Volume 74, No. 4
December, 2017

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

Indian Journal of Horticulture

Estd. 1942

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

The Horticultural Society of India (Regd.)

[Founded in January 1942]

The main object of the Society is to cultivate and promote research, education and development of Horticulture and allied branches of science.

Executive Council for 2017

Office Bearers

President : Dr K.L. Chadha, New Delhi
Immediate Past President : Dr S.N. Rao, Hyderabad
Senior Vice-President : Dr Kirti Singh, Jaunpur

Vice-Presidents

Dr M.L. Chadha, Hisar | Dr S.P. Ghosh, New Delhi | Dr Nazeer Ahmed, Srinagar

Secretary : Dr S.K. Singh, New Delhi
Joint Secretary : Dr Jai Prakash, New Delhi
Editor-in-Chief : Dr S.K. Singh, New Delhi
Editors : Dr Manish Srivastav, New Delhi
Dr T.K. Behera, New Delhi
Treasurer : Dr T.K. Behera, New Delhi

Executive Councillors

Dr Y.R. Chanana, Ludhiana
Dr M.S. Dhaliwal, Ludhiana
Dr W.S. Dhillon, New Delhi
Dr M.R. Dinesh, Bengaluru
Dr T. Janakiram, New Delhi
Dr Pritam Kalia, New Delhi
Dr S.K. Malhotra, New Delhi
Dr R.K. Pal, Solapur
Dr V.B. Patel, Bhagalpur
Dr K.V. Prasad, Pune
Dr S. Rajan, Lucknow
Dr B.M.C. Reddy, West Godavari
Dr P.L. Saroj, Puttur
Dr Balraj Singh, Jodhpur
Dr Bijender Singh, Varanasi
Dr Desh Beer Singh, Srinagar
Dr D.R. Singh, Gangtok
Dr S. Uma, Trichy

— — — — — — — — — — — — — —

International Advisory Board

Dr B.S. Ahloowalia, Austria
Dr Maurizio Lambardi, Italy
Dr R.E. Litz, USA
Dr S. Kanlayanarat, Thailand
Dr K.V. Raman, USA
Dr Md. Abdur Rahim, Bangladesh
Dr A.K. Yadava, USA
Dr Zora Singh, Australia
Dr David M. Spooner, USA

Editorial Board

Dr K. Nirmal Babu, Kozhikode
Dr M.I.S. Gill, Ludhiana
Dr B.V.C. Mahajan, Ludhiana
Dr R.L. Misra, New Delhi
Dr D.P. Waskar, Parbhani
Dr Vishal Nath, Muzaffarpur
Dr Subhash Chander, New Delhi
Dr (Ms.) Indu Sawant, Pune
Dr R.R. Sharma, New Delhi
Dr Major Singh, Pune
Dr Brahma Singh, New Delhi
Dr P.M. Haldankar, Dapoli

The Indian Journal of Horticulture is the official publication of the Horticultural Society of India. The journal is published every quarter, *i.e.*, during March, June, September and December. The journal publishes original research papers on all horticultural crops. The subscription rates are follows:

Category	Soft copy (₹)	Hard copy (₹)	Overseas (US $)
Patron		1,00,000	—
Corporate membership		5,00,000	—
Life membership	7,500	10,000	—
Annual membership	1,500	2,000	US$ 100 (Soft copy)
Annual subscription	10,000	15,000	US$ 450 (add US$ 40 for postage and handling)

Membership / subscription may be sent to the **Secretary / Treasurer, F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi - 110 012, India** through bank demand draft drawn in favour of **Horticultural Society of India** payable at **New Delhi**. **Syndicate Bank, NASC Complex, Pusa Campus, New Delhi 110012, A/c No.: 91532010004372, IFSC Code : SYNB0009153**

The article for publication may please be sent in duplicate to **the Editor, Horticultural Society of India** to its Secretariat address: **F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi 110 012, India**. Old address: **C/o Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110 012, India**. Any other correspondence about the Horticultural Society of India and its activities may be sent on the above address.

Tel. No. 91(011) 25842127; **E-mail**: hsi42@rediffmail.com; **Website**: www.hsi1942.in

Online version available at: www.indianjournals.com

DOI : 10.5958/0974-0112.2017.00094.9

Indian J. Hort. 74(4), December 2017: 479-485

Assessment of water relation traits during different phenological stages in mango (*Mangifera indica* L.)

Pooja Saxena, V.K. Singh* and S. Rajan

ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow 226101, Uttar Pradesh

ABSTRACT

In mango, water stress plays an important role in flowering & fruiting regulation as it provides the floral induction signal. Pressure volume curve (P-V curve) was used to derive basic water relation parameters, *viz.,* osmotic potential, symplastic and apoplastic water content, solute potential at full turgor, turgor loss point, water content at turgor loss point and elasticity modulus in plant system. In the present paper Amrapali and Langra mango cultivars having regular and irregular bearing habit, were studied in order to understand the leaf responses to different water status. The effect of paclobutrazol (PP_{333}), a growth retardant having anti-gibberellin activity and induce the flowering even in the 'Off' year on these parameters was also studied. During flower bud differentiation (FBD), Amrapali had more osmotic potential (-3.39 MPa) than Langra (-5.38 MPa) without any significant shift with paclobutrazol treatment. However, osmotic potential increased markedly in trees treated with paclobutrazol (2 g a.i./ tree) at flower bud burst and panicle emergence stages as compared to control. Langra exhibited lower turgor loss point (-5.56 MPa) than Amrapali (-5.41 MPa) at FBD, which indicate early turgor loss in leaves of Langra than Amrapali during the critical period of flowering. Amrapali showed lower turgor loss point (TLP) in subsequent stages of flower development, which may signify that with lower TLP it may be able to maintain osmoregulation at lower leaf water potentials. Sap flow also varied significantly in these cultivars as Amrapali had higher range of sap flow (6.76-18.99 kg/h) than Langra (6.91-13.11 kg/h) in different flower developmental stages. The results of this study showed that adjustment for water stress may be greater in Amrapali than Langra sharing same habitat but having different bearing pattern. Better osmoregulation may be helpful for Amrapali to outgrow better than Langra under subtropical conditions.

Key words: *Mangifera indica* L., relative water content, osmotic adjustment, water potential.

INTRODUCTION

Water stress is one of several factors which affect mango production (Balley *et al*., 2). Information on water relations and irregular bearing pattern influenced by water stress in mango is limited. Pressure-volume analysis is used to determine various water relation parameters of the plants (Lenz *et al*., 6) such as the osmotic and pressure potentials of the symplast and apoplast, the bulk modulus of elasticity (E) and Turgor loss point (TLP). Application of this technique requires starting the plant tissue at near zero water potential (Ngugi *et al*., 9). The leaves collected from the tree are generaly at water potential considerably less than zero, and therefore it must be rehydrated for further study. Pressure volume curve (P-V curve) is a plot of inverse water potential, which by definition declines linearly with RWC below the Turgor loss point (TLP) (Tyree and Hammel, 14). From P-V curve, symplastic water fraction (R's) is estimated by extrapolating the straight-line section with large negative water potential and TLP is estimated as the point where the line becomes non-linear (Sobrado, 11). These traits may differ between varieties and individual plants can adjust them over time.

Sap flow is generally used to study the movement of water through conductive xylem of tree. Its pattern may not be homogenous across the tree at different phenological stages therefore investigation on its profile during different flower developmental phenology in mango is important to study. Among plant growth retardant, paclobutrazol is considered as one of the important plant growth retardant which restricts vegetative growth, induces flowering and fruiting in many fruit species including mango (Abdel *et al*., 1; Singh and Singh, 10). Paclobutrazol treated plants may maintain high leaf turgor at high traspirational demand and better able to withstand stress conditions (Chaves *et al*., 4). Keeping this in view, the inter-relationship of paclobutrazol with estimated water related parameters in mango was studied.

MATERIALS AND METHODS

The present investigations were carried out with full bearing trees of 'Langra' (biennial) and 'Amrapali' (regular) mango cultivars during year 2010-11 and

*Corresponding author's E-mail: singhvk_cish@rediffmail.com

2011-12 at ICAR-CISH, Rehmankhera, Lucknow located at 26.54°N Latitude, 80.45°E Longitude and 127 m. above mean sea level. The soil is mixed hyperthermic family of typical Ustochrepts with sandy loam texture. The range of average maximum and minimum temperatures was 18.5-38.6 and 5.0-25.3, respectively during experimental period. The rainfall recorded ranged between 3.7-17.5 mm during the same period and relative humidity was in the range of 71.7-88.9% with mean daily pan evaporation ranged from 1.3-11 mm. A single soil drenching of paclobutrazol @ 2, 4, 6 and 8 g a.i. per tree in the root zone at 15 cm depth was applied during the month of September. Both under paclobutrazol treated and control, four trees were taken. Untreated trees were kept as control. About 50 potential healthy shoots were tagged from each direction and recently fully matured leaves adjacent to apical meristem having same age and orientation as standardized earlier (Yadava and Singh, 15) were collected. Sampling of leaves for assessment of different water relationship parameters was done at different stages of flowering, *i.e.* before flower bud differentiation, flower bud differentiation, bud burst and panicle elongation. The experiment was performed in a complete randomized design with five replications.

The plant water status was determined by simultaneous measurements of water potential (Ψw) and leaf water content (LWC). Each excised leaf was immediately put inside zip pouch for Ψw measurement. All predawn and midday Ψw measurements were made with water potential measurement system (WP4 & WP4-T Dewpoint Meters, Decagon Devices, USA).Values of LWC were determined as: LWC = 100 (FM-DM)/ FM. Where FM is leaf fresh mass and DM is leaf dry mass. Dry mass was determined after drying the leaf samples at 80°C for 24 h. Values of LWC were expressed as relative water content (RWC) by determining FM, DM and saturated mass (SM) as RWC = (FM−DM)/ (SM−DM).

For determination of water potential petioles was cut underwater and leaves were hydrated in potable tap water (room temperature and kept in the dark for 24 h); after equilibration (2.5 h) of leaf sample a disc (approx. 100 mg) was taken from mid portion of leaf and Ψw was measured. Leaf discs were left to dry on the bench between measurements and were weighed after every 10 min interval (till it reaches at weighed difference of 0.001 g) immediately before and after water potential was measured with a C-52 thermocouple psychrometer. Water potential was measured using standard procedures (Turner, 13). Standard solution of known water potential (Ψw) was always run with samples and values corrected to a temperature of 25°C. After equilibration the water potential (Ψw) was measured after 10 min. interval as the leaf disc loses 2-3 mg water. This was repeated until 10-12 measurements had been made on each sample, and the plants had reached a RWC of approx.16% and 5% and a Ψw of approx. -7.0 and -5.0 MPa for Amrapali and Langra, respectively.

For the P-V curves, five replicate of P-V curves were measured for both the varieties. Water relation parameters of these two varieties were calculated from pressure-volume isotherms. P-V curve was drawn on the basis of RWC and reciprocal of Ψw on × and y axis, respectively as described earlier. Turgor-loss points were obtained by subjecting previously rehydrated leaves to a series of paired measurements of WP and relative water content (RWC) as they were allowed to air-dry. TLP was determined from the start of the straight line, from plots of inverse balance pressure vs. shoot fresh mass. Calculation of the other water relation traits from p-v curve was followed by the standard procedure. Sap flow at different stages of flowering was measured by Sap Flow System EMS in both the varieties with five replications in each direction of the tree. The measuring principle is based on the tissue heat balance method (THB) with internal heating and sensing (Cermak *et al*., 3). The sap flow value of shoots was calculated as per the method described by Tatarinov *et al.* (12). All measurements were expressed as mean of five measurements (±SE) from each tree per treatment. Significant differences were detected at $p = 0.05$, according to the Student's *t* test.

RESULTS AND DISCUSSION

The Table 1 (A, B, C, D) presents important parameters, *viz*. osmotic potential (OP), symplastic (R's) and apoplastic water content (R'a), solute potential at full turgor (SPAFT), turgor loss point (TLP), water content at turgor loss point (WCTLP) and elasticity modulus (E) at different phenophases which were derived from P-V curve (Fig. 1A & 1B) at FBD stage for two cultivars (P-V curve for rest of the stages were not shown). Parameters in the table clearly revealed that during FBD Amrapali had more OP (-3.39 MPa) than Langra (-5.38 MPa) without any significant change with paclobutrazol. However OP increased markedly in trees treated with paclobutrazol (2 g a.i./ tree] at flower bud burst and panicle emergence stages as compared to control. Osmotic potential increases in paclobutrazol treated trees (Amrapali = -3.23 MPa; Langra = -5.18 MPa) as compared to control one (Amrapali = -3.39 MPa; Langra = -5.38 MPa). This finding showed that paclobutrazol had osmoregulatory capacity in order to maintain water status of tree during active and stressed stage of flower bud development. Symplastic

Table 1A. Changes in water relation parameters of Amrapali (A) and Langra (L) at flower bud differentiation with paclobutrazol treatments.

Parameter	Control		2 g a.i.		4 g a.i.		6 g a.i.		8 g a.i.	
	A	L	A	L	A	L	A	L	A	L
OP	-3.39 ± 0.11	-5.38 ± 0.23	-3.23 ± 0.12	-5.18 ± 0.41	-3.23 ± 0.13	-5.18 ± 0.63	-3.39 ± 0.11	-5.05 ± 0.78	-3.23 ± 0.10	-5.13 ± 0.56
R's %	76.00 ± 5.66	60.00 ± 6.52	89.00 ± 8.71	73.00 ± 7.11	92.00 ± 6.23	75.00 ± 7.23	87.00 ± 8.41	75.00 ± 7.14	83.00 ± 6.33	72.50 ± 5.64
R'a %	24.00 ± 1.23	40.00 ± 3.63	11.00 ± 1.21	27.00 ± 3.22	8.00 ± 0.98	25.00 ± 2.33	13.00 ± 1.21	25.00 ± 2.45	17.00 ± 1.86	27.50 ± 3.44
SPAFT	-3.39 ± 0.11	-5.38 ± 0.23	-3.23 ± 0.12	-5.18 ± 0.41	-3.23 ± 0.13	-5.18 ± 0.63	-3.39 ± 0.11	-5.05 ± 0.78	-3.23 ± 0.10	-5.13 ± 0.56
TLP	-5.41 ± 0.47	-5.56 ± 0.89	-5.26 ± 0.87	-5.52 ± 0.95	-5.56 ± 0.87	-5.56 ± 0.75	-5.41 ± 0.66	-5.78 ± 0.89	-5.56 ± 0.91	-5.49 ± 0.93
WCTLP	33.00 ± 5.66	48.00 ± 7.11	26.00 ± 2.33	31.00 ± 6.33	20.00 ± 1.22	34.00 ± 2.63	11.90 ± 1.24	25.00 ± 2.21	14.00 ± 1.11	45.00 ± 7.11
E	2.21 ± 0.91	5.12 ± 0.41	2.75 ± 0.23	0.66 ± 0.09	3.27 ± 0.78	2.02 ± 0.23	2.42 ± 0.41	0.87 ± 0.08	2.80 ± 0.25	0.55 ± 0.04

E = Elasticity modulus, OP = Osmotic potential, R's = Symplastic water content, R'a = Apoplastic water content, SPAFT = Solute potential at full turgor, TLP = Turgor loss point, WCTLP = Water content at turgor loss point; Data are expressed as a pooled mean ± standard deviation

Table 1B. Change in water relation parameters of Amrapali (A) and Langra (L) at bud burst stage with paclobutrazol treatments.

Parameter	Control		2 g a.i.		4 g a.i.		6 g a.i.		8 g a.i.	
	A	L	A	L	A	L	A	L	A	L
OP	-6.49 ± 0.91	-6.62 ± 0.33	-4.55 ± 0.23	-6.45 ± 0.45	-6.37 ± 0.56	-6.29 ± 0.44	-6.41 ± 0.65	-6.25 ± 0.41	-6.67 ± 0.77	-0.61 ± 0.44
R's %	78.00 ± 7.41	82.80 ± 9.71	81.00 ± 8.99	78.80 ± 9.88	81.00 ± 8.79	76.70 ± 8.45	76.00 ± 9.77	73.40 ± 8.75	66.00 ± 6.78	80.50 ± 8.11
R'a %	22.00 ± 4.56	17.20 ± 2.33	19.00 ± 8.74	21.20 ± 2.33	19.00 ± 4.11	23.30 ± 3.41	24.00 ± 2.63	26.60 ± 3.22	34.00 ± 2.52	19.50 ± 3.11
SPAFT	-6.49 ± 0.91	-6.62 ± 0.33	-4.55 ± 0.23	-6.45 ± 0.45	-6.37 ± 0.56	-6.29 ± 0.44	-6.41 ± 0.65	-6.25 ± 0.41	-6.67 ± 0.77	-0.61 ± 0.44
TLP	-6.99 ± 0.74	-6.62 ± 0.66	-7.09 ± 0.74	-6.94 ± 0.85	-7.14 ± 0.99	-6.15 ± 0.78	-7.04 ± 0.91	-6.94 ± 0.66	-6.67 ± 0.71	-6.62 ± 0.23
WCTLP	36.00 ± 2.33	27.00 ± 1.22	29.00 ± 2.33	20.00 ± 3.66	26.00 ± 4.41	33.00 ± 3.66	25.00 ± 2.63	26.00 ± 1.42	31.00 ± 1.56	22.00 ± 1.11
E	0.89 ± 0.11	0.55 ± 0.09	1.15 ± 0.23	0.97 ± 0.14	0.99 ± 0.11	0.25 ± 0.09	0.96 ± 0.08	1.00 ± 0.04	0.80 ± 0.07	0.58 ± 0.04

E = Elasticity modulus, OP = Osmotic potential, R's = Symplastic water content, R'a = Apoplastic water content, SPAFT = Solute potential at full turgor, TLP = Turgor loss point, WCTLP = Water content at turgor loss point; Data are expressed as a pooled mean ± standard deviation

Table 1C. Changes in water relation parameters of Amrapali (A) and Langra (L) mango at panicle emergence with application of paclobutrazol.

Parameter	Control		2 g a.i.		4 g a.i.		6 g a.i.		8 g a.i.	
	A	L	A	L	A	L	A	L	A	L
OP	-6.49 ± 0.52	-6.29 ± 0.66	-4.55 ± 0.71	-6.45 ± 0.56	-6.33 ± 0.87	-6.41 ± 0.11	-6.33 ± 0.56	-6.29 ± 0.47	-6.41 ± 0.44	-6.25 ± 0.56
R's %	76.00 ± 4.11	80.00 ± 5.56	81.00 ± 4.89	78.10 ± 7.11	65.00 ± 6.63	76.60 ± 4.56	79.00 ± 5.89	77.40 ± 6.61	77.00 ± 5.56	83.80 ± 6.63
R'a %	24.00 ± 2.33	20.00 ± 4.56	19.00 ± 1.22	21.90 ± 2.63	35.00 ± 4.56	23.40 ± 2.66	21.00 ± 3.21	22.60 ± 2.54	23.00 ± 3.14	16.20 ± 2.56
SPAFT	-6.49 ± 0.52	-6.29 ± 0.66	-4.55 ± 0.71	-6.45 ± 0.56	-6.33 ± 0.87	-6.41 ± 0.11	-6.33 ± 0.56	-6.29 ± 0.47	-6.41 ± 0.44	-6.25 ± 0.56
TLP	-6.94 ± 0.87	-6.62 ± 0.56	-7.09 ± 0.78	-6.99 ± 0.98	-7.14 ± 0.45	-6.62 ± 0.63	-7.04 ± 0.84	-7.04 ± 0.85	-6.90 ± 0.66	-6.62 ± 0.87
WCTLP	44.00 ± 4.11	30.00 ± 8.74	25.90 ± 2.33	31.00 ± 6.33	17.00 ± 1.22	25.00 ± 2.54	21.00 ± 3.85	10.00 ± 1.24	32.00 ± 2.88	25.00 ± 3.74
E	0.81 ± 0.09	0.55 ± 0.01	1.15 ± 0.44	1.00 ± 0.23	0.99 ± 0.12	0.57 ± 0.07	0.87 ± 0.08	1.00 ± 0.25	0.79 ± 0.33	0.60 ± 0.41

E = Elasticity modulus, OP = Osmotic potential, R's = Symplastic water content, R'a = Apoplastic water content, SPAFT = Solute potential at full turgor, TLP = Turgor loss point, WCTLP = Water content at turgor loss point; Data are expressed as a pooled mean ± standard deviation

Table 1D. Change in water relation parameters of Amrapali (A) and Langra (L) mango at panicle elongation with paclobutrazol application.

