation was carried out at Regional Research Station, Gayeshpur, B.C.K.V., Nadia, West Bengal, during the years 2009-2011. The site of the experiment is situated at 22p 57^1 N latitude and 89p 34^1 E longitude, at an average altitude of 9.75m above mean sea level. The experiment was laid out in Randomised Block Design (RBD) in five replications. Age of the trees was seven years, at a spacing of 10m x 10m. The experiment consisted of 10 treatments, viz., T_1: 1000:500:500g NPK/tree (Control), T_2: T_1 + Zn (0.5%) + B (0.2%) + Mn (1%) + Ca (0.6%) as foliar application, twice (Aug & Oct); T_3: T_1 + Organic mulching (10cm thick layer of dry leaves); T_4: T_2 + Organic mulching (10cm thick layer of dry leaves); T_5: ½ T_1 + 50kg FYM + 250g *Azospirillium*; T_6: ½ T_1+ 50kg FYM + 250g *Azospirillium*; T_7: ½ T_1 + 250g *Azotobacter* + 250g

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Azospirillium; T_8: ½ T_1 + 50kg FYM + 250g *Azotobacter*; T_9: ½ T_1 + 50kg FYM + 250g *Pseudomonas florescence*; T_{10}: ½ T_1 + 50kg FYM + 250g *Pseudomonas florescence* + 250g *Trichoderma*. Every plant treated was supplemented with the dose set for each treatment from the month of March after flowering. Treatments, along with mulches (dry wheat-straw leaves), were applied at a thickness of 8-10cm and retained in the field for three years for soil moisture conservation and increased organic matter in soil. Nutrient fertilizers (N, P and K) were provided in the form of urea (46% N), single super phosphate (16% P_2O_5) and potassium sulphate (50% K_2O), respectively, and applied in two split doses in March (at the marble stage of fruit development) and July (after harvest). Vegetative growth parameters were recorded after harvest (in June) and, again, before initiation of the next flowering (December). Yield parameters were also recorded. Irrigation was applied after the fertilizer and, subsequently, as and when required (depending upon the rainfall). Irrigation was stopped 7-10 days before harvest.

Plant growth parameters showed significant variation under different treatments (Table 1, 2, 3 & 4). Plants grown under 500:250:250g NPK/tree + 50kg FYM + 250g *Azospirillium* (T_6), showed improved vegetative growth parameters compared to other treatments. However, T_2 + Organic mulching (10cm thick layer of dry leaves) (T_4) caused the maximum total increment in canopy height, closely followed by 500:250:250g NPK/tree + 50kg FYM + 250g *Azospirillium* (T_6). These findings are similar to those of Sivakumar (2001) and Shulka *et al* (2009). Further, Gautam *et al* (2012) found in mango cv. Sunderja, that application of 500:250:250g N:P:K/tree + 50kg FYM + 10kg Vermicompost registered maximum plant height, canopy height, plant spread (N-S and E-W) and tree volume compared to the Control 500:250:250g N:P:K/tree. Vegetative parameters were superior in the treatment with nitrogen fixing bacteria, viz., *Azotobacter* and *Azopirillium.* This could be due to the higher nitrogen content in soil, essential for growth of the plant system. Subba Rao *et al* (1980) also reported inoculation of *Azotobacter* and *Azospirillium* in several non-legumes crops as contributing

Table 1. Effect of integrated nutrient management (INM) on plant height in mango cv. Himsagar