Parameter	Control		2 g a.i.		4 g a.i.		6 g a.i.		8 g a.i.	
	A	L	A	L	A	L	A	L	A	L
OP	-6.21 ± 0.52	-6.67 ± 0.74	-6.37 ± 0.66	-6.67 ± 0.53	-6.90 ± 0.56	-6.67 ± 0.56	-6.13 ± 0.74	-6.41 ± 0.56	-6.29 ± 0.89	-6.45 ± 0.74
R's%	80.00 ± 7.41	86.20 ± 9.66	68.00 ± 8.74	75.90 ± 7.56	90.00 ± 7.45	90.00 ± 8.11	77.00 ± 7.41	79.30 ± 8.66	69.00 ± 7.56	79.30 ± 7.41
R'a%	20.00 ± 2.33	13.80 ± 1.22	32.00 ± 2.56	24.10 ± 3.41	10.00 ± 2.56	10.00 ± 2.11	23.00 ± 2.32	20.70 ± 2.41	31.00 ± 2.66	20.70 ± 2.45
SPAFT	-6.21 ± 0.52	-6.67 ± 0.74	-6.37 ± 0.66	-6.67 ± 0.53	-6.90 ± 0.56	-6.67 ± 0.56	-6.13 ± 0.74	-6.41 ± 0.56	-6.29 ± 0.89	-6.45 ± 0.74
TLP	-7.35 ± 1.22	-7.14 ± 2.12	-7.41 ± 2.36	-7.09 ± 1.45	-7.81 ± 3.11	-7.41 ± 1.22	-6.80 ± 2.41	-7.09 ± 1.22	-7.04 ± 2.36	-6.99 ± 1.32
WCTLP	35.00 ± 3.66	26.00 ± 4.74	45.00 ± 5.11	27.00 ± 1.22	11.00 ± 0.89	20.00 ± 0.74	45.00 ± 5.22	34.00 ± 1.23	51.00 ± 6.89	37.00 ± 2.63
E	1.89 ± 0.08	0.97 ± 0.11	1.75 ± 0.23	0.98 ± 0.07	1.61 ± 0.11	1.38 ± 0.05	1.61 ± 0.08	0.99 ± 0.11	0.91 ± 0.04	0.86 ± 0.11

E = Elasticity modulus, OP = Osmotic potential, R's = Symplastic water content, R'a = Apoplastic water content, SPAFT = Solute potential at full turgor, TLP = Turgor loss point, WCTLP = Water content at turgor loss point; Data are expressed as a pooled mean ± standard deviation

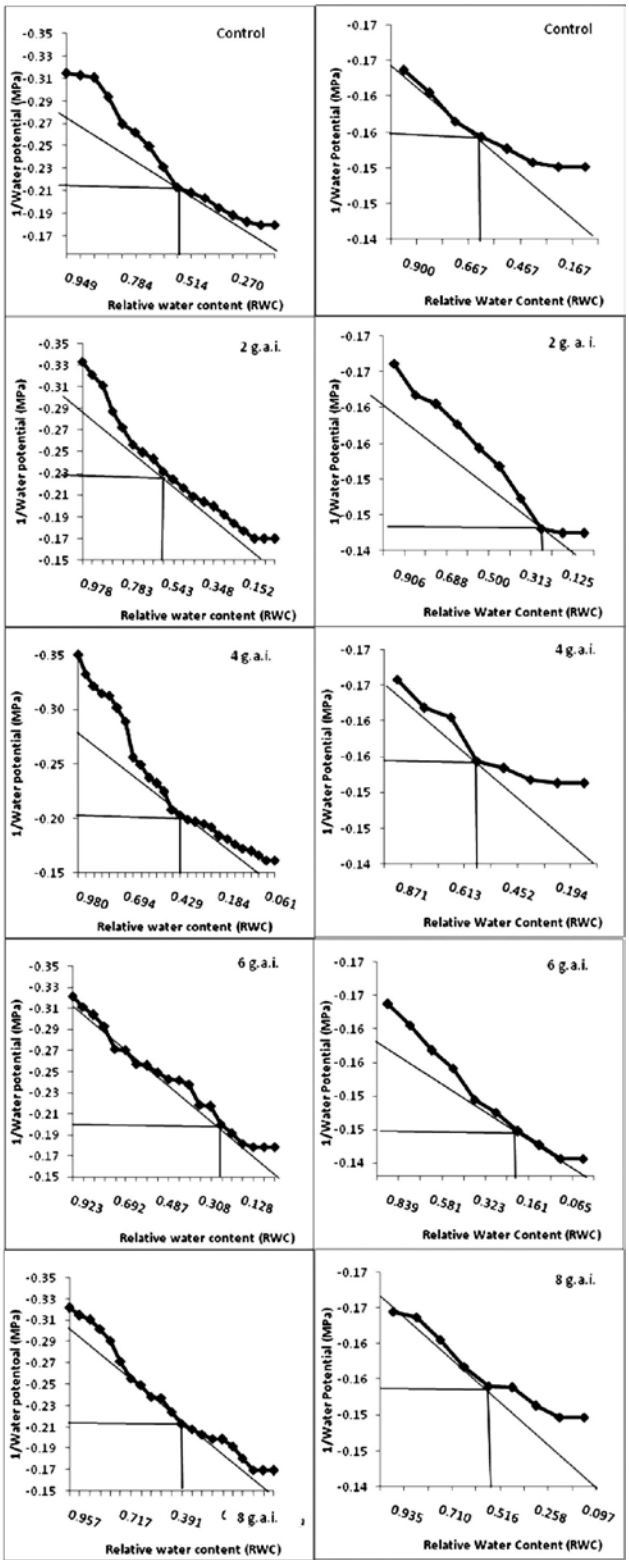
content (R's) also found more in Amrapali (76.00%) than Langra (60.00%) at FBD as a result apoplastic content (R'a) appeared reverse pattern among two varieties as indicated by lower value in Amrapali (24.00%) than Langra (40.00%). Paclobutrazol showed positive response and increased symplastic content after FBD in both the varieties. Its value was found higher in Langra (73.40-82.80%) than Amrapali (66.00-78.00%) after FBD, which ultimately increases R'a in Amrapali (32.44-44.00%) than Langra (17.20-26.60%). On the other hand at FBD Langra exhibited lower TLP (-5.56 MPa) than Amrapali (-5.41 MPa), which indicate early turgor loss in leaves of Langra during the critical period of flowering process. Turgor loss point increases (Amrapali = -5.26; Langra = -5.52) with treatment as compared to control (Amrapali = -5.41; Langra = -5.56) condition. Bulk modulus of elasticity was found 2.21 and 5.21 MPa for Amrapali and Langra, respectively at flower bud differentiation (FBD), which showed that former had more elasticity than later at FBD. It was found that during flowering period Amrapali reached to -9.0 MPa and recovered more completely after panicle elongation (-3.1 MPa) while on other hand Langra reached at -7.0 MPa and could not recover completely. Elasticity decreased during the time of flower bud differentiation (Amrapali = 1.15; Langra = 0.97) and increased at onset of panicle emergence (Amrapali = 0.79; Langra = 0.60) in both the varieties than control (Amrapali = 0.89; Langra = 0.55) indicating maintenance of turgor in treated trees as a function of the dynamic process of cell wall adjustment, as reflected by marked reductions in both the saturated and turgor-loss volumes and by maintenance of elastic coefficients of the tissues.

Sap flow measurements were also made on two varieties (Table 2), which showed that Amrapali (6.76-18.99 kg/h) had higher sap flow than Langra (6.91-13.11 kg/h) in different flower developmental stages being maximum (Amrapali = 12.88 ± 1.89, Langra = 6.91 ± 0.99 kg/h) at panicle elongation and minimum (Amrapali = 6.76 ± 0.88, Langra = 6.91 ±

Table 2. Sap flow in mango Amrapali (A) and Langra (L) mango genotypes.

Stage	Sap flow (kg/ h)	
	Amrapali	Langra
Flower bud differentiation (FBD)	10.12 ± 1.21	9.11 ± 1.99
Bud burst (BB)	6.76 ± 0.88	6.91 ± 0.78
Panicle emergence (PEe)	12.67 ± 2.11	8.80 ± 0.99
Panicle elongation (PE)	12.88±1.89	8.32±0.96

Data are expressed as a pooled mean ± standard deviation



(A) **(B)**

Fig. 1. (A) P-V curve for Amrapali and (B) Langra mango genotypes. Each point represents mean ± s.d. of five replicates.

0.89 kg/h) at bud burst stage of flower development (Fig. 2). The result confirms that the value of P-V curve obtained by thermocouple psychrometer as an alternative or supplement to other methods for evaluating water relations parameters of Langra and Amrapali, including some quantities that cannot be estimated satisfactorily in any other way. The plot onto a graph of 1/ψ against water content is a curved line, which is concave to the y-axis. RWC in a measured P-V curve is the sum of this (curvilinear) apoplast component and the (theoretically linear) symplast component. P-V curve clearly indicates that total water content of saturated Amrapali tends to be higher than Langra. Amrapali contains more apoplastic water content. A possible explanation for this may be that Amrapali tends to have higher contents of cell wall uronic acids, which can readily bind water. The higher water-holding capacity of Amrapali will allow it to continue metabolism for longer than Langra. While this could be viewed as a form of 'desiccation avoidance', Amrapali also recovers faster than Langra during rest period suggesting that they have higher inherent tolerance. This may help them to persist in stress exposed duration. In contrast, Langra lacks such strong avoidance and tolerance mechanisms. Amrapali showed lower TLP in subsequent stages of flower development, which may signify that with lower TLP Amrapali should be able to maintain osmoregulation at lower leaf water potentials. Varieties with lower TLP should be able to continue growth at lower leaf water potential (Ingram and Bartels, 5). Paclobutrazol showed positive response and increased Symplastic content after FBD in both the varieties. R's value was found higher

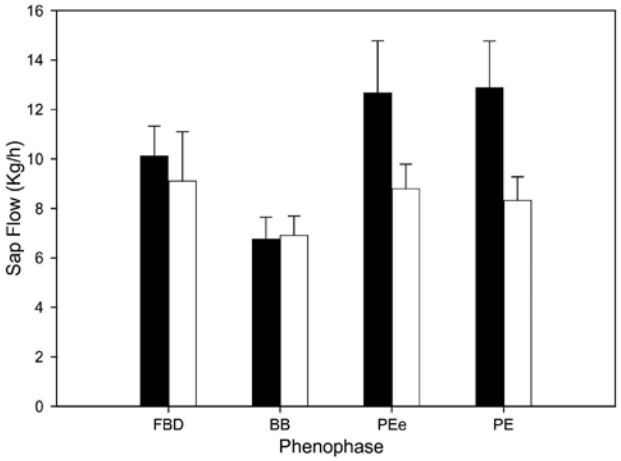


Fig. 2. Sap flow of Amrapali (■) and Langra (□) at different phenophases. FBD = flower bud differentiation, BB = bud burst, Pee = panicle emergence and PE = panicle elongation.

in Langra after FBD this increases R'a in Amrapali, which is an indicator of highly adjusting nature of former variety against water deficit during flowering than later one as high R'a value supports stress tolerant capacity. Turgor regulation at reduced water contents was closely associated with changes in the elastic quality of the cell walls. An understanding of sap dynamics in mango cultivars is very important in devising a strategy for irrigation in mango. The greater sap flow at panicle elongation than flower bud differentiation might be due to the high water content or turgor pressure, which declined with increased stress as with the increase in stress loss of water through transpiration decreased the sap quantity. Langra had more rigid cell walls than Amrapali and as a result during flowering period Langra lost more water before turgor started dropping (at higher RWC) than Amrapali and at less negative osmotic potential during stress as a result PSII started declining earlier. Increased elasticity (decreasing E, *i.e.* decreasing the slope between full turgor and TLP) was associated with less negative solute potential in Amrapali than Langra.

Paclobutrazol-treated trees showed marked increases in stress resistance, and pressure-volume analysis confirmed that water stress was ameliorated during stress period. Turgor was maintained in the paclobutrazol-treated trees despite water contents near or below the turgor-loss volumes of well-watered controls. The maintenance of turgor in these trees was in large part a function of the dynamic process of cell wall adjustment, as reflected by marked reductions in both the saturated and turgor-loss volumes and by large increases in the elastic coefficients of the tissues. In this study, the plants treated with PBZ appear to have been more resistant to stress than those without PBZ treatment. Similar results have been reported for bean, jack pine, white spruce and black spruce (Marshall *et al*., 7). The treatment-induced reductions in water contents enabled trees to maintain turgor with tissue volumes close to, or below, the turgor-loss volume of untreated trees. The role of paclobutrazol in lowering water consumption and reduced water loss in treated plants was already been recorded (Navarro *et al*., 8). Sap flow data showed decreasing pattern during successive stages of flower bud differentiation, which confirms internal stress during these stages. After panicle elongation both varieties started regaining their water status which was faster in Amrapali than Langra. The results of the present study clearly showed that desiccation tolerance is greater in Amrapali than Langra sharing same habitat. Desiccation tolerance can help Amrapali to outgrow the competition of Langra that normally grow in association with it.

ACKNOWLEDGEMENTS

The authors acknowledge Dr U.V. Pathre, Ex-Senior Principal Scientist, NBRI, Lucknow for critical suggestion and going through the article and Director, ICAR-CISH, Rehmankhera, Lucknow for providing facilities.

REFERENCES

1. Abdel, Rahim, A.O.S., Elamin, O.M. and Bangerth, F.K. 2008. Effects of paclobutrazol on floral induction and correlated phyto-hormonal changes in grafted seedlings of different mango (*Mangifera indica* L.) cultivars. *Sudan J. Agric. Res.* **11**: 111-20.

2. Balley, I.S.E., Haris, M. and Whiley, A.W. 2000. Effect of water stress on flowering and yield of 'Kensington Pride' mango (*Mangifera indica* L.). *Acta Hort.* **509**: 277-81.

3. Cermak, J., Kucera, J. and Nadezhdina, N. 2004. Sap flow measurements with some thermodynamic methods, flow integration within trees and scaling up from sample trees to entire forest stands. *Trees*, **18**: 529-46.

4. Chaves, M.M., Maroco, J.P. and Pereira, J.S. 2003. Understanding plant responses to drought: From genes to the whole plant. *Func. Plant Biol.* **30**: 239-64.

5. Ingram, J. and Bartels, D. 1996. The molecular basis of dehydration tolerance in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **47**: 377-403.

6. Lenz, I., Tanja, Wright, J. Ian and Westoby, Mark 2006. Interrelations among pressure-volume curve traits across species and water availability gradients. *Physiol. Plant.* **127**: 423-33.

7. Marshall, J.G., Rutledge, R.G., Blumwald, E. and Dumbroff, E.B. 2000. Reduction in turgid water volume in jack pine, white spruce and black spruce in response to drought and paclobutrazol. *Tree Physiol.* **20**: 701-07.

8. Navarro, A., Jesús, M., Blanco, S. and Banon, S. 2007. Influence of paclobutrazol on water consumption and plant performance of *Arbutus unedo* seedlings. *Scientia Hort.* **111**: 133-39.

9. Ngugi, M.R., Doley, D., Hunt, M.A., Dart, P. and Ryan, P. 2003. Leaf water relations of *Eucalyptus cloeziana* and *Eucalyptus argophloia* in response to water deficit. *Tree Physiol.* **23**: 335-43.

10. Singh, V.K. and Singh, A. 2003. Effect of paclobutrazol on regularity of bearing in mango (*Mangifera indica* L.). *Physiol. Mol. Biol. Plants*, **9**: 239-48.

11. Sobrado, M.A. 1986. Aspects of tissue water relations and seasonal changes of leaf water potential components of evergreen and deciduous species coexisting in tropical dry forests. *Oecologia*, **68**: 413-16.

12. Tatarinov, A., Fyodor, Kucera, J. and Cienciala, E. 2005. The analysis of physical background of tree sap flow measurement based on thermal methods. *Meas. Sci. Tech.* **16**: 1157-69.

13. Turner, N.C. 1981. Techniques and experimental approaches for the measurements of plant water status. *Plant Soil*, **58**: 339-66.

14. Tyree, M.T. and Hammel, H.T. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *J. Exp. Bot.* **23**: 267-82.

15. Yadav, R.B.R. and Singh, V.K. 1995. Selection of leaf and time for measurement of photosynthesis in mango trees (*Mangifera indica* L.). *Indian J. Plant Physiol.* **38**: 186-87.

Received : July, 2016; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00095.0

Indian J. Hort. 74(4), December 2017: 486-490

Tree architecture influenced productivity and quality attributes in apple under HDP

K.K. Srivastava*, Dinesh Kumar, J.I. Mir and S.R. Singh
ICAR-Central Institute of Temperate Horticulture, Rangreth, Srinagar 190 007 (J&K)

ABSTRACT

The present experiment was conducted to develop efficient tree architecture in apple under high density planting systems. In this trial vertical axis tree and cordon tree architecture were validated first time in India viewing the success in horticultural advanced countries. Maximum annual extension growth (102.34 cm), trunk cross sectional area (23.66 cm^2), the highest yield per tree (10.92 kg), yield per hectare (84.63 t/ha) and yield efficiency (0.45 $kg/cm^{2)}$ were recorded in cultivar Coe Red Fuji under vertical axis tree architecture. Maximum fruit weight was observed in cultivar Coe Red Fuji under cordon tree architecture (167.14 h). The red colour intensity was recorded maximum (*a) (20.16) in cultivar Coe Red Fuji under vertical axis tree architecture, while, maximum yellow colour intensity (*b**) (28.44) were found in Granny Smith fruits under same architecture. Cultivar Coe Red Fuji has excelled on the overall vegetative growth, yield and quality attributes under HDP in vertical axis system.**

Key words: Apple, vertical axis, cordon tree, tree architecture, HDP, yield efficiency.

INTRODUCTION

Apple (*Malus domestica* Borkh.) is an important temperate fruit crop and its cultivation site falls geographically between 25-52°N or S latitudes. The apple productivity depends on canopy management, scion and rootstock behavior, fertilization, disease and pest management. Among all these factors canopy management plays a very vital role in production function. In apple, traditionally training system, *i.e.*, central leader, modified centre leader and open centre leader are being adopted with the advent of new growth controlling scion and rootstocks. The concept of high density led the development of modern tree architecture like vertical axis tree architecture (VATA) and cordon tree architecture (CTA), which had been found efficient in giving high yield and quality apple production. Training systems are the key factors for high yield and quality (Celik *et al*., 6). However, the selection of tree architecture depends on previous conducted studies (Celik *et al*., 5). Trees must be trained and pruned to achieve a manageable uniform size, a balance between growth and regular yield and also to permit good light and spray solution penetration to the inner most points of the centre canopy (Malavoeta and Cross, 13). Over the last 30-40 years, several tree architectures for the apple have been developed to attain high yields and quality (Ferree and Warrington, 8). Modern orcharding systems are based on high tree densities with a range from 1,000 to 6,000 trees and some upto 10,000 trees $hectare^{-1}$ (Robinson, 14). Tree architecture involves the manipulation of planting arrangement and canopy geometry to improve the light interception and distribution of photosynthetic active radiation for the purpose of optimizing fruit quality and yield. Greene (9) reported the desired growth and yield of Granny Smith and Starking Delicious trees trained on 8 different ways and found no differences in leaf area and yield but systems did affect in fruit size and colour, the incidence of certain fruit defects and yield efficiency. Differences in fruit qualities were associated with difference in light interception and aeration in side canopy (Greene, 9; Ferree *et al*., 7).

MATERIALS AND METHODS

The present experiment was carried out during 2009-2013 at ICAR-CITH, Srinagar, Jammu and Kashmir situated at 34°, 45' N latitude, 74°, 50' E longitude at altitude of 1640 m above mean sea level. The average maximum and minimum temperature 19.63° and 6.52°C, respectively with total precipitation of 60.72 cm and relative humidity of 58.35%. Treatment comprised of three apple varieties, *i.e.*, Coe Red Fuji (V1), Granny Smith (V2) and Spartan (V3) on M9 rootstock on two architectures, namely, Vertical axis tree architecture (VATA) (T-1) and cordon tree architecture (CTA) (T-2) planted at 0.75 m × 1.5 m spacing. The experiment was laid in factorial randomized block design with three replications; uniform cultural practices were applied in all the trees under study. Data with respect to annual extension growth was measured by selecting four shoots randomly from each

*Corresponding author's present address, ICAR-Central Institute for Subtropical Horticulture, Rehmankhera, Kakori, Lucknow 226101; E-mail: kanchanpom@gmail.com

quadrant of the experimental tree and the average trunk cross-sectional area (TCA) was also worked out as per the procedure given by Westwood (19). The fruit weight was recorded using digital electronic balance and fruit yield was calculated at the time of harvesting and expressed as kg tree^{-1} and projected in tonnes hectare^{-1}. The yield efficiency was calculated by using the formula given by Westwood (19).

Colour measurements were recorded using Hunter Color Lab having the head 15 mm diameter and were expressed in the units of *L**, *a** and *b**. Chroma (*C**) and hue angle (*h°1*) were computed from L^*,a^* and b^* values. The colour meter was calibrated using the manufacturers' standard white tile (Lancaster and Lister, 12). The data were analyzed using SPSS statistical software.

RESULTS AND DISCUSSION

The data presented in Table 1 revealed that maximum trunk circumference (20.33 cm) and trunk cross sectional area (33.19 cm^2) was recorded in cultivar Coe Red Fuji, which was significantly superior to all other cultivars. Whereas, minimum trunk circumference (14.35 cm) and trunk cross sectional area (16.76 cm^2) were noticed in cultivar Granny Smith. Maximum annual shoot extension growth was noticed in cultivar Coe Red Fuji (98.55 cm), which was significantly superior to Granny Smith (84.17 cm). The effect of tree architecture on annual shoot extension growth was observed to be significant and annual shoot extension growth was recorded maximum (94.20 cm) with CTA, followed by VATA (90.72 cm). Interaction effect of cultivar and tree architecture on annual growth extension was observed to be significant. Cultivar Coe Red Fuji on VATA exhibited significantly maximum annual shoot growth (102.34 cm), which was superior to other architecture (Table 1). The system might be due to proper light penetration in canopy leading better to photosynthetic production. These findings are in accordance with those of Szczygiel and Mika (18) better on apple.

Tree vigour and growth habit varied, depending on the tree architecture (Table1), maximum trunk circumference (16.18 cm) and trunk cross sectional area (21.20 cm^2) were recorded in cultivar Coe Red Fuji, followed by Granny Smith. Significant effect of tree architecture was observed on trunk circumference and trunk cross sectional area. Maximum trunk circumference (14.32 cm), trunk cross sectional area (17.34 cm^2) were recorded in VATA. Interaction effect of cultivar and tree architecture on trunk circumference and trunk across sectional area were found to be non-significant. However, maximum trunk circumference (16.70 cm) and trunk cross sectional area (23.66 cm^2) were noticed in cultivar Coe Red Fuji

Table 1. Effect of cultivar and tree architecture on growth and yield of apple under HDP (pooled data 2011-13).

Cultivar	Annual extension growth (cm)	Trunk circum. (cm)	TCA (cm^2)	Yield (kg/ tree)	Yield (t/ ha)	Yield efficiency (kg/ cm^2)	Fruit wt. (g)
Coe Red Fuji (V1)	98.55	16.18	21.20	8.68	70.84	0.39	164.02
Granny Smith (V2)	84.17	13.72	15.46	3.19	28.34	0.18	137.73
Spartan (V3)	94.62	12.26	12.02	1.94	17.42	0.15	144.79
CD (P = 0.05)	5.57	0.81	2.67	2.88	8.88	0.08	20.82
Tree architecture							
VATA (T1)	90.72	14.32	17.34	5.97	48.96	0.31	143.74
CTA (T2)	94.20	13.97	15.12	3.26	28.72	0.17	153.95
CD (P = 0.05)	2.41	0.24	2.18	2.35	5.41	0.07	1.05
Interaction effect of cultivar and tree architecture							
Coe Red Fuji (V1) × VATA (T1)	102.34	16.70	23.66	10.92	84.63	0.45	160.91
Coe Red Fuji (V1) × CTA (T2)	94.77	15.66	18.75	6.44	57.06	0.33	167.14
Granny Smith (V2) × VATA (T1)	76.08	14.07	16.48	4.05	36.01	0.23	116.82
Granny Smith (V2) × CTA (T2)	94.27	13.39	17.43	2.32	20.61	0.13	158.64
Spartan (V3) × VATA (T1)	95.68	12.20	1.87	2.93	26.24	0.24	153.49
Spartan (V3) × CTA (T2)	93.56	12.33	12.18	0.96	8.60	0.06	136.09
CD (P = 0.05)	6.43	N.S	N.S	1.42	3.96	0.02	19.44

under VATA. The above findings are in consonance with Szczygiel and Mika (18) who reported stronger vegetative growth of trees expressed as trunk cross sectional area on M9. However, growth intensity was not significantly affected by the training and planting density of trees, although some tendency to stronger growth of trees planted at a lower density was observed. The cultivars had significant effect on yield attributes (Table 1). Maximum yield tree^{-1} (8.68 kg), yield hectare^{-1} (70.84 t/ha) and yield efficiency (0.39 kg cm^{-2}) were noticed in cultivar Coe Red Fuji. Similarly, the yield attributes were significantly influenced by the tree architecture with maximum yield tree^{-1} (5.97 kg tree^{-1}), yield hectare^{-1} (48.96 t ha^{-1}) and yield efficiency (0.31 kg cm^{-2}) under VATA, which was statistically superior to CTA. The interaction effects of cultivar and tree architecture on yield attributes were observed to be significant. Maximum yield tree^{-1} (10.92 kg), yield hectare^{-1} (84.63 /ha) and yield efficiency (0.45 cm^{-2}) were noted in Coe Red Fuji under VATA, which was significantly superior to all other systems. Yield efficiency was positively correlated with trunk cross sectional area, fruit weight, yield tree^{-1}; yield per hectare and yield efficiency. Yield is linearly related to light interception, which suggest that increase in light interception by tree architecture resulted in increased yields (Robinson, 15). Further, Antognozzi *et al. (*2) reported that the highest yield was obtained in slender spindle architecture having M9 and M26 as rootstocks for Gloden Delicious and M9 rootstock for Starking Delicious. Bielicki and Rozpara (3) obtained increased total yield in cherry grown on spindle architecture. Szczygiel and Milka (18) also reported that highest yield from tree on M9, trained on vertical axis tree architecture.

Maximum fruit weight was noticed in Coe Red Fuji (164.02 g), which was significantly superior to Granny Smith (137.73 g) but statistically at par with Spartan (144.79 g). Effect of tree architecture on fruit weight was observed to be significant (Table 1). Highest fruit weight was recorded on CTA (153.95 g). Combined effect of cultivar and tree architecture on fruit weight was found to be significant, maximum fruit weight (167.14 g) was observed in cultivar Coe Red Fuji under CTA, which was superior to Spartan under same architecture (136.09 g) and Granny Smith VATA (116.82 g). These results corroborate with the findings of Shengrui *et al.* (16) who obtained increased average fruit weight in apple cultivars on trellis architecture. Similarly, Bielicki and Rozpara (3) and Aleksander (1) noted higher, fruit weight in cherry after using the efficient tree architecture.

Interaction effect of cultivar and tree architecture was found to be significant. Maximum annual extension growth (102 cm) and minimum noted in Coe- Red Fuji and Granny Smith on VATA, respectively. Similarly TCA, yield tree^{-1}, yield, t ha^{-1} and yield efficiency were recorded highest in Coe Red Fuji in VATA, whereas fruit weight was found highest in same cultivar but in CTA (Table 1).

Maximum red colour intensity (*a**) was recorded in cultivar Spartan (17.25) followed by Coe Red Fuji (15.70), however, it was negative (-*a**) in cultivar Granny Smith (-7.45). The positive *b**values were observed in all the cultivars and was maximum (27.46) in Granny Smith. Tree architecture had positive impact on red colour intensity (*a**) in all the treatments with maximum (9.68) in VATA. However, effect of tree architecture on positive (*b**) value (yellow colour intensity) on fruit was observed to be non-significant. Interaction effect of cultivar and tree architecture on red colour intensity (*a**) was maximum (20.16) in Coe Red Fuji on VATA, followed by Spartan on CTA (17.29) and Spartan under VATA (16.22). Interaction effect of cultivar and tree architecture on yellow colour intensity (*b**) on fruits were observed to be highest (28.44) in cultivar Granny Smith under VATA, which was significantly superior to other system (Table 2). Shengrui *et al*. (16) reported that the light penetration in the canopy was increased by tree architecture, which thereby improved colour of fruits with uniform size in apple. These results are in accordance with Hampson *et al.* (2002) who reported that fruit colour was improved on various tree architectures in apple.

Tree architecture for HDP is most influential factor for yield and quality. As the tree density increases, initially tree gives good quality yield, but with advancement in age shading affects both. For successful orcharding under HDP, suitable tree architecture is inevitable, which helps to control tree shape and canopy for better penetration of solar light, aeration and ease in targeted spray. The growth, productivity and quality were found superior in Coe Red Fuji trained on vertical axis tree architecture under intensive orchard system in apple.

REFERENCES

1. Aleksander, G. 2011. Effect of tree training system on yield and fruit quality of sweet cherry 'Korda'. *J. Fruit Orn. Pl. Res*. **19**: 79-83.

2. Antognozzi, E., Prcetts, P. and Famiani, F. 1993. Effects of rootstocks and training system on growth and yield of two apple cultivars. *Acta Hort*. **349**: 187-90.

3. Bielicki, P. and Rozpara, E. 2010. Growth and yield of 'Kordia' sweet cherry trees with various

Table 2. Effect of cultivar and tree architecture on colouring pattern of fruits under HDP (pooled data 2011-13).

Cultivar	Color intensity/ Kind of colour				
	*L**	*a**	*b**	*Hue*	*Chroma*
Coe Red Fuji (V1)	54.25	15.70	20.91	57.97	25.62
Granny Smith (V2)	61.25	-7.45	27.46	-74.1	28.65
Spartan (V3)	43.35	17.25	12.00	69.60	20.46
CD (P = 0.05)	1.22	1.13	1.07	5.58	0.29
Tree architecture					
Vertical axis (T1)	54.43	9.68	19.75	24.22	25.65
Cordon tree architecture (T2)	54.31	5.65	20.49	11.40	24.17
CD (P = 0.05)	1.00	0.92	0.87	4.56	0.23
Interaction of cultivar and tree architecture					
Coe Red Fuji (V1) × VATA (T1)	56.70	20.16	19.27	43.61	27.71
Coe Red Fuji (V1) × CTA (T2)	56.35	7.25	22.56	72.34	23.53
Granny Smith (V2) × VATA (T1)	62.81	-7.33	28.44	-74.60	29.61
Granny Smith (V2) × CTA (T2)	65.11	-7.58	26.48	-73.68	27.61
Spartan (V3) × VATA (T1)	43.78	16.22	11.56	103.66	19.62
Spartan (V3) × CTA (T2)	41.46	17.29	12.44	35.55	21.31
CD (P = 0.05)	1.73	1.60	1.52	7.90	0.41

rootstock and inter-stem combinations. *J. Fruit Orn. Pl. Res*. **18**: 45-50.

4. Byers, R.E., Carbaugh, D.H., Parsley, C.N. and Wolf, T.K. 1991. The influence of low light on apple fruit abscission. *J. Hort. Sci* .**66**: 7-17.

5. Celik, H., Agaoglu, Y.S., Agaoglu, Y.S., Fidan, Y.S., Fidan, Y., Marasali, B. and Soylemezoglu, G. 1998. *General Viticulture*, Fersa Press, Ankara, 253 p.

6. Celik, H., Marasali, B., Soylemezoglu, G., Baydar, N.G. and Ilbay, A.K. 1999. Determination of trellis height and training system for Hasandede cultivar in Ankara. *3rd National Horticultural Congress of Turkey*, September 14-17, Ankara, pp. 569-73.

7. Ferree, D.C., Greene, K.A. and Bishop, B. 1993. Influence of orchard management system of canopy composition, light distillation, net photosynthesis and transpiration of apple trees. *J. Hort. Sci*. **68**: 377-92.

8. Ferree, D.C. and Warrington, I.J. 2003. *Apples: Botany, Production and Uses*, CABI Publishing Co., pp. 660.

9. Greene, K.A. 1993. Influence of orchard management system on yield, quality and vegetative characteristics of apple tree. *J. Hort. Sci*. **68**: 365-76.

10. Hoampson, C.R., Quamme, H.A. and Brownlee, R.T. 2002. Canopy growth, yield and fruit quality of 'Royal Gala' apple trees grown for eight year in five tree training systems. *HortSci.* **37**: 627-31.

11. Kondo, S. and Takahashi, Y. 1987. Effects of high temperature in the night time and sheding in the daytime on the early drop of apple fruit 'Starking Delicious'. *J. Japan Soc. Hort. Sci*. **56**: 142-50.

12. Lancaster, J.E. and Lister, E.L. 1997. Influence of pigment composition on skin colour in a wide range of fruits and vegetable. *J. Amer. Soc. Hort. Sci*. **22**: 594-98.

13. Malavoeta, C. and Cross, J. 2009. Guidelines for integrated production of pome fruits. 10 *BC/wprs Bulletin*, **47**: 1-13.

14. Robinson, T.L. 2003. Apple orchard planting systems pp. 345-407. **In**: *Apples*, D.C., Ferree and I.J. Warrington, CABI Publishing, Wallingford, U.K.

15. Robinson, T.L. 2007. Recent advances and future directions in orchard planting. *Acta Hort*. **732**: 367-82.

16. Shengrui, Y., Walser, R. and Martin, C. 2012. Evaluation of eight apple cultivars and two training systems in Northen Mexico. *New Mexico Bull.*, **803**: 1-8.

17. Strikic, F., Radunic, M. and Rosin, J. 2007. Apricot growth and productivity in high density orchard. *Acta Hort*. **732**: 495-500.

18. Szczygiel, A. and Mika, A. 2003. Effects of high density planting and two training methods of dwarf apple trees grown in Sub-Carpathian region. *J. fruit Orn. Pl. Res*. **11**: 45-51.

19. Westwood, M.N. 1993. *Temperate Zone Pomology: Physiology and Culture* (3rd edn.), Portland, Oregon, Timber Press. 523 p.

Received : November, 2015; Revised : September, 2017; Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00096.2

Indian J. Hort. 74(4), December 2017: 491-497

Physiological and biochemical alterations due to low temperature stress in papaya genotypes

Satyabrata Pradhan, A.K. Goswami[*], S.K. Singh, Jai Prakash, Suneha Goswami[**], Chinnusamy V.[***], Akshay Talukdar[***], Vartika Srivastava[****] and Arun Kumar[***]

Division of Fruits and Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

ABSTRACT

Papaya (*Carica papaya* L.) being a typical tropical plant, is highly sensitive to low temperature stress. The present experiment was conducted under completely controlled conditions of National Phytotron Facility, ICAR-IARI, New Delhi to investigate the effect of different low temperature regimes on antioxidant enzymes and other physiological and biochemical parameters in five papaya genotypes and one distant relative *i.e.*, cold tolerant genus *Vasconcellea cundinamarcensis*. The outcomes suggested a genotype-specific substantial increase of antioxidant enzymes activities, *viz*., superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR) under the cold treatment regimes. The highest per cent increase of SOD, APX, GPX and GR activities were found in the tolerant genotype *V. cundinamarcensis*, while the highest increase in CAT activity was observed in P-7-9 at the 20˚/10˚C (day/night) temperature regime over the control. Low temperature regimes led to increase in membrane injury in papaya leaves possibly through the phase transition or oxidative damage of cell membrane due to ROS generation in the photosystem because of disruption in the photosynthetis process. The highest increase in the membrane injury index was noted in genotype Red Lady (79.93%). The photosynthetic rate was severely reduced under the low temperature regimes. The total sugars and total soluble proteins content in papaya leaves were observed to increase under the low temperature regimes may be due to cold induced osmotic stress.

Key words: Antioxidant enzymes, low temperature regimes, membrane injury, papaya, photosynthesis.

INTRODUCTION

Low temperature stress is one of the major factors affecting the growth and development of tropical plants like papaya (*Carica papaya* L.). Chilling and freezing injury ensue from low temperatures affect the plant in two ways, *i.e*., by transition of the cell membrane into solid gel and dehydration of cell due to ice nucleation in the intercellular spaces inducing osmotic stress. Photosynthesis is a highly synchronized process and is vulnerable to any change in environmental conditions, as it needs to balance the absorbed light energy of photosystems with the energy consumed by metabolic processes of the plant. Low temperature stress aggravates an imbalance between the source of energy and the metabolic process, thus generating the reactive oxygen species (ROS) leading to oxidative stress. Antioxidant enzymes (SOD, CAT, APX, GPX and GR) are compounds which can retard or overcome the process oxidative stress inhibiting the initiation or multiplication of oxidizing reactions (Ruelland *et al*., 11).

Although the distant relatives of cultivated papaya (*C. papaya* L.), *viz*., *Vasconcellea cundinamarcensis*, *V. pennata* and *V. pentagona* are resistant to frost but almost every commercial variety of papaya is highly sensitive to low temperature stress, which limits its successful cultivation in the sub-tropical areas. Reports showed that the optimum temperature range for the growth and development of papaya is 21º to 33ºC, while winter temperatures below 12-14ºC can hamper the growth and production considerably (Ram, 10). Irrespective of the above facts, neither the germplasm nor the physiology behind the cold stress tolerance in papaya has been studied in depth. The aim of the present research was to perceive the interactions between the antioxidant enzymes and low temperature regimes in papaya and to identify a low temperature tolerant papaya genotype.

MATERIALS AND METHODS

Plant material for the experiment included five *C. papaya* L. genotypes (Red Lady, Pusa Nanha, P-7-15, P-7-9 and P-9-5) and one cold tolerant genotype (*Vasconcellea cundinamarcensis*). Evaluation for low temperature stress was undertaken at the controlled environment conditions in the National Phytotron Facility, ICAR-IARI, New Delhi during

*Corresponding author's E-mail: amit.tkg@gmail.com
**Division of Biochemistry, ICAR-IARI, New Delhi 110012
***Division of Genetics, ICAR-IARI, New Delhi 110012
***National Phytotron Facility
****ICAR-NBPGR, Pusa Campus, New Delhi 110012

2016-17. The seeds of above mentioned genotypes were sown in trays containing the growing medium comprising of perlite, vermiculite, cocopeat and vermicompost (1:1:1:1) and then transplanting was done 8 week after sowing, into plastic pots filled with same potting medium under the growth chamber. All other recommended standard operations were performed at proper growth stage of the plants. A temperature regime of 28/18ºC (day/night) along with a photoperiod of 12 h 30 min. (L/D) and relative humidity of 70 ± 5% during day and 85-90% during the night and irradiance of 700-800 µmol $m^{-2}s^{-1}$ at leaf level. After proper establishment of the transplanted seedlings, the temperature treatments were induced by lowering the temperature in the growing chamber by 2ºC per two day from 26/ 16ºC (day/ night) to 20/ 10ºC (day/ night) up to 8 days. In total, three replications comprising of 9 plants per replication for each genotype were maintained. In control (T_0), three plants for each genotype were maintained at 28/ 18ºC (day/ night) regime. The details of temperature treatments are portrayed in Table 1. The observations on leaf physiological and biochemical parameters were recorded after each low temperature treatment. Five matured leaves from three plants per each treatment were selected and their photosynthesis rate (µmol m^{-2} s^{-1}) was measured by using an infrared gas analyser, while leaf membrane injury index (MII) was estimated following the method of Blum and Ebercon (2).

Total soluble proteins content in the leaf tissue was estimated by the method given by Bradford (3), while total soluble sugars were estimated using anthrone reagent method (Sadasivam and Manickam, 12). Leaf anti-oxidant enzyme assays were conducted in stress exposed and control plants. The catalase (CAT, EC: 1.11.1.6) assay was conducted as per method of Aebi (1), superoxide dismutase (SOD, EC: 1.15.1.1) as per Dhindsa *et al*. (4), glutathione peroxidase (GPX, EC 1.11.1.9) as per Srivastava and Huystee (13), ascorbate peroxidase (APX, EC 1.11.1.11) as per method of Nakano and Asada (8) and glutathione reductase (GR, EC 1.6.4.2) activity was estimated by the method of Halliwell and Foyer (5).