Treatment	Dec 2008 (m)	June 2009 (m)	Increase (cm)	Dec 2009 (m)	Increase (cm)	June 2010 (m)	Increase (cm)	Dec 2010 (m)	Increase (cm)	June 2011 (m)	Increase (cm)	Total increase (cm)
T_1	5.02	5.16	14.00	5.31	15.00	5.44	13.00	5.57	13.00	5.73	16.00	71.00
T_2	4.86	5.03	17.00	5.19	16.00	5.36	17.00	5.52	16.00	5.71	19.00	85.00
T_3	4.65	4.83	18.00	4.99	16.00	5.16	17.00	5.31	15.00	5.50	19.00	85.00
T_4	4.95	5.13	18.00	5.30	17.00	5.48	18.00	5.64	16.00	5.82	18.00	87.00
T_5	4.76	4.99	23.00	5.16	17.00	5.34	18.00	5.48	14.00	5.67	19.00	91.00
T_6	5.30	5.52	22.00	5.72	20.00	5.91	19.00	6.08	17.00	6.38	30.00	108.00
T_7	4.78	4.96	18.00	5.13	17.00	5.30	17.00	5.46	16.00	5.65	19.00	87.00
T_8	4.46	4.65	19.00	4.84	19.00	5.02	18.00	5.21	19.00	5.39	18.00	93.00
T_9	5.05	5.23	18.00	5.42	19.00	5.59	17.00	5.76	17.00	5.93	17.00	88.00
T_{10}	4.83	5.02	19.00	5.19	17.00	5.36	17.00	5.52	16.00	5.73	21.00	90.00
SE±m	0.15	0.15	-	0.12	-	0.15	-	0.13	-	0.12	-	-
CD *(P=0.05)*	0.43	0.34	-	0.34	-	0.44	-	0.39	-	0.35	-	-

Table 2. Effect of integrated nutrient management (INM) on tree volume of mango cv. Himsagar

Treatment	Dec 2008 (m^3)	June 2009 (m^3)	Increase (m^3)	Dec 2009 (m^3)	Increase (m^3)	June 2010 (m^3)	Increase (m^3)	Dec 2010 (m^3)	Increase (m^3)	June 2011 (m^3)	Increase (m^3)	Total increase (m^3)
T_1	67.30	74.38	7.08	82.98	8.60	92.41	9.43	101.91	9.50	112.88	10.97	45.58
T_2	75.02	85.61	10.59	96.54	10.93	108.60	12.06	121.31	12.71	136.72	15.41	61.70
T_3	57.88	67.26	9.38	77.16	9.90	87.09	9.93	99.98	12.89	113.80	13.82	55.92
T_4	76.07	87.29	11.22	99.46	12.17	117.50	18.04	127.45	9.95	147.36	19.91	71.29
T_5	76.07	92.24	16.17	106.93	14.69	118.20	20.75	135.53	17.33	151.90	16.37	75.83
T_6	99.53	116.09	16.56	132.58	16.49	150.81	15.23	166.68	15.87	185.48	18.80	85.95
T_7	56.02	64.36	8.34	73.46	9.10	83.35	9.89	94.73	11.38	107.63	12.90	51.61
T_8	67.71	78.84	11.13	90.92	12.08	105.29	14.37	118.69	13.40	134.84	16.15	67.13
T_9	81.90	92.93	11.03	105.11	12.18	117.75	12.46	131.93	14.18	146.83	14.90	64.93
T_{10}	70.99	80.92	9.99	91.33	10.41	107.20	15.87	117.50	10.30	131.45	13.95	60.46
SE±m	5.72	7.21	—	6.55	—	7.44	—	11.35	—	8.70	—	—
CD *(P=0.05)*	16.27	20.5	—	18.61	—	21.14	—	32.24	—	24.73	—	—

Table 3. Effect of integrated nutrient management (INM) on plant-spread (North – South) in mango cv. Himsagar