Table 1. Details of controlled temperature regimes maintained under growth chamber.

Treatment No.	Day temp. (°C)	Night temp. (°C)
T_0 (control)	28± 0.1	18 ± 0.1
T_1	26 ± 0.1	16 ± 0.1
T_2	24 ± 0.1	14 ± 0.1
T_3	22 ± 0.1	12 ± 0.1
T_4	20 ± 0.1	10 ± 0.1

The statistical analysis of the data which comprised of five treatments including control (T_0) and three replications were analysed in factorial completely randomized block design using statistical analysis system software, SAS package (9.3 SAS Institute, Inc, and USA) followed by t-test (LSD). P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

The data presented clearly demonstrated that papaya plants had marked changes due to exposure to low temperature regimes with a uniform and substantial increase in the leaf dry weight, MII, leaf total soluble proteins, total soluble sugars content and activity of antioxidant enzymes, while decline in the leaf fresh weight and photosynthetic rate.

Amongst the genotypes, *V. cundinamarcensis* (872.13 mg) maintained the highest mean leaf FW, which was statistically at par with P-9-5 (854.80 mg) (Table 2). Amongst the G × T interaction, Red Lady × T_4 (228.00 mg) exhibited the lowest leaf FW, while the highest was in *V. cundinamarcensis* × T_0 (919.00 mg). The highest reduction in leaf FW within the genotypes from the T_0 to T_4 was observed in the genotype P-7-15 (15.48%), while the lowest was in Pusa Nanha (5.39%). Amongst the temperature regimes, the mean leaf DW was observed to be highest in T_4 (77.50 mg), which was 25.90% higher than the T_1 (61.56 mg) (Table 2). The genotype P-9-5 (129.60 mg) exhibited the highest leaf DW. Amongst all the possible G × T combinations, the highest leaf DW was in P-9-5 × T_3 (149.00 mg). Although the highest mean fresh weight was noted in *V. cundinamarcensis* (872.13 mg) followed by P-9-5 (854.80 mg) but the highest mean leaf dry weight was noted in P-9-5 (129.60 mg) followed by *V. cundinamarcensis* (104.93 mg), which may be due to higher moisture content in the fresh leaves of *V. cundinamarcensis* owing to its castor-like with more thickness.

Amongst the mean value of different treatments, T_4 plants had significantly higher total protein content (2.17 µg protein $µl^{-1}$) than all others and it was observed to be 35.51% higher than the T_0 (1.60 µg protein $µl^{-1}$) (Table 3). Amongst the possible G × T combinations, P-9-5 × T_4 (2.71 µg protein $µl^{-1}$) and P-9-5 × T_3 (2.51 µg protein $µl^{-1}$) had statistically similar and higher protein content. The highest increase from T_0 to T_4 was noted in *V. cundinamarcensis* (79.06%), while the lowest was in the genotype Pusa Nanha (8.78%). In this study, leaf FW was observed to decrease under the low temperature regimes, while leaf DW reflected the opposite trend. The relative water content was also observed to decrease under the low temperature stress (data not presented). The results suggested a significant increase in the protein

Table 2. Effect of different temperature regimes on leaf fresh and dry weights in papaya genotypes under controlled phytotron conditions.

Genotype	Leaf fresh weight (mg)						Leaf dry weight (mg)					
Temp. regime	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
Red Lady	251.67^{ijk}	251.00^{jk}	245.67^{jk}	232.67^{k}	228.00^{k}	241.80^{e}	25.00^{mno}	24.00^{no}	23.00^{o}	41.33^{lm}	40.00^{lmn}	30.67^{e}
Pusa Nanha	420.33^{e}	408.00^{ef}	407.33^{ef}	385.00^{efg}	397.67^{efg}	403.67^{c}	48.67^{lk}	47.00^{lk}	47.67^{lk}	52.33^{ljk}	50.00^{lk}	49.13^{d}
P-7-15	355.33^{efg}	350.33^{efg}	335.67^{hg}	315.00^{ih}	300.33^{ihj}	331.33^{d}	55.67^{lijk}	24.00^{no}	50.33^{lk}	47.33^{lk}	61.67^{hijk}	47.80^{d}
P-7-9	580.67^{d}	579.33^{d}	584.00^{d}	547.00^{d}	538.00^{d}	565.80^{b}	55.67^{lijk}	41.00^{lm}	68.67^{hij}	72.00^{hi}	72.33^{gh}	61.93^{c}
P-9-5	899.33^{a}	899.33^{a}	883.00^{ab}	813.33^{c}	779.00^{c}	854.80^{a}	130.00^{b}	108.00^{ed}	126.33^{bc}	149.00^{a}	134.67^{ab}	129.60^{a}
V. cund.	919.00^{a}	912.00^{a}	879.33^{ab}	827.67^{bc}	822.67^{bc}	872.13^{a}	93.33^{ef}	125.33^{bc}	111.00^{cd}	88.67^{fg}	106.33^{ed}	104.93^{b}
Mean	571.06^{a}	566.67^{a}	555.83^{a}	520.11^{b}	510.94^{b}		68.06^{bc}	61.56^{c}	71.17^{ab}	75.11^{a}	77.50^{a}	
LSD (P ≤ 0.05)												
Genotype (G)						28.46						7.36
Temp. regime (T)						25.98						6.72
G × T						63.63						16.45

Table 3. Influence of different temperature regimes on leaf total soluble proteins and total soluble sugars content in papaya genotypes grown under controlled phytotron conditions.

Genotype	Total soluble proteins (µg protein µl^{-1} enzyme extract)						Leaf total soluble sugars (mg g^{-1})					
Temp. regime	T_0	T_1	T_2	T_3	T_4	Mean	T_0	T_1	T_2	T_3	T_4	Mean
Red Lady	1.43^{jkl}	1.53^{ijk}	1.56^{ijk}	1.58^{ijk}	1.79^{ghi}	1.58^{cd}	29.54^{pq}	31.57^{op}	36.40^{mno}	39.57^{lmn}	42.97^{kl}	36.01^{e}
Pusa Nanha	2.20^{cde}	2.23^{bcd}	2.36^{bc}	2.37^{bc}	2.39^{bc}	2.31^{a}	36.50^{mno}	40.16^{lmn}	54.38^{fg}	74.70^{c}	135.58^{a}	68.26 ^{a}
P-7-15	1.82^{fghi}	2.28^{bcd}	2.30^{bcd}	2.34^{bc}	2.36^{bc}	2.22^{ab}	34.44^{nop}	40.72^{klm}	44.05^{ijkl}	46.37^{hijk}	66.53^{d}	46.42^{d}
P-7-9	1.40^{kl}	1.44^{jkl}	1.54^{ijk}	1.56^{ijk}	1.67^{hijk}	1.52^{d}	41.47^{klm}	43.95^{jkl}	49.25^{ghij}	57.22^{ef}	65.65^{d}	51.51^{c}
P-9-5	1.59^{ijk}	1.79^{ghi}	2.01^{defg}	2.51^{ab}	2.71^{a}	2.12^{b}	50.58^{gh}	56.47^{ef}	59.21^{ef}	62.09^{de}	75.98^{c}	60.87^{b}
V. cund.	1.17^{l}	1.63^{hijk}	1.70^{hij}	1.90^{efgh}	2.10^{cdef}	1.70^{c}	24.51^{q}	49.57^{ghij}	49.80^{ghi}	61.11^{de}	84.47^{b}	53.89^{c}
Mean	1.60^{d}	1.82^{c}	1.91^{c}	2.04^{b}	2.17^{a}		36.17^{e}	43.74^{d}	48.85^{c}	56.84^{b}	78.53^{a}	
LSD (P ≤ 0.05)												
Genotype (G)						0.13						2.57
Temp. regime (T)						0.12						2.35
G × T						0.30						5.76

Temperature regime: T_0 (control) = 28/18°C (day/ night); T_1 = 26/16°C (day/ night); T_2 = 24/14°C (day/ night); T_3 = 22/12°C (day/ night); T_4 = 20/10°C (day/ night)

content of low temperature treated plants, which may be due to decrease in leaf water content. Lee and Lee (7) also noted increase in the leaf protein content in cucumber under chilling stress (4ºC, 12 h) and also attributed the same reason of decrease in leaf relative water content for higher protein accumulation.

Although a little change in mean MII in T_1 (5.67%) was observed over the control but the mean MII of T_4 plants (0.44) was found to be 57.65% higher than the control plants (0.28) (Fig. 1A). Amongst the mean MII of genotypes, the lowest value was noted in P-7-15 (0.31) followed by *V. cundinamarcensis* (0.33). Amongst the possible G × T combinations, Red Lady × T_4 had the highest MII (0.57), while the lowest was observed in *V. cundinamarcensis* × T_0 (0.23) followed by P-7-9 × T_0 (0.25). The per cent increase in MII from T_0 to T_4 was highest in the genotype Red Lady (79.93%). In the present study, the ROS generation may have initiate membrane lipid peroxidation (data not shown), weaken membrane lipid unsaturation, trigger membrane protein polymerization, and resulted in an increase in membrane permeability, which was evident from the higher MII. The findings of the present study agree with the results of Pennycooke *et al*. (9), which showed that membrane is the primary site of chilling or freezing injury in plants.

It was reported that low temperatures affect different aspects of photosynthesis process. It reduces the activity of different enzymes involved in the Calvin cycle and ROS-scavenging systems resulting in ROS generation in PSI and PSII. The change in redox poise imposed by ROS accumulation, resulted in reduction of photosynthetic rate (Ruelland *et al*., 11). In the present study, a severe decline in the photosynthetic rate was observed in the papaya genotypes under the cold stress. The control (T_0) plants showed the highest (2.512 µmol m^{-2} s^{-1}) mean photosynthetic rate (*A*) value, while temperature treatment reduced it (Fig. 1B). Amongst the genotypes, P-7-15 exhibited the maximum mean *A* (2.05 µmol $m^{-2}s^{-1}$). Amongst G × T interactions, plants of Red Lady × T_0 expressed the highest *A* (3.073 µmol $m^{-2}s^{-1}$), while the lowest was expressed in Red Lady × T_4 (0.550 µmol $m^{-2}s^{-1}$). As compared to the control the highest per cent decrease in the *A* at T_4, was observed in Red Lady (82.10%), while lowest in P-7-9 (24.26%). Jeyakumar *et al*. (6) through their study on the physiological performance of papaya genotypes under abiotic stress conditions reported that leaf relative water content had significant influence on photosynthetic rate. In the present study, also both the leaf FW and photosynthetic rates were observed to decrease under the low temperature regimes.

The highest mean total sugars content in the leaves was observed in T_4 (78.53 mg g^{-1}), which is 117.10% higher than the control (36.17 mg g^{-1}) (Table 3). Amongst the six genotypes, Pusa Nanha exhibited the highest mean total sugars content (68.26 mg g^{-1}), while the lowest was noted in Red Lady (36.01 mg g^{-1}). Amongst all the possible combinations of G × T interactions, the highest total sugars content was maintained by the plants of Pusa Nanha × T_4 (135.58 mg g^{-1}). A dramatic increase (81.50%) in the total sugars content was observed in Pusa Nanha at the 20/10°C (day/ night) over 22/12°C (day/ night) temperature regimes. It was noted that the highest increase from T_0 to T_4 was in Pusa Nanha (271.47%) followed by *V. cundinamarcensis* (244.70%). The

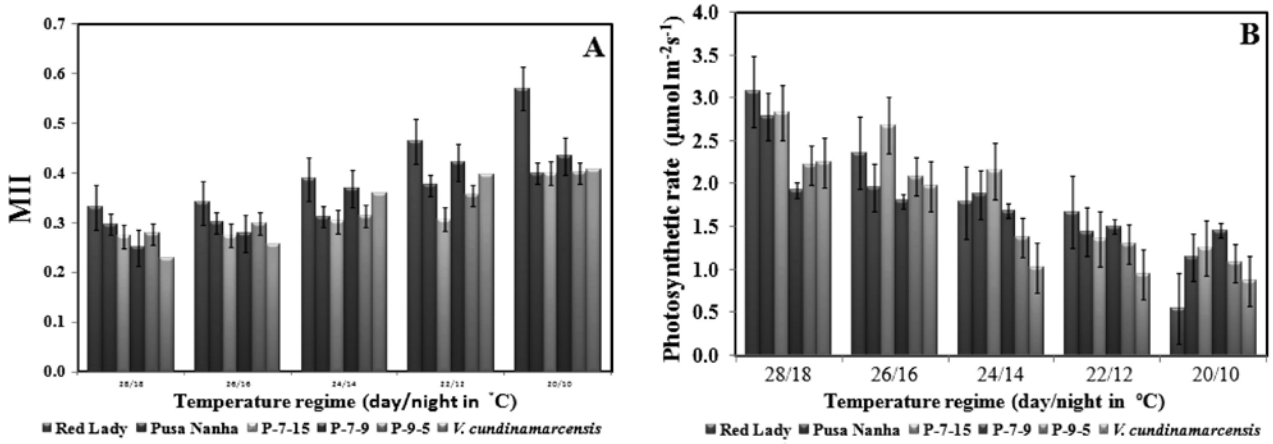


Fig. 1. Effect of different temperature regimes on membrane injury index (MII) and photosynthetic rate of papaya genotypes grown under controlled phytotron conditions; Temperature regime: T_0 (control) = 28/18°C (day/night); T_1 = 26/16°C (day/night); T_2 = 24/14°C (day/night); T_3 = 22/12°C (day/night); T_4 = 20/10°C (day/night), Vertical bars indicate ± SE mean.

increase of leaf total soluble sugars content may be due to the fact that sugars change the osmotic potential of the cell and consequently diminish the difference in water potential between the ice formed in the apoplastic space and the solution within the cell. As a result, the rate at which water is withdrawn from the cell is reduced and the cell membrane becomes more stable to resist the effect of the stress generated by the low temperature. Our results are in agreement to those of Stushnoff *et al*. (14) on Red Delicious apple, where correlation was obtained between cold hardiness in cortical tissues and buds with sorbitol, total sugars and raffinose family oligosaccharides (RFO).

In this study, plants of T_4 (4.08 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}) was observed to have the highest mean CAT activity, which is 141.76% higher than the control plants (1.69 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}) (Fig. 2A). Of the possible G × T interactions, highest increase in the activity of CAT was recorded in Red Lady × T_4 (5.36 µmoles H_2O_2 hydrolysed mg^{-1} TSP min^{-1}). Decreased temperature regimes from T_0 to T_4 caused the highest per cent increase in CAT activity in the genotype P-7-9 (207.40%), while it was lowest in *V. cundinamarcensis* (87.71%). The highest mean SOD activity was observed in the T_4 (8.27 unit mg^{-1} TSP min^{-1}) leaves, which was 47.13% higher than the control (5.26 unit mg^{-1} TSP min^{-1}) (Fig. 2B). Of the various interactions, the highest increase in SOD activity was recorded in *V. cundinamarcensis* × T_4 (9.38 unit mg^{-1} TSP min^{-1}), while the lowest activity was recorded in plants of P-7-15 × T_4 (4.98 unit mg^{-1} TSP min^{-1}). The highest per cent increase in SOD activity (from T_0 to T_4) was observed in *V. cundinamarcensis* (83.85%), while it was lowest in Red Lady (29.96%). Plants under T_4 (43.47 unit mg^{-1} TSP min^{-1}) were observed to have the highest mean GPX activity, which was 44.14% higher than the control plants (30.16 unit mg^{-1} TSP min^{-1}) (Fig. 2C). Amongst the mean GPX activity of genotypes, the highest value was noted in *V. cundinamarcensis* (42.83 unit mg^{-1} TSP min^{-1}), while amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest GPX activity (54.54 unit mg^{-1} TSP min^{-1}). As compared to the control. The highest per cent increase in GPX activity in T_4, was observed in *V. cundinamarcensis* (74.99%). APX activity was found to be significantly increased under low temperature regimes. The highest mean APX activity was found in T_4 plants (4.41 unit mg^{-1} TSP), which was 237.88% higher than the control (1.31 unit mg^{-1} TSP) (Fig. 2D). The genotype *V. cundinamarcensis* was found to have the highest mean APX activity (3.91 unit mg^{-1} TSP), while lowest in Pusa Nanha (2.13 unit mg^{-1} TSP). Amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest APX activity (6.18 unit mg^{-1} TSP). Temperature treatments also increased the APX activity within the genotypes from T_0 to T_4, which was highest in the genotype *V. cundinamarcensis* (295.52%). The highest mean GR activity was found in T_4 plants (4.76 unit mg^{-1} TSP), which was 126.90% higher than the control (2.10 unit mg^{-1} TSP) (Fig. 2E). The genotype *V. cundinamarcensis* was found to have the highest mean GR activity (4.08 unit mg^{-1} TSP), while amongst the possible G × T combinations, *V. cundinamarcensis* × T_4 had the highest GR activity (6.36 unit mg^{-1} TSP). The per cent increase in GR activity from T_0 to T_4 was the highest in genotype *V. cundinamarcensis* (200.11%).

An explanation for differences in the susceptibility of plants to chill-temperature photo-inhibition is that for chilling-tolerant plants the ROS produced in different cellular organelles is efficiently scavenged by the antioxidant enzymes, *viz*., CAT, SOD, APX, GR, GPX *etc.* Here also, an increase in activity of all the above antioxidant enzymes was noted. In the present study, the highest increase in activities of SOD, APX, GPX and GR was found in the tolerant genotype *V. cundinamarcensis*, while the highest increase in CAT was observed in P-7-9. Amongst the *C. papaya* genotypes, P-9-5 registered the highest enhancement in activities of APX, GPX and GR. The results of our study are in agreement with that of Wang and Li (15), Pennycooke *et al*. (9) and Lee and Lee (7). It was striking to note that, the cold tolerant genotype, *V. cundinamarcensis* registered the lowest increase in CAT at 20/ 10ºC (day/ night) regime over the control. Although the genotype Red Lady was observed to be susceptible to low temperature stress based on most of the other parameters but it registered the highest mean CAT activity. It may be due to the fact that CAT is produced in mitochondria and peroxisomes but absent in chloroplast, which is one of the important site of H_2O_2 generation under stress conditions. Compared to APX, it is also considered as a less efficient system of H_2O_2 scavenging due its higher Km value for H_2O_2 than APX.