Treatment	Dec 2008 (m)	June 2009 (m)	Increase (cm)	Dec 2009 (m)	Increase (cm)	June 2010 (m)	Increase (cm)	Dec 2010 (m)	Increase (cm)	June 2011 (m)	Increase (cm)	Total increase (cm)
T_1	5.09	5.24	15.00	5.39	15.00	5.55	16.00	5.75	20.00	5.91	16.00	82.00
T_2	5.29	5.47	18.00	5.66	19.00	5.82	16.00	6.01	19.00	6.21	20.00	0.92
T_3	4.73	4.92	19.00	5.11	19.00	5.26	15.00	5.55	29.00	5.69	14.00	0.96
T_4	4.99	5.17	18.00	5.36	19.00	5.56	20.00	5.75	19.00	5.93	18.00	0.94
T_5	5.5	5.69	19.00	5.87	18.00	6.07	20.00	6.29	22.00	6.49	20.00	0.99
T_6	5.68	5.89	16.00	6.10	19.00	6.29	17.00	6.54	25.00	6.73	19.00	1.05
T_7	5.41	5.57	16.00	5.76	19.00	5.93	17.00	6.14	21.00	6.30	16.00	0.89
T_8	5.30	5.50	20.00	5.70	20.00	5.88	18.00	6.08	20.00	6.29	21.00	0.99
T_9	5.50	5.68	18.00	5.85	17.00	6.02	17.00	6.22	20.00	6.39	17.00	0.89
T_{10}	5.35	5.55	20.00	5.72	17.00	5.90	18.00	6.09	19.00	6.26	17.00	0.91
SE±m	0.25	0.26	—	0.25	—	0.25	—	0.27	—	0.26	—	—
CD *(P=0.05)*	NS	NS	—	NS	—	NS	—	0.78	—	0.76	—	—

Table 4. Effect of integrated nutrient management (INM) on plant-spread (East – West) in mango cv. Himsagar

reatment	Dec 2008 (m)	June 2009 (m)	Increase (cm)	Dec 2009 (m)	Increase (cm)	June 2010 (m)	Increase (cm)	Dec 2010 (m)	Increase (cm)	June 2011 (m)	Increase (cm)	Total increase (cm)
T_1	4.64	4.82	18.00	4.988	16.00	5.17	19.00	5.34	17.00	5.49	15.00	85.00
T_2	5.15	5.34	19.00	5.524	18.00	5.71	19.00	5.89	18.00	6.10	21.00	95.00
T_3	4.74	4.94	20.00	5.138	19.00	5.39	26.00	5.56	17.00	5.75	19.00	101.00
T_4	5.61	5.84	23.00	6.042	20.00	6.24	20.00	6.43	19.00	6.64	21.00	103.00
T_5	5.52	5.74	22.00	5.892	15.00	6.13	24.00	6.32	19.00	6.54	22.00	102.00
T_6	5.67	5.96	29.00	6.204	24.00	6.48	28.00	6.68	20.00	6.90	22.00	123.00
T_7	4.11	4.31	20.00	4.482	17.00	4.69	21.00	4.86	17.00	5.06	20.00	95.00
T_8	5.02	5.27	25.00	5.488	21.00	5.76	28.00	5.95	19.00	6.16	21.00	114.00
T_9	5.00	5.18	18.00	5.364	18.00	5.57	21.00	5.75	18.00	5.93	18.00	93.00
T_{10}	4.89	5.07	18.00	5.252	18.00	5.51	26.00	5.69	18.00	5.85	16.00	96.00
SE±m	0.26	0.24	—	0.26	—	0.26	—	0.27	—	0.27	—	—
CD *(P=0.05)*	0.74	0.70	—	0.75	—	0.76	—	0.76	—	0.76	—	—

Table 5. Effect of integrated nutrient management (INM) on yield in mango cv. Himsagar

Treatment	No. of fruits / tree				Average fruit weight (g)				Fruit yield (kgme)			
	2009	2010	2011	Pooled	2009	2010	2011	Pooled	2009	2010	2011	Pooled
T_1	21.00	178.00	158.00	119.00	224.506	231.28	232.38	229.38	5.05	38.58	40.41	28.02
T_2	32.25	267.00	240.00	175.43	233.8	239.30	234.20	235.76	7.51	61.77	53.15	40.81
T_3	80.25	245.20	246.00	180.05	226.35	246.76	248.06	232.57	21.29	63.71	55.29	46.76
T_4	50.00	271.40	246.00	189.13	239.45	245.98	246.94	244.12	10.01	65.97	58.82	44.93
T_5	60.60	262.20	275.00	199.26	222.50	244.08	240.02	235.77	17.74	65.48	62.78	48.66
T_6	74.66	294.00	333.70	234.12	243.00	255.58	290.74	263.10	21.90	71.95	81.85	58.56
T_7	55.00	196.75	216.00	153.91	222.6	239.332	255.98	239.30	13.72	47.57	44.65	35.31
T_8	78.00	280.75	261.75	206.83	244.22	250.62	265.82	253.55	20.81	65.91	63.18	49.97
T_9	25.00	278.00	245.80	177.60	235.15	239.40	255.70	243.41	9.04	62.95	61.77	44.59
T_{10}	51.00	259.00	254.00	194.33	237.00	247.02	260.62	248.27	13.78	68.10	61.38	47.75
SE±m	8.40	8.32	10.16	6.49	4.02	5.63	10.25	3.90	2.76	3.60	7.65	1.37
CD *(P=0.05)*	23.88	23.63	28.87	18.45	11.42	NS	29.11	11.09	7.76	8.70	2.69	3.91