In conclusion, exposure of papaya genotypes to low temperature stress can result in reduction of photosynthetic rate leading to higher antioxidant enzyme (SOD, CAT, APX, GPX and GR) activities to counteract the effect of reactive oxygen species generated in response to oxidative stress of low temperature regimes. The low temperature stress also affected the cell membrane, which is evident from higher membrane injury index. The concomitant increase in total soluble sugars and proteins content were noted in response to the osmotic stress induced due to reduction in the leaf fresh weight under the

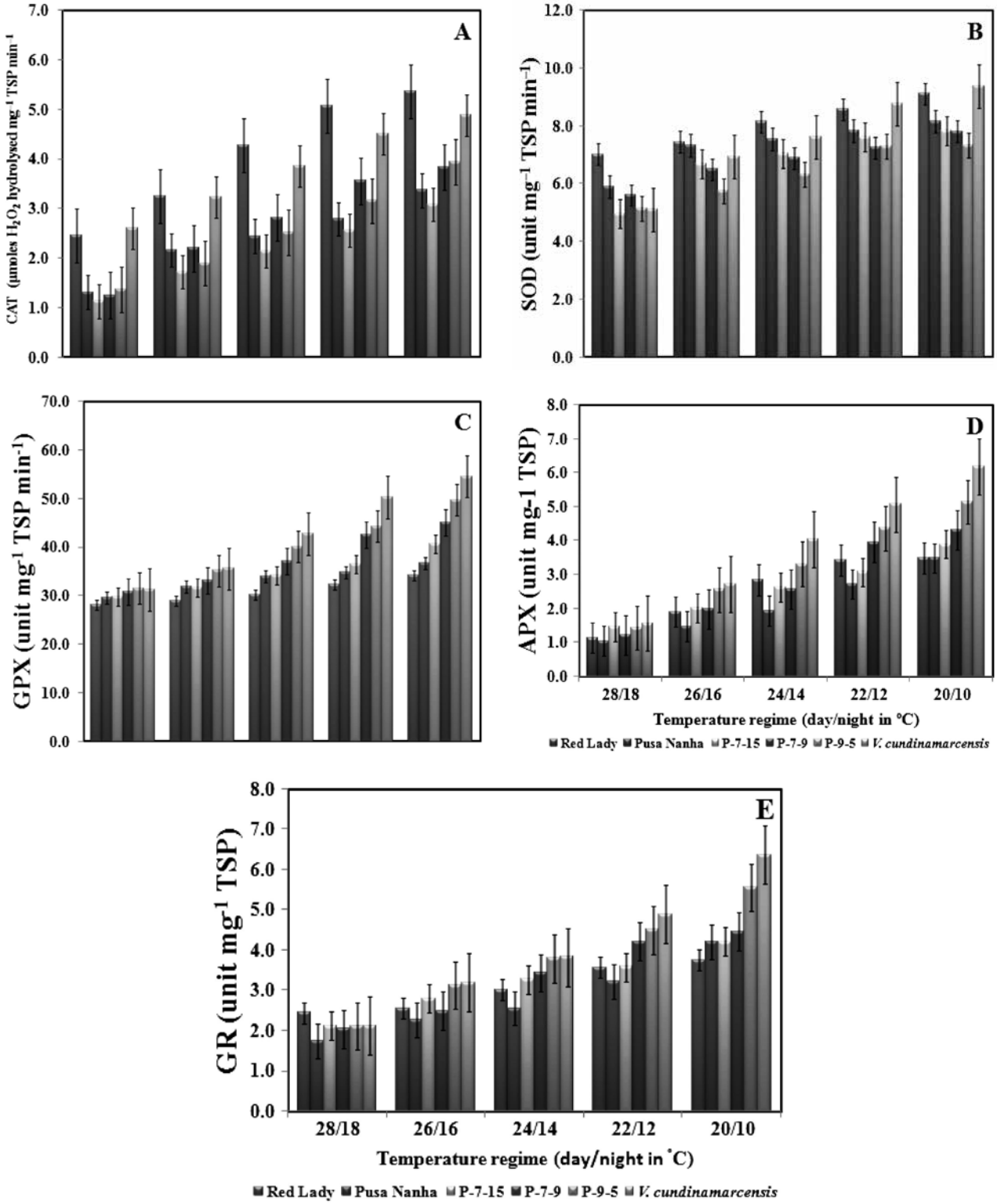


Fig. 2. Effect of different temperature regimes on antioxidant activities in papaya genotypes raised under controlled phytotron conditions; Temperature regime: T_0 (control) = 28/18°C (day/night); T_1 = 26/16°C (day/night); T_2 = 24/14°C (day/night); T_3 = 22/12°C (day/night); T_4 = 20/10°C (day/night); Vertical bars indicate ± SE mean.

low temperature regimes. Based on the results, the genotypes P-9-5 and P-7-9 can be regarded as tolerant to low temperature stress, compared to the tolerant genotype (*Vasconcellea cundinamarcensis*).

ACKNOWLEDGEMENTS

The authors are grateful to ICAR-Indian Agricultural Research Institute, New Delhi, for financial assistance and Scientist In-charge, National Phytotron Facility, ICAR-IARI, New Delhi for the facilities.

REFERENCES

1. Aebi, H. 1984. Catalase *in vitro*. *Method. Enzymol.* **105**: 121-26.
2. Blum, A. and Ebercon, A. 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat. *Crop Sci.* **21**: 43-47.
3. Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248-54.
4. Dhindsa, R.S., Plumela-Dhindsa, P. and Thorpe, T.A. 1981. Leaf senescence and lipid peroxidation. *J. Exp. Bot.* **123**: 93-101.
5. Halliwell, B. and Foyer, C.H. 1978. Properties and physiological functions of a glutathione reductase purified from spinach leaves by affinity chromatography. *Planta*, **139**: 9-17.
6. Jeyakumar, P., Kavino, M., Kumar, N. and Soorianathasundaram, K. 2005. Physiological performance of papaya cultivars under abiotic stress conditions. **In**: *International Symposium on Papaya. Acta Hort.* **740**: 209-15.
7. Lee, D.H. and Lee, C.B. 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. *Plant Sci.* **159**: 75-85.
8. Nakano, Y. and Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* **22**: 867-88.
9. Pennycooke, J.C., Cox, S. and Stushnoff, C. 2005. Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (*Petunia* × *hybrida*). *Env. Exp. Bot*. **53**: 225-32.
10. Ram, M. 2005. *Papaya*, Indian Council of Agricultural Research, New Delhi, 78 p.
11. Ruelland, E., Vaultier, M.N., Zachowski, A. and Hurry, V. 2009. Cold signalling and cold acclimation in plants. *Adv. Bot. Res*. **49**: 35-150.
12. Sadasivam, S. and Manickam, A. 1992. **In**: *Biochemical Methods for Agricultural Science*, Wiley Eastern Limited, New Delhi, pp. 11-12.
13. Srivastava, O.P. and Van Huystee, R.B. 1977. An inter-relation-ship among peroxidase, IAA oxidase, and polyphenol oxidase pea nut cells. *Canadian J. Bot.* **55**: 2630-35.
14. Stushnoff, C., Remmele Jr, R.L., Essensee, V. and McNeil, M. 1993. Low temperature induced biochemical mechanisms: implications for cold acclimation and de-acclimation. **In**: *Interacting Stresses on Plants in a Changing Climate*, pp. 647-57.
15. Wang, L.J. and Li, S.H. 2006. Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. *Plant Sci*. **170**: 685-94.

Received : February, 2017; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00097.4

Indian J. Hort. 74(4), December 2017: 498-504

Diagnosis of nutrient imbalance by Diagnostic and Recommended Integrated System in pomegranate growing soils of south-western Maharashtra

A.B. Gosavi*, A.N. Deshpande and A. Maity**

Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra

ABSTRACT

Diagnostic and Recommended Integrated System (DRIS) approach was employed to develop soil diagnostic norms of major, secondary and micronutrients for pomegranate cv. Bhagwa. For this purpose, pomegranate growing 150 orchards having Bhagwa variety of 4-to 6-year-old under drip irrigation with *hasta bahar* were selected from south western Maharashtra (Solapur, Satara and Sangli districts). The DRIS norms developed for soil nutrients along with DRIS indices and order of nutrient requirement in the selected orchards at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages of pomegranate are presented and discussed in this paper. The DRIS norms developed for very low, low, optimum, high and very high status of soil nutrients in pomegranate at various crop growth stages can serve as nutrient guide and diagnose the nutrient deficiency and excess or imbalance of nutrients. In this study, the DRIS indices indicated that calcium was the most needed plant nutrient by pomegranate at 50 per cent flowering, fruit development and even at Ist harvesting stage.

Key words: Pomegranate, soil nutrients, DRIS norm, indices.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is the important horticultural crop of semi-arid and arid regions of India. History, for origin of the crop, indicates that pomegranate is originated from Iran and then shifted to Afghanistan, Pakistan, India, and United States (Sarkhosh *et al*.,11; Navale, 7). The fruit is symbolic of plenty and very much liked for its cool, refreshing juice and valued for its medicinal properties. The juice has sugar content of 12 to 16 per cent, phosphorous 70 mg, magnesium 12 mg, iron 0.3 mg, sodium 0.3-1.8 mg per 100 mg of edible part (Shulman *et al.*, 12).

Since, pomegranate is a hardy crop, it thrives well under light soils as well limited irrigation conditions in Maharashtra (Deshpande and Patil, 4). They reported that the status of N, P, K, Ca and Mg in soils of pomegranate orchards from Solapur district of Maharashtra is sufficiently high. The distribution of all the nutrients (N, P and K) decreased with increasing depth of soils in both the profiles and both types of gardens. The higher values of EC, $CaCO_3$, Ex. Mg content and lower values of organic carbon, available N, P, K and Ex. Ca were observed in neglected gardens as compared with well managed gardens.

In a review paper on DRIS, Bangroo *et al.* (1) concluded that DRIS norms should be developed for specific conditions, in which all other factors to be correlated with yield or quality (or any other variable) be known and isolated: cultivar, climate, soil and crop management, productivity *etc.*, attaining the specific objectives. After establishment of new promising variety Bhagwa, in south western Maharashtra, separate recommendation regarding nutrition of this variety is necessary for precision farming and hence the study was undertaken.

MATERIALS AND METHODS

A survey 150 cultivators of growing pomegranate from the south western Maharashtra (Solapur, Satara and Sangli districts) was undertaken during 2011-12. The selection of farmers in each district was done on the basis of area under pomegranate in the district as compared to the total area in south western Maharashtra. The farmers having Bhagwa variety of 4- to 6-year-old, grown on drip irrigation in *Hasta bahar* were selected for study. Soil samples from 0-30 cm depth at *bahar*, 50 per cent flowering, fruit development and Ist harvest stage of pomegranate were collected and brought to the laboratory. They were air-dried in shade, ground with mortar and pestle and passed through 2 mm sieve for analysis. Sieved soil samples were stored in cloth bags with proper labeling for analysis. The soil analysis was done by following the standard procedures.

For DRIS analysis the orchards were grouped into two different classes on the basis of yield as per the standard method given by Beaufils (2). The highest yield of pomegranate was recorded 25.98 Mg ha^{-1}. The yield of 20.00 Mg ha^{-1} was 77 per

*Corresponding author's E-mail: gosaviavi@rediffmail.com

**ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra

cent of the highest yield. This yield was identified as a line of demarcation between the high and low yielding groups. The procedure as initially developed by Beaufils (2), modified by Bhargava (3) was used through a computer based programme for development of DRIS norms.

Following equation was developed for calculation of DRIS indices of soil nitrogen.

N = 1/9 {(f (N/P) + f (N/K) + f (N/S) + (N/Na) + f (N/B) + f (N/Mg) + f (N/Fe) + f (N/Mn) + f (N/Cu) + f (N/Zn)}

Similarly for P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, B and Mo, equations were developed.

Where $f(N/P) = \left\{\frac{N/P}{n/p}\right\}\left\{\frac{1000}{CV}\right\} - 1$ when N/P>n/p

and

$fc(N/P) = 1 - \left\{\frac{N/P}{n/p}\right\}\left\{\frac{1000}{CV}\right\}$ when N/P<n/p

Where, N/P is the actual value of the ratio of N and P in the soil under diagnosis. The n/p is the value of the norm (which is the mean value of high yielding plots) and CV is the coefficient of variation for population of high yielding plots.

The norms for classification of nutrients in leaves were derived using them as mean of high yielding orchards as the mean for optimum. The range of mean was the value derived from mean -4/3 standard deviation to +4/3 standard deviation. The range of low was obtained by calculating -4/3 standard deviation to -8/3 standard deviation and the values below mean -8/3 standard deviation were considered as deficient. The value from mean +4/3 standard deviation to mean +8/3 standard deviation was considered as an excesses (Bhargava, 3).

RESULTS AND DISCUSSION

The DRIS norms developed for other chemical properties of soil (Tables 1 to 4) revealed that, optimum level for pH were 7.78 to 8.88, 7.79 to 8.85, 7.86 to 8.87 and 7.92 to 8.89 at *bahar*, 50 per cent flowering, fruit development and Ist harvest stages, respectively. While for electrical conductivity the norms were 0.20 to 0.46, 0.19 to 0.48, 0.19 to 0.50 and 0.21 to 0.50 dSm^{-1}, for organic carbon those were 4.1 to 9.7, 4.3 to 9.6, 4.5 to 9.8 and 4.6 to 9.8 g kg^{-1} and for calcium carbonate the DRIS norms are 86.6-156.3, 91.8-155.5, 91.5-148.5 and 86.8-136.6 g kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages respectively. Raghupathi and Bhargava (8) observed the range of pH from 8.1 to 8.8 with the optimum DRIS norms of pH from 8.2 to 8.6, whereas for electrical conductivity the norms were 0.08 to 0.31 dS m^{-1} in the pomegranate orchards of Bijapur district of northern Karnataka. Deshpande and Patil (4) reported the range of pH from 7.6 to 9.4, electrical conductivity from 0.10 to 0.95 dSm^{-1} and organic carbon from 0.40 to 1.21 per cent for soils of the pomegranate growing orchards in Solapur area.

DRIS norms for available nitrogen at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages were 161.50 to 255.50, 152.20 to 241.90,

Table 1. DRIS norms for soil available nutrients at *bahar* stage of pomegranate.

Parameter	Very low	Low	Optimum	High	Very high
pH (1:2.5)	<7.21	7.21-7.77	7.78-8.88	8.88-9.44	>9.44
EC (dS m^{-1})	<0.06	0.06-0.19	0.20-0.46	0.47-0.59	>0.59
OC (g kg^{-1})	<01.1	1.1-4.0	4.1-9.7	9.8-12.6	>12.6
$CaCO_3$ (g kg^{-1})	<51.6	51.6-86.5	86.6-156.3	156.4-191.2	>191.2
Av. N (kg ha^{-1})	<114.3	114.3-161.4	161.5-255.5	255.6-302.5	>302.5
Av. P (kg ha^{-1})	<6.14	6.14-11.77	11.78-23.01	23.02-28.63	>28.63
Av. K (kg ha^{-1})	<127.9	127.9-232.2	232.3-440.9	441-545.2	>545.2
Ex. Ca (cmol (p^+) kg^{-1})	<18	18-27.64	27.65-46.92	46.93-56.55	>56.55
Ex. Mg (cmol (p^+) kg^{-1})	<5.26	5.26-10.80	10.81-21.89	21.9-27.43	>27.43
Ex. Na (mg kg^{-1})	<0.11	0.11-0.22	0.23-0.45	0.46-0.56	>0.56
S (mg kg^{-1})	<1.92	1.92-6.93	6.94-16.95	16.96-21.96	>21.96
DTPA Cu (mg kg^{-1})	<0.14	0.14-2.11	2.12-6.06	6.07-8.03	>8.03
DTPA Fe (mg kg^{-1})	<1.12	1.12-3.70	3.71-8.85	8.86-11.43	>11.43
DTPA Mn (mg kg^{-1})	<3.59	3.59-7.71	7.72-15.96	15.97-20.08	>20.08
DTPA Zn (mg kg^{-1})	<0.31	0.31-0.51	0.52-0.92	0.92-1.12	>1.12
B (mg kg^{-1})	<0.04	0.04-0.16	0.17-0.40	0.41-0.52	>0.52
Mo (mg kg^{-1})	<0.01	0.01-0.05	0.06-0.13	0.14-0.18	>0.18

153.60 to 228.80 and 141.70 to 222.00 kg ha^{-1}, respectively. The highest requirement of nitrogen was observed at *bahar* stage (206.83 kg ha^{-1}) for pomegranate. Raghupathi and Bhargava (9) reported the optimum range 44 to 103 mg kg^{-1} for available soil nitrogen. Deshpande and Patil (4) observed the range of available nitrogen from 113 to 310 kg ha^{-1} in the pomegranate orchards of Solapur region.

Table 2. DRIS norms for soil available nutrients at flowering stage of pomegranate.

Parameter	Very low	Low	Optimum	High	Very high
pH (1:2.5)	<7.25	7.25-7.78	7.79-8.85	8.86-9.38	>9.38
EC (dS m^{-1})	<0.04	0.04-0.18	0.19-0.48	0.49-0.62	>0.62
OC (g kg^{-1})	<1.5	1.5-4.2	4.3-9.6	9.7-12.4	>12.4
$CaCO_3$ (g kg^{-1})	<59.9	59.9-91.7	91.8-155.5	155.6-187.3	>187.3
Av. N (kg ha^{-1})	<107.2	107.2-152.1	152.2-241.9	242.0-286.8	>286.8
Av. P (kg ha^{-1})	<8.09	8.09-13.4	13.5-23.9	24-29.2	>29.2
Av. K (kg ha^{-1})	<199	199-310	311-533	534-644	>644
Ex. Ca (cmol (p$^+$)kg^{-1})	<22.2	22.2-30.1	30.2-46.0	46.1-53.9	>53.9
Ex. Mg (cmol (p$^+$)kg^{-1})	<8.2	8.2-13.2	13.3-23.2	23.3-28.2	>28.2
Ex. Na (mg kg^{-1})	<0.13	0.13-0.23	0.24-0.43	0.44-0.54	>0.54
S (mg kg^{-1})	<2.16	2.16-7.18	7.19-17.24	17.25-22.27	>22.27
DTPA Cu (mg kg^{-1})	<0.74	0.74-2.78	2.79-6.85	6.86-8.88	>8.88
DTPA Fe (mg kg^{-1})	<0.62	0.62-3.01	3.02-7.77	7.78-10.15	>10.15
DTPA Mn (mg kg^{-1})	<5.03	5.03-9.73	9.74-19.12	19.13-23.82	>23.82
DTPA Zn (mg kg^{-1})	<0.22	0.22-0.47	0.48-0.96	0.97-1.21	>1.21
B (mg kg^{-1})	<0.06	0.06-0.21	0.22-0.50	0.51-0.60	>0.60
Mo (mg kg^{-1})	<0.01	0.01-0.05	0.06-0.14	0.14-0.18	>0.18

Table 3. DRIS norms for soil available nutrients at fruit development stage of pomegranate.

Parameter	Very low	Low	Optimum	High	Very high
pH (1:2.5)	<7.34	7.34-7.85	7.86-8.87	8.88-9.38	>9.38
EC (dS m^{-1})	<0.02	0.02-0.18	0.19-0.50	0.51-0.66	>0.66
OC (g kg^{-1})	<1.7	1.7-4.4	4.5-9.8	9.8-12.4	>12.4
$CaCO_3$ (g kg^{-1})	<60.9	60.9-90	91.5-148.5	149-177	>177
Av. N (kg ha^{-1})	<115.8	115.8-153.5	153.6-228.8	228.9-266.5	>266.5
Av. P (kg ha^{-1})	<7.59	7.59-13	13.1-24.1	24.2-29.6	>29.6
Av. K (kg ha^{-1})	<149.3	149.3-311.2	311.3-635.5	635.6-797.6	>797.6
Ex. Ca (cmol (p$^+$) kg^{-1})	<22.2	22.2-29.6	29.7-44.3	44.4-51.7	>51.7
Ex. Mg (cmol (p$^+$) kg^{-1})	<6.7	6.7-11.8	11.9-22.1	22.2-27.3	>27.3
Ex. Na (mg kg^{-1})	<0.13	0.13-0.23	0.24-0.43	0.44-0.53	>0.53
S (mg kg^{-1})	<2.19	2.19-7.35	7.36-17.69	17.7-22.86	>22.86
DTPA Cu (mg kg^{-1})	<0.77	0.77-2.88	2.89-7.11	7.12-9.22	>9.22
DTPA Fe (mg kg^{-1})	<1.92	1.92-3.53	3.54-6.74	6.75-8.35	>8.35
DTPA Mn (mg kg^{-1})	<6.59	6.59-10.24	10.25-17.52	17.53-21.17	>21.17
DTPA Zn (mg kg^{-1})	<0.24	0.24-0.49	0.50-1.00	1.01-1.25	>1.25
B (mg kg^{-1})	<0.1	0.1-0.23	0.24-0.50	0.51-0.63	>0.63
Mo (mg kg^{-1})	<0.01	0.01-0.05	0.06-0.13	0.14-0.17	>0.17

Table 4. DRIS norms for soil available nutrients at first harvesting stage of pomegranate.