about 25kgN / ha through fixation in soil, leading to better plant growth and 5-15% higher yield.

Results also revealed that yield parameters (Table 5) such as number of fruits/tree, average fruit weight and yield (kg/tree) increased under different combinations of integrated nutrient management compared to that in Control (T_1) (1000:500:500g N:P:K/tree). Significantly high cumulative yield was obtained in ½ T_1+ 50kg FYM + 250g *Azospirillium* (T_6), followed by ½ T_1 + 50kg FYM + 250g *Azotobacter* (T_8), while significantly lower value was seen in Control. These finding are in line with those of Patel *et al* (2005). Hasan *et al* (2009) too observed maximum flowering and fruiting in trees supplied with 50% recommended dose

Fig. 1. Observations on girth and plant-spread

Fig. 2. Observations on plant height

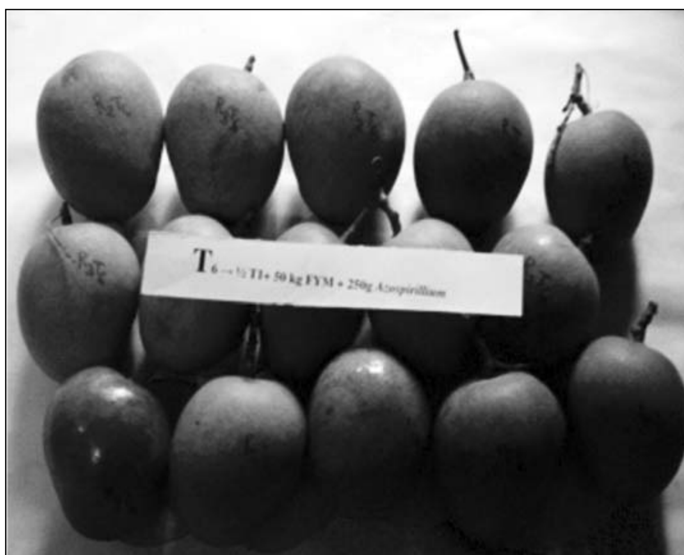

Fig. 3. Harvested fruit of mango cv. Himsagar under treatment T_6

Fig. 4. Heavy bearing under treatment T_6

of nutrients along *Azospirillium* and VAM inoculation. Further, Yadav *et al* (2011) reported in mango cv. Amrapali that the recommended NPK + Vermicompost + *Azotobacter* + PSB + Zn + Fe + Paclobutrazol application recorded optimum yield compared to that in Control (recommended NPK/tree). Similarly, Gautam *et al* (2012) found that application of 500:250:250g N:P:K/tree + 50kg FYM + 10kg Vermicompost registered maximum number of fruits/tree compared to Control (500:250:250g N:P:K/tree). Therefore, it can be concluded that integration of inorganic fertilizer with biofertilizers improves vegetative growth and yield in mango, without affecting fruit quality. This can be recommended for sustainable mango production with minimal use of fertilizer under the alluvial zone of West Bengal.

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(Registration No BLU- S 1089-2005-06 dated 21-01-2006)

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4. Brain Storming Session on Problems in Marketing of Fruits, Vegetables, Flowers and Medicinal Plants
5. Group Meeting on National Scenario on Management of Sucking Pests
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