Parameter	Very low	Low	Optimum	High	Very high
pH (1:2.5)	<7.42	7.43-7.91	7.92-8.89	8.90-9.38	>9.38
EC (dS m^{-1})	<0.05	0.06-0.20	0.21.-0.50	0.51-0.65	>0.65
OC (g kg^{-1})	<1.9	2.0-4.5	4.6-9.8	9.9-12.5	>12.5
$CaCO_3$ (g kg^{-1})	<61.7	61.8-86.7	86.8-136.6	136.7-161.5	>161.5
Av. N (kg ha^{-1})	<101.4	101.5-141.6	141.7-222	222.1-262.0	>262.0
Av. P (kg ha^{-1})	<7.44	7.45-12.71	12.72-23.25	23.26-28.52	>28.52
Av. K (kg ha^{-1})	<78.40	78.5-245.6	245.7-580.5	580.6-747.8	>747.8
Ex. Ca (cmol (p^+) kg^{-1})	<21.7	21.8-28.4	28.5-41.6	41.7-48.2	>48.2
Ex. Mg (cmol (p^+) kg^{-1})	<5.9	6.0-10.1	10.2-18.8	18.9-23.1	>23.1
Ex. Na (mg kg^{-1})	<0.10	0.11-0.20	0.21-0.40	0.41-0.50	>0.50
S (mg kg^{-1})	<2.26	2.27-7.35	7.35-17.53	17.54-22.62	>22.62
DTPA Cu (mg kg^{-1})	<0.77	0.78-2.63	2.64-6.37	6.38-8.23	>8.23
DTPA Fe (mgk g^{-1})	<0.52	0.53-2.34	2.35-5.89	5.9-7.66	>7.66
DTPA Mn (mg kg^{-1})	<4.37	4.38-8.50	8.51-16.77	16.78-20.90	>20.90
DTPA Zn (mg kg^{-1})	<0.23	0.24-0.47	0.48-0.95	0.96-1.19	>1.19
B (mg kg^{-1})	<0.01	0.02-0.15	0.16-0.44	0.45-0.59	>0.59
Mo (mg kg^{-1})	<0.01	0.02-0.05	0.06-0.13	0.14-0.17	>0.17

The DRIS norms for available phosphorus were 11.78 to 23.01, 13.50 to 23.90, 13.10 to 24.10 and 12.72 to 23.25 kg ha^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages respectively. The highest P requirement of the crop was observed at 50 per cent flowering and fruit development stage (19.52 and 19.28 kg ha^{-1}, respectively). Raghupathi and Bhargava (9) reported the optimum P requirement 10.7 to 20.7 mg kg^{-1} for Ganesh variety. The potassium DRIS norms at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages were 232 to 440, 311 to 533, 311 to 635 and 245 to 580 kg ha^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages, respectively. Highest requirement of potassium was observed at fruit development stage (416.2 kg ha^{-1}) since more potassium was essential for higher and quality fruit production (Raghupathi and Bhargava, 8; Raghupathi and Bhargava, 9; Gimenez *et al*., 6; Bhargava, 3). The DRIS norms reported by Raghupathi and Bhargava (9) for potassium were 73 to 115 mg kg^{-1}.

The optimum DRIS norms for exchangeable calcium at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages were 27.65 to 46.92, 30.2 to 46.0, 29.7 to 44.3 and 28.5 to 41.6 cmol (p+) kg^{-1}, respectively. For exchangeable magnesium, the norms were 10.8 to 21.9, 13.3 to 23.2, 11.9 to 22.1 and 10.2 to 18.8 mg kg^{-1} at respective crop growth stages. The DRIS norms for sodium are also developed and those were 0.23 to 0.45, 0.24 to 0.43, 0.24 to 0.43 and 0.21 to 0.40 mg kg^{-1}, whereas the norms for sulpher were 6.94 to 16.95, 7.19 to 17.24, 7.36 to 17.69 and 7.35 to 17.53 mg kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages, respectively. Raghupathi and Bhargava (9) reported the optimum S requirement 87 to 209 mg kg^{-1} for pomegranate.

Regarding micronutrients, the DRIS norms for DTPA copper were 2.12 to 6.06, 2.79 to 6.85, 2.89 to 7.11 and 2.64 to 6.37 mg kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages, respectively. Raghupathi and Bhargava (9) reported the optimum copper requirement 0.85 to 1.91 mg kg^{-1} for Ganesh pomegranate. The DRIS norms for DTPA iron at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages were 3.71 to 8.85 3.02 to 7.77, 3.54 to 6.74 and 2.35 to 5.89 mg kg^{-1}, respectively. Raghupathi and Bhargava (9) reported the optimum DRIS norms for iron from 0.25 to 0.70 mg kg^{-1} for Ganesh variety.

Regarding DTPA extractable manganese, the DRIS norms developed were 7.72 to 15.96, 9.74 to 19.12, 10.25 to 17.52 and 8.51 to 16.77 mg kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages, respectively. The optimum DRIS norms for DTPA zinc at respective crop growth stages were 0.52 to 0.92, 0.48 to 0.96, 0.50 to 1.00 and 0.48

to 0.95 mg kg^{-1}, respectively. Higher requirement of zinc among various crop growth stages was observed at fruit development stage. Raghupathi and Bhargava (9) reported the optimum DRIS norms for zinc from 0.29 to 1.09 mg kg^{-1}.

The optimum DRIS norms for boron were 0.17 to 0.40, 0.22 to 0.50, 0.24 to 0.50 and 0.16 to 0.44 mg kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages. While, DRIS norms for molybdenum were 0.06-0.13, 0.06-0.14, 0.06-0.13 and 0.06-0.13 mg kg^{-1} at *bahar*, 50 per cent flowering, fruit development and Ist harvesting stages respectively.

The DRIS indices developed for soil nutrients of pomegranate at *bahar* stage, 50% flowering, fruit development and Ist harvesting stage are presented in Tables 5 and 6. More the negative index more is the imbalance of that nutrient at that crop growth stage (Beaufils, 2; Bhargava, 3; Francisco, 5; Raghupathi *et al.*, 10). The DRIS indices indicated that even those nutrients in most of the orchards were under optimum category, there was imbalance of those nutrients at fruit development stage, which should be given proper attention.

The data for mean DRIS indices at *bahar* stage revealed that in major nutrients, the indices for nitrogen, phosphorous and potassium were -10.37, -5.00 and -1.84, respectively. This indicates even 85 per cent orchards were at optimum level of nitrogen at *bahar* stage, the ratios of nitrogen with other nutrients were not in balance state, which shows negative values of DRIS index which is the superiority of DRIS norms over critical level values. At 50 per cent flowering stage, indices for nitrogen, phosphorous and potassium were -12.10, -8.20 and -1.32, respectively. The non application of nitrogenous fertilizers by farmers at flowering stage would be the reason for its higher requirement and imbalance among the major nutrients. The data on DRIS indices revealed that, the indices for nitrogen, phosphorous and potassium were -10.99, -12.06 and -0.61, respectively at fruit development stage. The results revealed that the mean DRIS indices for available nitrogen, phosphorous and potassium contents were -7.24 -2.79 and -0.47, respectively. At harvesting stage, higher imbalance in nitrogen content was observed, whereas potassium was mostly at balanced state over other nutrients.

For secondary nutrients, at *bahar* stage, the indices for calcium, magnesium, sodium and sulphur were -8.42, 16.13, -6.33 and 0.47, respectively. This indicates the requirement of calcium and sodium at *bahar* stage. The magnesium was in excess state whereas sulphur was mostly in balanced state. At 50% flowering stage, the DRIS indices for calcium, magnesium, sodium and sulphur contents of the soil were -13.25, 21.07, -9.60 and 0.94, respectively. Higher calcium requirement for the flowering of pomegranate is reported by many research workers which is justified here by DRIS indices. The sulphur content was almost at balanced state at this stage. The

Table 5. Mean, minimum and maximum of DRIS indices for soil nutrients at *bahar* and 50% flowering stage of pomegranate.

Nutrient	*Bahar* stage			50% flowering stage		
	Mean	Min.	Max.	Mean	Min.	Max.
Av. N	-10.37	-84.79	30.30	-12.10	-120.33	31.65
Av. P	-5.00	-51.88	63.01	-8.20	-49.38	29.03
Av. K	-1.84	-122.28	51.95	-1.32	-87.42	32.03
Ex. Ca	-8.42	-84.28	24.37	-13.25	-109.43	36.93
Ex. Mg	16.13	-31.89	190.18	21.07	-28.81	318.24
Ex. Na	-6.33	-48.16	54.64	-9.60	-85.92	39.83
S	0.47	-60.02	70.30	0.94	-49.75	50.56
DTPA Cu	0.80	-47.49	68.60	4.52	-58.16	74.86
DTPA Fe	1.65	-51.49	122.36	-4.86	-68.52	87.33
DTPA Mn	-3.63	-57.63	37.80	-2.95	-34.36	43.14
DTPA Zn	5.56	-25.41	102.61	4.52	-45.63	52.61
B	-0.33	-85.80	63.62	-11.15	-154.12	107.41
Mo	0.13	-35.69	48.55	0.24	-52.23	77.43

Table 6. Mean, minimum and maximum of DRIS indices for soil nutrients at fruit development and Ist harvesting stage of pomegranate.

Nutrient	Fruit development stage			Ist harvesting stage		
	Mean	Min.	Max.	Mean	Min.	Max.
Av. N	-10.99	-101.18	32.29	-7.24	-75.23	39.75
Av. P	-12.06	-96.26	31.83	-2.79	-37.66	24.00
Av. K	-0.61	-61.24	35.34	-0.47	-86.43	39.48
Ex. Ca	-12.87	-111.91	38.03	-15.77	-98.67	19.75
Ex. Mg	19.42	-24.78	223.39	19.09	-39.00	175.86
Ex. Na	-9.85	-76.90	24.49	-7.74	-56.29	30.17
S	1.83	-36.59	48.04	1.72	-56.24	55.12
DTPA Cu	1.73	-67.55	56.56	-0.05	-81.58	75.20
DTPA Fe	-3.62	-99.12	38.80	-3.64	-68.41	45.77
DTPA Mn	0.22	-35.33	71.72	-4.65	-103.13	56.37
DTPA Zn	3.57	-28.25	39.96	2.22	-29.36	50.44
B	-3.48	-55.71	27.61	-3.64	-72.74	51.85
Mo	-0.18	-63.89	59.81	-0.22	-53.26	42.13

DRIS indices at fruit development stage for calcium, magnesium, sulphur and sodium were -12.87, 19.42, 1.83 and -9.85, respectively. It indicated that among the secondary nutrients, sodium followed by iron were the imbalanced nutrients, whereas magnesium was the abundant nutrient and at Ist harvesting stage, the mean DRIS indices for calcium, magnesium, sulphur and sodium were -15.77 and 19.09, 1.72 and -7.74, respectively indicating higher calcium requirements at this stage.

In micronutrients content, the DRIS indices at *bahar* stage for copper, iron, manganese, zinc, boron and molybdenum were 0.80, 1.65, -3.83, 5.56, -0.33 and 0.13, respectively. This indicates the requirement of manganese at this stage, whereas molybdenum was mostly at balanced state. At 50% flowering stage the indices for copper, iron, manganese, zinc, boron and molybdenum were 4.52, -4.86, -2.95, 4.52, -11.15 and 0.24, respectively indicating the requirement of iron, manganese and boron at this stage, while molybdenum status particularly showed balanced nutrition at this stage. The DRIS indices at fruit development stage for copper, iron, manganese, zinc, boron and molybdenum were 1.73, -3.62, 0.22, 3.57, -3.48 and -0.18, respectively indicated the requirement of iron and boron. However, copper, manganese and molybdenum were practically at balanced state, whereas at Ist harvesting stage, DRIS indices for copper, iron, manganese, zinc, boron and molybdenum were -0.05, -3.64, -4.65, 2.22, -3.64 and -0.22, respectively. This indicated the imbalance of micronutrients, *viz.*, iron, manganese and boron, while, copper was mostly at balanced state over rest of the nutrients.

The order of nutrient requirement of pomegranate at different crop growth stages using soil DRIS indices indicated that calcium was the most needed plant nutrient by pomegranate at 50 per cent flowering, fruit development and even at Ist harvesting stage. The soil nutrients, *viz.* calcium and nitrogen should be looked seriously for supply through fertilizers from beginning to harvesting of pomegranate. It was interesting to note that, role of sodium as a beneficial nutrient for pomegranate at each sampling stage was pointed out by DRIS indices. Supplement of phosphorous manganese and potassium was necessary at *bahar*, 50 per cent flowering stage and iron was needed at 50 per cent flowering, fruit development and Ist harvesting stages. At *bahar*, iron and zinc were at sufficient levels because most of the farmers apply $FeSO_4$ and $ZnSO_4$ as a basal dose, however as the time proceeds, iron becomes deficient due to faster oxidation in soil or otherwise requirement of iron by pomegranate is increased as the *bahar* proceeds further. Sulphur and molybdenum remained in balanced state and magnesium was always in excess quantity throughout the *bahar* period. The reason behind sulphur sufficiency was application of single super phosphate as a basal dose. The sufficiency level of magnesium might be due to application of dolomite through fertilizers as well as high status of magnesium and molybdenum in soil and low requirement of magnesium, copper and molybdenum by pomegranate showed these nutrients either in balance level or in excess.

Table 7. Order of nutrient requirement of pomegranate at different growth stages using soil DRIS indices.

Growth stage	Order of nutrient requirement
At *bahar* stage	N>Ca>Na>P>Mn>K>B>Mo>S>Cu>Fe>Zn>Mg
50% flowering	Ca>N>B>Na>P>Fe>Mn>K>Mo>S>Zn>Cu>Mg
Fruit development	Ca>P>N>Na>Fe>B>K>Mo>Mn>Cu>S>Zn>Mg
Ist harvesting	Ca>Na>N>Mn>Fe>B>P>K>Mo>Cu>S>Zn>Mg

REFERENCES

1. Bangroo, S.A., Bhat, M.I., Tahir, A., Aziz, M.A., Bhat, M.A. and Wani, M.A. 2010. Diagnosis and Recommendation Integrated System (DRIS): A Review. *Inter. J. Curr. Res.* **10**: 84-97.
2. Beaufils, E.R. 1973. Diagnosis and recommendation integrated system (DRIS). A general scheme for experimentation and calibration based on principles developed from research in plant nutrition. Pietermararitzburg, University of Natal, *Soil Sci. Bull.* **1**: 132.
3. Bhargava, B.S. 2002 Leaf analysis for nutrient diagnosis, recommendation and management in fruit crops. *J. Indian Soc. Soil Sci.* **50**: 352-73.
4. Deshpande, A.N. and Patil, G.D. 2011. Nutritional survey of pomegranate orchards from Solapur district of Maharashtra. *J. Agric. Res. Tech.* **36**: 263-69.
5. Francisco, D. and Assis Alves Mourão, Filho. 2004. DRIS: concepts and applications on nutritional diagnosis in fruit crops. USP/ESALQ Depto. de Produção Vegetal, 9 - 13418-900 - Piracicaba, SP Brasil.
6. Gimenez, M., Oltra, M.A., Melgarejo, P., Martinez, J., Martinez, J.J. and Ferrandez, M. 2000.

Computer program for calculating pomegranate drip fertigation. Production, processing and marketing of pomegranate in the Mediterranean region: Advances in research and technology. *Proceedings of the Symposium jointly organized by CIHEAM and Escuela Politecnica Superior de Orihuela of the Universidad Miguel Hernandez* (*EPSO-UMH*), Orihuela, Spain, 15-17 October, 1998.

7. Navale, A.M. 2007. Mineral nutrient status of pomegranate orchards in Western Maharashtra, India. *Asian J. Hort.* **2**: 294-97.

8. Raghupathi, H.B. and Bhargava, B.S. 1998a. Diagnosis of nutrient imbalance in pomegranate by diagnosis and recommendation integrated system and compositional nutrient diagnosis, *Comm. Soil Sci. Plant Anal*. **29**: 2881-92.

9. Raghupathi, H.B. and Bhargava, B.S. 1998b. Leaf and soil nutrient diagnostic norms for pomegranate (*Punica granatum* L). *J. Indian Soc. Soil Sci*. **46**: 412-16.

10. Raghupathi, H.B., Reddy, Y.T.N., Rein, M.K. and Bhargava, B.S. 2004. Diagnosis of nutrient imbalance in mango by DRIS and PCA approach. *J. Plant Nutr.* **27**: 1131-48.

11. Sarkhosh, A., Zamani, Z., Fatahi, R. and Ebadi, A. 2006. RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Scientific Hort.* **111**: 24-29.

12. Shluman, Y., Fainberstein, Z. and Lavee, S. 1984. Pomegranate fruit development and maturation. *J. Hort. Sci.* **59**: 265-74.

Received : March, 2015; Revised : July, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00098.6

Indian J. Hort. 74(4), December 2017: 505-509

Effect of different rootstocks on growth, leaf sclerophylly and chlorophyll fractions of Kinnow mandarin

Sunil Kumar, O.P. Awasthi*, A.K. Dubey and R.M. Sharma

Division of Fruits & Horticultural Technology, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

ABSTRACT

A study was undertaken to evaluate growth performance, leaf sclerophylly and chlorophyll content of Kinnow mandarin budded on different rootstocks. In the pre-bearing stage, the magnitude of vegetative growth revealed stimulated increase during April-September compared to September-December. The vegetative growth of Kinnow on *Jatti khatti* was better in terms of per cent increase in plant height, plant spread and canopy volume. Reduced vegetative growth was observed in Kinnow trees on Troyer citrange rootstock. Maximum increment in scion (88.37%) and root diameter (83.64%) was recorded on *Jatti khatti* between April-September. Congenial relationship was observed between scion and rootstock and had mean values for this relationship close to 1.0. Leaf sclerophylly studies exhibited higher leaf area (166.24 cm^2), fresh mass (4.36 g) and dry matter (1.85 g) on *Jatti khatti* with higher density of foliar tissue (433.70 g^{-1} kg) on *Karna khatta*. Minimum leaf area (85.07 cm^2), fresh mass (2.50 g) and dry matter (0.92 g) was recorded on rough lemon, but leaves were significantly more succulent. Chlorophyll fractions, *viz*., Chlorophyll '*a*' (1.60 mg g^{-1} FW), total chlorophyll (1.87 mg g^{-1} FW) and chlorophyll *a:b* (6.20) were significantly higher in leaf of Kinnow on rough lemon and minimum on Carrizo citrange rootstock.

Key words: Chlorophyll, Kinnow, growth, leaf sclerophylly, rootstock.

INTRODUCTION

Kinnow mandarin cultivation is being successfully done in the arid and semi-arid tracts of the Indian subcontinent. Due to higher productivity per unit area and consumers preference, its cultivation in the recent past has also extended to non-traditional areas, such as national capital regions of Delhi, Haryana, U.P., M.P., Chhattisgarh *etc*. The rootstocks commonly used for raising Kinnow mandarin is *Jatti khatti*. The monopolised cultivation of Kinnow on *Jatti khatti* however, cannot be considered as an ideal rootstock for all set of agro-climatic conditions. Even in the traditional areas, diversification in rootstock is essential keeping in view the climate change, biotic and abiotic stresses. The significance of rootstock in citrus industry needs no emphasis, because rootstocks have perhaps contributed more than any factors to the success or failure of citrus orcharding. Several studies have indicated profound influence of rootstocks on scion cultivars including plant stature, physiological parameters, yield and leaf level nutrients (Aviles *et al*., 1; Awasthi *et al*., 2; Goswami *et al*., 4; Sharma *et al*., 9). Given that the rootstocks are unlike genotypes and may modify growth and other growth related parameters, the present study was aimed to evaluate the effect of different rootstocks on Kinnow tree growth and also to understand the variations in leaf sclerophylly and chlorophyll fractions due to rootstocks in a pre-bearing three-year-old orchard of Kinnow.

*Corresponding author's E-mail: awasthiciah@yahoo.com

MATERIALS AND METHODS

The present work was carried out during 2014-2015 at the Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi (77°12' E longitude, 28°40'N latitude, 228.6 m asl). The climate is categorized as semi-arid, sub-tropical with hot dry summer (41-44°C) and cold winter (3-7°C). The average annual rainfall during the period of experimentation was 719 mm and more than 60% rainfall received during July, August and September. Plant materials for the experiment consisted of three-year-old non-bearing plants of Kinnow budded on seven rootstocks, *viz*., rough lemon (*Citrus jambhiri* Lush.), *Karna khatta* (*Citrus karna* Raf.), Carrizo citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* [L.] Raf.), Rangpur lime (*Citrus limonia* [L] Osb.), Troyer citrange (*Citrus sinensis* [L.] Osb. × *Poncirus trifoliata* (L.) Raf.], *Jatti khatti* (*Citrus jambhiri* Lush.) and sour orange (*Citrus aurantium* L.). The budded plants were planted during the year 2011. Plant growth in terms of plant height (m), stock diameter (mm), scion diameter (mm) and canopy volume (m^3) were recorded two months after the emergence of spring (February), rainy (July) and autumn (October) season flush, *i.e*., April, September and December.

Plant height was determined by measuring the distance from the ground to the top of the plant with the help of measuring scale. Plant spread [N-S (D1)] and [E-W (Dr)] was recorded with the help of metre scale and canopy volume (V) was determined from individual measurements of tree height (H) and width in parallel (D1) and perpendicular (Dr) by the formulae V = (π/6) × H × D1 × Dr (Zekri, 11). Scion diameter was taken at fixed height 10 cm above the graft union and trunk diameter 10 cm below the graft union. The positions were marked with black paint for recurrent observations. The scion: rootstock ratio was calculated by dividing the scion value with rootstock value. The data recorded on different vegetative parameters were compared in terms of per cent increase by calculating the growth difference between September-April and December-September. Observation on leaf sclerophlly was taken using ten mature leaves from rainy season (August) flush, collected from Kinnow trees grown on different rootstocks during October. The parameters examined were leaf area (LA), using a LI-COR, LI 3100 area meter (LI-COR, USA), fresh mass (FM) and dry mass (DM) per leaf. The leaves were weighed immediately after harvest to determine their fresh mass. The leaves were then oven dried at 70°C for 48 h and their dry mass was determined. Several indices of leaf physiological parameters were calculated by the formulae suggested by Ennajeh *et al*. (3). These included Specific leaf area (SLA = LA/DM: in $cm^2 g^{-1}$ DW), specific leaf weight (SLW = DW/LA: in g cm^2 LA), density of foliar tissue (D = DW/FW × 1000: in g kg^{-1}) and succulency [S = (FW-DW)/LA: in mg H_2O cm^{-2}. The leaf chlorophyll content (chlorophyll *a*, *b* and total ll) were extracted from leaves collected during September and determined the following the method suggested by Hiscox and Israelstam (5). The experiment was conducted in randomised block design with four replications. Data for all the parameters were subjected to analysis using statistical analysis system software (SAS version 9.3).

RESULTS AND DISCUSSION

Rootstock influenced the growth behaviour of Kinnow significantly (P≤ 0.05). Irrespective of rootstock, the overall per cent increase in the growth parameters, *i.e.,* plant height, canopy spread and canopy volume was more between April-September as compared to the period between September-December (Table 1). The difference in plant height between April-September and September-December revealed that Kinnow trees on *Jatti khatti* was most vigorous thus exhibiting an increase of 49.42 and 20.83%, respectively, followed by rough lemon rootstock, which recorded an increase of 44.45 and 19.0% between the said period. Minimum increase in the plant height between April-September (12.31%) and September-December (2.76%) was recorded in trees on Troyer citrange. Similar to plant height, per cent increase in canopy volume was also significantly higher on *Jatti khatti* during both the periods, while it was minimum in trees on Troyer rootstock. The findings of the present study clearly suggest the differential response of the rootstock on the scion variety may be due to the inherent genetic character of the rootstocks. In the present study, *Jatti khatti* and rough lemon (both *Citrus jambhiri*) showed its superiority in terms of plant height including enhanced canopy volume of Kinnow on *Jatti khatti* rootstock over other rootstocks, thus indicating their well adapted nature to soil conditions with efficient root system that might have resulted in higher accumulation of nutrients. Reduced growth

Table 1. Seasonal increase in plant height, canopy spread and canopy volume of Kinnow mandarin budded on different rootstocks.

Rootstock	Plant height (%)		Canopy spread (%)				Canopy volume (%)	
	Rainy	Winter	Rainy (N-S)	Winter (N-S)	Rainy (E-W)	Winter (E-W)	Rainy (N-S)	Winter (E-W)
Rough lemon	44.45^{b}	19.00^{a}	51.07^{c}	9.52^{c}	47.10^{dc}	9.23^{d}	222.56^{c}	42.36^{d}
Karna khatta	16.26^{e}	5.80^{cb}	39.77^{d}	23.07^{a}	35.20^{e}	21.43^{b}	119.75^{f}	58.10^{b}
Carrizo citrange	23.49^{d}	19.14^{a}	54.35^{c}	16.36^{b}	51.23^{c}	16.86^{cb}	188.30^{d}	61.97^{b}
Rangpur lime	35.32^{c}	7.17^{b}	63.87^{b}	6.40^{c}	64.32^{b}	7.56^{d}	264.71^{b}	22.66^{e}
Troyer citrange	12.31^{e}	2.76^{c}	54.06^{c}	6.94^{c}	44.55^{d}	6.71^{d}	150.09^{e}	17.27^{f}
Jatti khatti	49.42^{a}	20.83^{a}	73.62^{a}	29.25^{a}	70.82^{a}	31.47^{a}	341.58^{a}	105.58^{a}
Sour orange	24.04^{d}	19.50^{a}	64.21^{b}	12.08^{cb}	60.19^{b}	12.22^{cd}	226.73^{c}	50.33^{c}
LSD (P ≤ 0.05)	4.06	3.06	4.84	6.23	5.27	6.83	7.14	4.61

attributes in Kinnow trees on Troyer rootstock may be due to the weak nutrient accumulating behaviour of rootstock and higher accumulation of phenol content in the scion leaf which might have imparted low vigour to the scion variety. Variation in growth parameters of Kinnow due to rootstocks have also been reported earlier by Josan and Thatai (6) and Goswami *et al.* (4).

Scion and rootstock diameter equilibrium is very important for the compatibility of rootstocks with the scion. Significant differences were recorded with respect to the cumulated percentage increase in scion and root diameter during rainy (July) and autumn (October) over the summer (April) and rainy season (July) growth (Table 2). Maximum increment in scion (88.37%) and root diameter (83.64%) was recorded in *Jatti khatti* followed by Kinnow trees on Rangpur lime rootstock (76.06 and 73.70%, respectively) during April-September. Minimum increase in the scion (23.48%) and root (21.83%) diameter was recorded in Kinnow trees on Carrizo rootstock, which did not differ significantly with the trees on rough lemon. Contrary to the minimum increase in the scion and rootstock diameter on these rootstocks during September, it was maximum during December with a higher increase in the scion (8.89%) and root diameter (8.71%) in trees on rough lemon, followed by Kinnow trees on Carrizo rootstock. Although, significant differences in the scion and rootstock diameter was observed during different growth periods, the relationship between the scion and rootstock diameter above and below the budding line did not reflect any sign of incompatibility. Plants budded on different rootstocks had mean values for this relationship closest to 1.0, which is an ideal indication of congenial relationship.

The rootstock influence resulted in quite significant difference in the leaf sclerophylly parameters of Kinnow (Table 3). Kinnow trees budded on *Jatti khatti* rootstock exhibited higher leaf area (166.24 cm^2) and leaf fresh mass (4.36 g) followed by leaf area (147.72 cm^2) and leaf fresh mass (4.01 g) on sour orange rootstock. The minimum leaf area (85.07 cm^2) and fresh mass (2.50 g) was measured in scion leaf of Kinnow on rough lemon. Leaf dry matter was higher in Kinnow leaves on *Jatti khatti* (1.85 g) rootstock, while it was minimum (0.92 g) on rough lemon and Troyer citrange (1.05 g). Highest SLA (0.013 cm^2/g) was measured in Kinnow leaves on *Karna khatta* rootstock, followed by rough lemon and sour orange (0.011 cm^2/g) but the difference was not significant. SLW was recorded maximum (104.31 g/ cm^2) and minimum (92.39 g/cm^2) in Kinnow leaf on Carrizo and rough lemon respectively. Comparative study with respect to density of foliar tissue revealed higher density of foliar tissues (DFT) in scion leaf of Kinnow on *Karna khatta* rootstock (433.70 g/kg^{-1}) followed by *Jatti khatti* and sour orange rootstocks with similar statistical values. Minimum DFT was recorded in scion leaf of Kinnow on rough lemon and Carrizo rootstocks without any significant difference. Although leaf area, leaf fresh mass and dry matter was minimum in Kinnow leaf on rough lemon rootstock, leaf succulency (0.018 mg H_2O/cm^{-2}) was significantly higher on the said rootstock followed by leaf succulency (0.017 mg H_2O/cm^{-2}) in leaf of Kinnow on *Karna khatta*.

In the present study, higher leaf area and fresh mass in scion leaf of Kinnow on *Jatti khatti* and sour orange rootstock may be attributed to higher leaf area, thus accumulating moisture in proportion to the leaf area and *vice-versa* resulting in lower fresh mass of Kinnow leaves on rough lemon rootstock. It is interesting to note that although the leaf area was lower in Kinnow leaves on rough lemon rootstock, leaf succulency was significantly higher in leaves of Kinnow on *Jatti khatti* and sour orange rootstocks. The

Table 2. Seasonal increase in scion diameter, root diameter and scion/ stock ratio of Kinnow mandarin budded on different rootstocks.

Rootstock	Scion dia. (%)		Root dia. (%)		Scion/stock ratio	
	Rainy	Winter	Rainy	Winter	Rainy	Winter
Rough lemon	24.52^{e}	8.89^{a}	20.49^{e}	8.71^{a}	1.17^{a}	1.02^{b}
Karna khatta	64.39^{c}	2.15^{dc}	62.17^{c}	2.17^{dc}	1.03^{a}	0.98^{b}
Carrizo citrange	23.48^{e}	5.28^{b}	21.83^{e}	5.26^{b}	1.08^{a}	1.00^{b}
Rangpur lime	76.06^{b}	1.54^{de}	73.70^{b}	1.57^{e}	1.03^{a}	0.98^{b}
Troyer citrange	47.33^{d}	2.47^{c}	45.28^{d}	2.55^{c}	1.04^{a}	0.97^{b}
Jatti khatti	88.37^{a}	1.97^{dce}	83.64^{a}	1.79^{de}	1.05^{a}	0.98^{b}
Sour orange	23.48^{e}	1.36^{e}	58.58^{c}	1.38^{e}	1.11^{a}	0.98^{b}
LSD ($P \leq 0.05$)	7.25	0.63	5.41	0.56	0.15	0.05

Table 3. Leaf Sclerophylly characteristics of Kinnow mandarin budded on different rootstocks.

Rootstock	Leaf area (cm^2)	Fresh mass (g)	Dry matter (g)	SLA (cm^2/g)	SLW (g/cm^2)	DFT (g/kg^{-1})	Succulency (mg H_2O/cm^2)
Rough lemon	85.07^{f}	2.50^{f}	0.92^{f}	0.011^{b}	92.39^{d}	367.28^{d}	0.018^{a}
Karna khatta	139.14^{c}	4.25^{a}	1.72^{b}	0.013^{a}	75.47^{e}	433.70^{a}	0.017^{b}
Carrizo citrange	143.02^{cb}	3.79^{c}	1.37^{d}	0.010^{c}	104.31^{a}	361.95^{d}	0.017^{cb}
Rangpur lime	122.55^{d}	3.38^{d}	1.29^{d}	0.010^{cb}	94.59^{cd}	383.72^{c}	0.016^{cb}
Troyer citrange	104.03^{e}	2.72^{e}	1.05^{e}	0.010^{c}	98.77^{b}	387.07^{c}	0.016^{c}
Jatti khatti	166.24^{a}	4.36^{a}	1.85^{a}	0.010^{c}	96.62^{cb}	394.49^{b}	0.016^{c}
Sour orange	147.72^{b}	4.01^{b}	1.60^{c}	0.011^{b}	92.28^{d}	398.69^{b}	0.016^{cb}
LSD (P ≤ 0.05)	6.24	0.17	0.079	0.006	3.58	5.76	0.001

observation suggests anatomical modification, which might have been imparted by the rootstock. Higher leaf succulence also suggests the maintenance of high relative water content (RWC). Scion leaf of Kinnow on *Karna khatta* had the highest density of foliar tissue, which might be due to the thick cuticle layer. It is possible that leaves with high tissue density are able to survive a severe drought because of higher resistance to physical damage by desiccation (Mediavilla *et al*., 8).

Rootstock impact on chlorophyll fraction of Kinnow leaves was found significant (Fig. 1). Irrespective of rootstocks, chlorophyll *a* concentration was higher than chlorophyll *b* in Kinnow leaves on different rootstocks. Leaves of shoots budded on to rough lemon rootstock had the highest chlorophyll *a* (1.60 mg g^{-1} FW), total chlorophyll (1.87 mg g^{-1} FW) and chlorophyll *a:b* ratio (6.20) with similar trend in Kinnow leaves budded on sour orange rootstock. Leaves of shoots budded on to Carrizo rootstock had the lowest chlorophyll concentration and as compared to the maximum value recorded on rough lemon rootstock chlorophyll *a*, total chlorophyll and chlorophyll *a:b* ratio was reduced by 43.12, 35.82 and 49.03 per cent, respectively. Higher amount of chlorophyll fraction *a*, total chlorophyll and chlorophyll *a*:*b* ratio in scion leaf of Kinnow on rough lemon and sour orange rootstock may be due to effective absorption of micronutrients like Fe, because Fe is an important cofactor of many

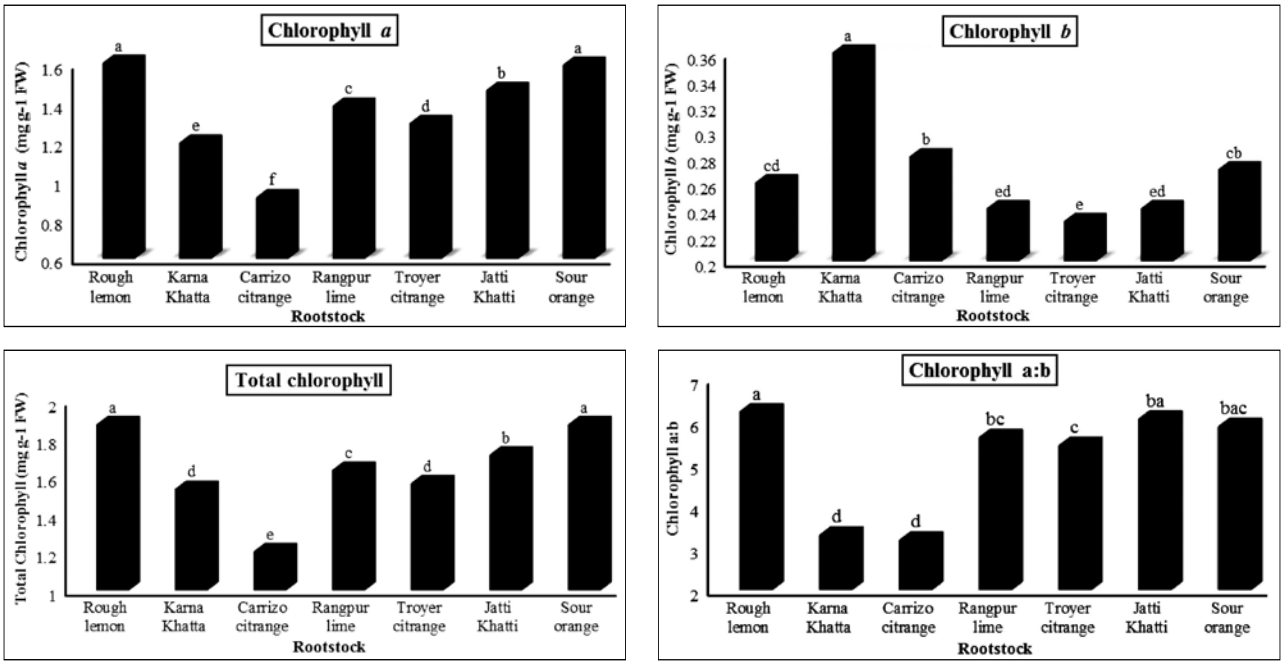


Fig. 1. Variation in chlorophyll fractions of Kinnow mandarin budded on different rootstocks.

enzymes, including those involved in the biosynthetic pathway of chlorophylls (Marschner, 7). The ability of sour orange rootstock to maintain high leaf Fe and chlorophyll concentrations have been documented previously (Sudahono *et al*., 10).

It can be concluded from the present study that overall Kinnow responded differently to the seven rootstocks tested. *Jatti khatti* was found vigorous and can be an ideal rootstock for both arid and semi-arid conditions. Succulent leaves of rough lemon and higher DFT in *Karna khatta* indicates their potential as a rootstock for drought prone areas. Carrizo proved to be an inferior rootstock in our conditions for most of the traits studied.

ACKNOWLEDGEMENTS

The senior author is grateful to the Indian Council of Agricultural Research, New Delhi for financial assistance in the form of Junior Research Fellowship and Head, Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi for the research facilities.

REFERENCES

1. Aviles, C.T., Filho, F.A.M., Stuchi, E.S., Silva, S.R. and Nunez, E.E. 2011. Horticultural performance of 'Folha Murcha' sweet orange onto twelve rootstocks. *Scientia Hort.* **129**: 259-65.

2. Awasthi, O.P., Dubey, A.K., Sharma, V.K., Jai Prakash., Goswami, A.K. and Sharma, R. 2015. Saline irrigation induced changes in growth, physiology and ionic relations in Kinnow budded on *Jatti khatti* and Sohsarkar. *Indian J. Hort.* **72**: 313-18.

3. Ennajeh, M., Vadel, A.M., Cochard, H. and Khemira, H. 2010. Comparative impacts of water stress on the leaf anatomy of a drought resistant and a drought sensitive olive cultivar. *J. Hort. Sci. Biotech.* **85**: 289-94.

4. Goswami, A.M., Sharma, R.R. and Saxena, S.K. 2001. High density planting in tropical and sub-tropical fruits. *Indian J. Hort.* **58**: 23-27.

5. Hiscox, J.D. and Israelstam, G.F. 1979. A method for extraction of chlorophyll from leaf tissue with maceration. *Canadian J. Bot.* **57**: 1332-34.

6. Josan, J.S. and Thatai, S.K. 2008. Studies on the evaluation of rootstocks for Kinnow mandarin under north Indian conditions. *Indian J. Hort.* **65**: 332-34.

7. Marschner, H. 1995. *Mineral Nutrition of Higher Plants* (2nd Edn.), London, Academic Press, 889 p.

8. Mediavilla, S., Escudero, A. and Heilmeier, H. 2001. Internal leaf anatomy and photosynthetic resource-use efficiency: interspecific and intraspecific comparisons. *Tree Physiol.* **21**: 251-59.

9. Sharma, J.N., Josan, J.S. and Thatai, S. 2002. Effect of rootstocks on tree health, yield and quality of Kinnow mandarin under arid-irrigated region of Punjab. *Indian J. Hort.* **59**: 373-77.

10. Sudahono, D.H.B., Byrne, D.H. and Rouse, R.E. 1994. Greenhouse screening of citrus rootstocks for tolerance to bicarbonate-induced iron chlorosis. *Hort. Sci.* **29**: 113-16.

11. Zekri, M. and Parsons, L.R. 1992. Salinity tolerance of citrus rootstocks. Effect of salt on root and leaf mineral concentrations. *Plant Soil*, **147**: 171-81.

Received : July, 2016; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00099.8

Indian J. Hort. 74(4), December 2017: 510-514

Effect of mulching and irrigation interval on fruit quality and yield of litchi cv. Dehradun

Ambika Bhandari, Arti Sharma*, V.K. Wali and Darpreet Kour

Division of Fruit Science, Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu 181202, Jammu & Kashmir

ABSTRACT

A study was conducted at the Research Farm, Division of Fruit Science, FOA Udheywalla, SKUAST, Jammu on 20-year-old trees of litchi cv. Dehradun. Trees were subjected to mulching with different types of materials (black polythene and paddy straw) and supplied with controlled irrigations @ 200 l/ tree at 3, 6 and 9 day intervals. Results revealed that the trees supplied with irrigation at 6 day interval and mulched with black polythene (T_8) showed least fruit cracking (10.15%) and maximum fruit yield (59.33 kg/tree). Fruit weight (18.27 g), fruit length (3.28 cm), fruit diameter (2.92 cm), pulp weight (10.45 g) and fruit firmness (2.51 kg/cm^2) were also found to be maximum in trees mulched with black polythene and irrigated at 6 day interval with highest benefit: cost ratio (2.77:1).

Key words: Litchi, mulching, irrigation, fruit cracking, yield.

INTRODUCTION

Litchi (*Litchi chinensis* Sonn.) is one of the important sub-tropical evergreen fruit tree and belongs to family Sapindaceae. Litchi is highly sensitive to water deficit, which aggravates the fruit cracking and shortens the post-harvest life. Further complexities in optimizing irrigation emerge due to different cultivars, plant sizes, and fruit developmental stages (Khurshid *et al*., 6). Several attempts have been made to standardize nutrient and water requirement of litchi tree in India. Irrigation intervals affect physico-chemical quality attributes and fruit cracking in litchi. Dehradun is an early variety of litchi, which matures in the 2nd week of June. Despite of its good qualities the litchi is severely affected by cracking, which drastically reduces the yield. General practice adopted by litchi growers is to avoid fruit cracking is by over irrigation. Due to climate change and dwindling water resources Indian Agriculture is suffering from water scarcity, thus the practice of over and excessive irrigation must be discouraged and irrigation requirements of individual fruit crops need to be optimized specially for the water sensitive crops like litchi. Thus, the present investigation was undertaken to study the effect of controlled irrigation at fixed intervals combined with an established practice of water conservation, *i.e.* mulching on yield and fruit quality of litchi.

MATERIALS AND METHODS

An experiment was conducted on 20-year-old litchi trees of cv. Dehradun. Trees of uniform vigour and size, maintained under uniform cultural practices growing at the Research Farm, Division of Fruit Science, FoA Udheywalla, SKUAST-Jammu during 2014-15 were selected for the study. The experimental site is situated at 32.73°N latitude and 74.87°E longitude at an elevation of 327 m from msl. Annual precipitation is about 1200 mm and about 70 percent of the rains are received during July to October. The annual mean maximum and minimum temperatures were 29.6° and 16.7°C, respectively. Summer months are hot with temperature and relative humidity ranging from 23.5° to 35.5°C and 53.0 to 73.5 per cent, respectively. The winter months experience mild to severe cold with average temperature ranging from 6.5° to 21.7°C. December is the coldest month, when minimum temperature reaches to 4.0°C. The highest temperature is recorded in the month of June (45.0°C). The daily maximum and minimum temperature and evaporation rate rise from March onwards. The soil type was sandy clay loam with pH 6.7, electrical conductivity 0.20 (dS m^{-1}) and 0.51% organic carbon. The soil had available nitrogen 225.5 (kg ha^{-1}), phosphorus 13.84 (kg ha^{-1}) and potassium 138 (kg ha^{-1}).

The treatment imposed were, T_1 = Irrigation at 3 day interval (control), T_2 = Irrigation at 6 day interval, T_3 = Irrigation at 9 day interval, T_4 = Paddy straw mulch + irrigation at 3 day interval , T_5 = Paddy straw mulch + irrigation at 6 day interval, T_6 = Paddy straw mulch + irrigation at 9 day interval , T_7 = Black polythene mulch + irrigation at 3 day interval, T_8 = Black polythene mulch + irrigation at 6 day interval, T_9 = Black polythene mulch + irrigation at 9 day

*Corresponding author's E-mail: drartisharma02@gmail.com

interval. Irrigation at 3 day interval was taken as control because it is the farmers practice in the region and is recommended by SKUAST-J. Total number of treatments taken was 9 and each treatment was replicated thrice. Selected trees were subjected to mulching with two types of materials (black polythene and paddy straw) and controlled irrigation @ 200 l/ tree at 3, 6 and 9 intervals. Regulated irrigations were applied from the fruit set till harvesting by applying measured water @ 200 l/ tree/ irrigation in the basin (rainfall was not taken into account).

Fruit set (%) and fruit cracking (%) were recorded on the selected tagged branches. Eight branches in each tree were randomly selected and tagged for recording data. Observations were recorded on fruit weight (g), fruit length (cm), fruit diameter (cm), pulp weight (g) and fruit firmness (kg/ cm^2). Fruit weight was measured by electronic balance and expressed in g. Fruit length (cm) and fruit diameter (cm) were measured using Vernier calipers. Pulp weight was obtained by subtracting the seed weight + peel weight from the total fruit weight and was expressed in g. The fruit firmness (kg/ cm^2) was determined by a pressure tester (penetrometer). Yield was recorded as yield per tree and expressed in kg/ tree. The economics of using different mulching materials and irrigation intervals in litchi orchard of cv. Dehradun have been worked out by calculating net returns for each treatment. Data was analysed by the method given by Panse and Sukhatme (7) by using two factor randomized block design.

RESULTS AND DISCUSSION

A significant response of application of mulch along with irrigation at 6 day interval on fruit cracking was observed over other treatments. Results revealed that on overall basis trees irrigated at 6 day interval and mulched with black polythene mulch (T_8) showed least fruit cracking (10.15%) and had the highest yield (59.33 kg /plant) compared to control. Whereas, T_9 (black polythene mulch + irrigation at 9 day interval) showed highest fruit cracking (22.53%), and lowest yield (42.60 kg /plant). Increased fruit yield in T_8 (6 day irrigation interval + black polythene mulching) could be attributed to induction of mild water stress due to increase in irrigation interval from 3 to 6 day and further significant decrease in fruit yield when irrigation interval was further increased to 9 day must be because of severe water stress. On mean value basis, irrigation at 3 day interval showed the minimum fruit cracking (13.26%) and was at par with irrigation at 6 day interval and black polythene mulch in terms of fruit yield (54.89%) (Table 1). On mean value basis, black polythene mulch was significantly effective in controlling fruit cracking (14.35%) and gave the maximum yield (53.48 kg/ plant). All the interactions were also found to be significant. These findings are supported by the work of Southwick (13) who stated the relationship between stress severity and the flowering response, however, when water stress is severe, flower disorders induce a heavy drop. Alaoui *et al*. (1) studied the impact assessment of deficit irrigation on yield and fruit quality in peach reported that higher yield and good quality can be obtained with less irrigation water and adequate frequency. A significant reduction of fruit cracking was recorded, as mulching and mild water stress are significantly effective in reducing fruit cracking in litchi. These results are in conformity with the findings of Joshi *et al*. (5) who reported that mulching helps to reduce the fluctuation in soil moisture in the cv. Rose Scented. Sandhu and Bal (12) worked on the effect of mulching and irrigation treatments on fruit cracking in Baramasi lemon and reported that use of black polythene mulch and irrigation intervals change the microclimate of the trees. Bal and Singh (4) working on the effect of

Table 1. Effect of mulching and irrigation interval on fruit cracking and yield in litchi cv. Dehradun.

Irrigation interval	Fruit cracking (%)				Fruit yield (kg/ tree)			
	No mulch	Paddy straw	Black polythene	Mean	No mulch	Paddy straw	Black polythene	Mean
3 day (control)	15.05	14.38	10.36	13.26	52.34	53.82	58.51	54.89
6 day	18.29	13.29	10.15	13.91	50.91	54.34	59.33	54.86
9 day	21.30	19.05	22.53	20.96	44.64	49.67	42.60	45.57
Mean	18.21	15.57	14.35		49.29	52.61	53.48	
CD at 5%								
Factor (A)		0.25				0.44		
Factor (B)		0.25				0.44		
Factor (A × B)		0.44				0.76		

mulching material on yield of *ber* reported that plants under black polythene mulch gave the maximum yield because of increased availability of soil moisture and control of weed growth. Similarly, Bakshi *et al*. (3) evaluated the effect of mulching material on yield of strawberry and reported that maximum yield per plant was under black polythene because of larger fruit owing to better hydrothermal regime of soil and complete weed-free environment. Various physical characteristics, *viz.*, fruit weight, fruit length, fruit diameter, pulp weight and fruit firmness significantly decreased when irrigation interval was increased without mulching. Reza *et al.* (11) while studying the effect of irrigation on fruit weight of litchi also reported that among the three irrigation treatments, *viz.*, No irrigation (I_0), one irrigation (I_1) and two irrigations (I_2), the maximum fruit weight was observed in I_2 followed by I_1 and I_0 days. However, when irrigation intervals were coupled with mulching all the physical characteristics recorded increase up to 6 day irrigation interval.

On overall basis, maximum fruit weight (18.27 g), fruit length (3.28 cm), fruit diameter (2.92 cm), pulp weight (10.45 g) and fruit firmness (2.51 kg/ cm^2) were observed in trees mulched with black polythene and irrigated at 6 day interval as compared to control. Whereas, minimum fruit weight (13.88 g), fruit length (2.60 cm), fruit diameter (2.38 cm), pulp weight (8.58 g) and fruit firmness (1.07 kg/cm^2) was found to be in treatment T_9 (black polythene mulch + irrigation at 9 day interval) (Tables 2 & 3). On mean value basis, irrigation at 3 and 6 day intervals were at *par* with each other in terms of fruit weight (17.33 and 17.44 g, respectively), fruit length (3.12 and 3.10 cm, respectively), fruit diameter (2.80 and 2.78 cm, respectively), pulp weight (9.91 and 9.96 g, respectively), fruit firmness (2.31 and 2.35 kg/cm^2, respectively) and specific gravity (1.060 and 1.064, respectively). On mean basis, all the mulching treatments were at par with each other in respect of fruit weight, fruit length, fruit diameter, pulp weight and specific gravity. The increase in

Table 2. Effect of mulching and irrigation interval on fruit characteristics of litchi cv. Dehradun.

Irrigation interval	Fruit wt. (g)				Fruit length (cm)				Fruit dia. (cm)			
	No mulch	Paddy straw	Black polythene	Mean	No mulch	Paddy straw	Black polythene	Mean	No mulch	Paddy straw	Black polythene	Mean
3 day (control)	16.78	17.33	17.88	17.33	3.40	3.08	3.20	3.12	2.72	2.81	2.87	2.80
6 day	16.42	17.63	18.27	17.44	2.92	3.12	3.28	3.10	2.61	2.83	2.92	2.78
9 day	15.51	14.53	13.88	14.64	2.85	2.72	2.60	2.72	2.54	2.42	2.38	2.44
Mean	16.24	16.49	16.67		3.05	2.97	3.02		2.61	2.68	2.70	
CD at 5%												
Factor (A)			0.19				0.15				0.12	
Factor (B)			0.19				0.16				0.15	
Factor (A × B)			0.34				0.25				0.20	

Table 3. Effect of mulching and irrigation intervals on pulp weight (g), fruit firmness (kg/ cm^2) and specific gravity of litchi cv. Dehradun.

Irrigation interval	Pulp wt. (g)				Fruit firmness (kg/ cm^2)				Specific gravity			
	No mulch	Paddy straw	Black polythene	Mean	No mulch	Paddy straw	Black polythene	Mean	No mulch	Paddy straw	Black polythene	Mean
3 day (control)	9.71	9.89	10.15	9.91	2.32	2.25	2.38	2.31	1.064	1.064	1.053	1.060
6 day	9.39	10.04	10.45	9.96	2.20	2.33	2.51	2.35	1.065	1.063	1.062	1.064
9 day	9.28	8.56	8.58	8.80	2.17	1.13	1.07	1.46	1.066	1.071	1.073	1.070
Mean	9.46	9.49	9.72		2.23	1.90	1.99		1.065	1.06	1.06	
CD at 5%												
Factor (A)			0.12				0.11				0.002	
Factor (B)			0.28				0.11				0.002	
Factor (A × B)			0.49				0.20				0.005	

fruit weight under black polythene mulch could be due to better soil moisture conservation and better soil temperature. According to Baba *et al*. (2), fruit weight was found significantly higher from plants mulched with black polythene. Further decrease in all these physical characteristics when irrigation interval was increased to 9 day can be explained in the light of fact that even mulching cannot overcome the negative effect of severe water stress induced by irrigation at 9 day interval. Earlier Rab and Haq (9) have also reported that specific gravity increased with increasing irrigation intervals. Benefit: cost ratio was found maximum in the treatment comprising of black polythene mulch and irrigation at 6 day interval (2.77:1) and minimum (1.01:1) in irrigation at 3 day interval. This may be attributed to higher yields and superior quality of fruits with different mulching treatments. These results are in confirmation with the results obtained by Prakash *et al*. (8) on litchi (Tables 4 & 5). With regard to increase in irrigation interval from 3 to 6 day resulted into high B:C ratio but when irrigation interval was further increased to 9 day a significant decrease in B:C was recorded. Reddy *et al*. (10) studied the effect of different types of irrigation and growing methods on growth, yield and water-use efficiency of tomato and recorded highest net returns (1,02,708 Rs./ha) and benefit cost ratio (2.41:1) recorded with furrow + black polythene mulch + trellising.

It was concluded that to get more income and better quality fruits in a litchi orchard, water management conditions must be improved by applying irrigations at 6 day interval and mulching trees with black polythene. This practice will result into increased on-farm crop water utilization and better fruit quality with less irrigation thereby conserving water.

Table 4. Average cost of cultivation (Rs.) of litchi cv. Dehradun using different mulching materials and irrigation intervals.

S. No.	Item	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
A.	Cost of inputs									
a)	Cost of FYM (₹)	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00	90.00
b)	Cost of Urea (₹)	24.57	24.57	24.57	24.57	24.57	24.57	24.57	24.57	24.57
c)	Cost of DAP (₹)	68.10	68.10	68.10	68.10	68.10	68.10	68.10	68.10	68.10
d)	Cost of MOP (₹)	17.07	17.07	17.07	17.07	17.07	17.07	17.07	17.07	17.07
e)	Cost of mulching material (₹)	-	-	-	220.00	220.00	220.00	300.00	300.00	300.00
	Total cost of inputs (A)	199.74	199.74	199.74	419.74	419.74	419.74	499.74	499.74	499.74
B.	Operational cost									
a)	Cost of basin preparation	82.41	82.41	82.41	82.41	82.41	82.41	82.41	82.41	82.41
b)	Cost of irrigation (₹)	2472.40	1236.20	741.72	2472.40	1236.40	741.72	2472.40	1236.20	741.72
c)	Tagging	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
d)	Weeding	329.65	329.65	329.65	164.82	164.82	164.82	-	-	-
e)	Fertilizer application	41.47	41.47	41.47	41.47	41.47	41.47	41.47	41.47	41.47
f)	Mulch application	-	-	-	41.47	41.47	41.47	41.47	41.47	41.47
g)	Harvesting	123.62	123.62	123.62	123.62	123.62	123.62	123.62	123.62	123.62
	Total operational cost (B)	3079.55	1843.35	1348.87	2956.19	1720.19	1225.51	2791.37	1555.17	1060.69
C.	Total cost (₹) C = (A + B)	3279.29	2043.09	1548.61	3375.93	2139.73	1645.25	3291.11	2054.91	1560.43
D.	Total cost/plant (₹)	1093.09	681.03	516.20	1125.31	713.24	548.41	1097.03	708.99	520.14
E.	Total cost/ha (₹)	109309.67	68103.00	51620.33	112531.00	71324.33	54841.67	109703.67	70899.00	52014.33

Table 5. Effect of mulching and irrigation interval on B: C ratio of litchi cv. Dehradun.

Treatment	Av. yield (kg/ tree)	Rate/ kg fruit (₹)	Gross return (₹)	Gross return/ ha (₹)	Cost of cultivation (B) (₹)	Net return C = (A-B) (₹)	Benefit: cost ratio
T_1 : Irrigation at 3 day interval (control)	52.34	42.00	2198.8	219828.00	109309.67	110518.33	1.01:1
T_2 : Irrigation at 6 day interval	50.91	43.00	2189.13	218913.00	68103.00	150810.00	2.21:1
T_3 : Irrigation at 9 day interval	44.64	35.00	1562.40	156240.00	51620.33	104619.67	2.03:1
T_4 : Paddy straw + Irrigation at 3 day interval	53.82	45.00	2421.90	242190.00	112531.00	129659.00	1.15:1
T_5 : Paddy straw + Irrigation at 6 day interval	54.34	43.00	2336.62	233662.00	71324.33	162337.67	2.28:1
T_6 : Paddy straw + Irrigation at 9 day interval	49.67	35.00	1738.45	173845.00	54841.67	119003.33	2.17:1
T_7 : Black Polythene + Irrigation at 3 day interval	58.51	45.00	2632.95	263295.00	109703.67	153591.33	1.40:1
T_8 : Black Polythene + Irrigation at 6 day interval	59.33	45.00	2669.85	266985.00	70899.00	196086.00	2.77:1
T_9 : Black Polythene + Irrigation at 9 day interval	42.60	35.00	1491.00	149100.00	52014.33	97085.67	1.87:1

REFERENCES

1. Alaoui, S.M., Abouatallah, A., Salghi, R., Amahmid, Z., Bettouche, J., Zarrouk, A. and Hammouti, B. 2013. Impact assessment of deficit irrigation on yield and fruit quality in peach orchard. *Der Pharma Chemica*, **5**: 236-43.
2. Baba, J., Kher, R., Bakshi, P. and Wali, V.K. 2010. Effect of planting time and mulching material on quality of strawberry. *J. Res. SKUAST-J*, **9**: 54-62.
3. Bakshi, P., Bhat, D.J., Wali, V.K., Sharma, A. and Iqbal, M. 2014. Growth, yield and quality of strawberry (*Fragaria* × *ananassa* Duch.) cv. Chandler as influenced by various mulching materials. *African J. Agric. Res.* **9**: 701-06.
4. Bal, J.S. and Singh, S. 2011. Effect of mulching material and herbicides on tree growth, yield and fruit quality of *ber*. *Indian J. Hort.* **68:**189-92.
5. Joshi, G., Singh, P.K., Srivastava, P.C. and Singh, S.K. 2012. Effect of mulching, drip irrigation scheduling and fertilizer levels on plant growth, fruit yield and quality of litchi (*Litchi chinensis* Sonn.). *Indian J. Soil Conserv.* **40**: 46-51.
6. Khurshid, S., Ahmad, I. and Anjum, M.A. 2004. Genetic diversity in different morphological characteristics of litchi (*Litchi chinensis* Sonn.*). Inter. J. Agri. Biol.* **6**: 1062-65.
7. Panse, V.G. and Sukhatme, P.V. 2000. *Statistical Methods for Agricultural Workers*, Publication and Information Division of ICAR, New Delhi.
8. Prakash, J., Singh, N.P. and Sankaran, M. 2007. Response of mulching on *in situ* soil moisture, growth, yield and economic return of litchi (*Litchi chinensis*) under rainfed condition in Tripura. *Indian J. Agri. Sci.* **77**: 762-64.
9. Rab, A. and Haq, I.U. 2011. Irrigation intervals affect physico-chemical quality attributes and skin cracking in litchi fruit. *Turkey J. Agri.* **36**: 553-62.
10. Reddy, S.G.V., Patil, D.V., Rao, B.S. and Nagendraprasad, B. 2015. Effect of different type of irrigation and growing methods on growth, yield and water use efficiency of tomato. *Bioscan*, **10**: 243-46.
11. Reza, M.M., Bhaduran, M.M., Hasan, M.A., Bari, M.S. and Hafiz, M.H.R. 200. Effects of irrigation and fertilization on yield and quality of litchi (*Litchi chinensis* Sonn.). *J. Sci. Tech.* **5**: 52-59.
12. Sandhu, S. and Bal, J.S. 2013. Quality improvement in lemon (*Citrus limon* (L.) Burm.) through integrated management of fruit cracking. *African J. Agri. Res.* **8**: 3552-57.
13. Southwick, S.M. and Davenport, T.L. 1986. Characterization of water stress and low temperature effect on flower induction in citrus. *Plant Physiol.* **81**: 26-29.

Received : March, 2016; Revised : July, 2017;
Accepted : September, 2017

DOI : 10.5958/0974-0112.2017.00100.1

Indian J. Hort. 74(4), December 2017: 515-519

Response of kiwifruit cultivars to deficit irrigation in terms of canopy temperature and water relations

Preet Pratima* and N. Sharma**

Department of Fruit Science, Dr Y.S. Parmar University of Horticulture & Forestry, Nauni, Solan 173 230, Himachal Pradesh

ABSTRACT

The present investigation was conducted with the comparative study of response of five kiwifruit cultivars, *viz*., Allison, Hayward, Abbott, Monty and Bruno to deficit irrigation. This experiment was carried out during 2011 and 2012 at Nauni, Solan (HP). These five cultivars were subjected to two irrigation levels, *i.e.*, standard irrigation (at 80% Field capacity) and deficit irrigation (at 60% Field capacity). The canopy temperature and stomatal resistance in leaves of kiwifruit cultivars were increased due to deficit irrigation treatment, whereas, the leaf water potential, chlorophyll content, transpiration rate and photosynthetic rate were decreased. The Hayward cultivar was proved to be more sensitive to deficit irrigation levels in terms of canopy temperature, leaf water potential, chlorophyll content, stomatal resistance, transpiration and photosynthetic rates, whereas the cultivar Bruno was proved to be least sensitive.

Key words: Kiwifruit, irrigation, water relation, canopy temperature, chlorophyll, photosynthetic rate.

INTRODUCTION

The kiwifruit (*Actinidia deliciosa*) is a large, woody, deciduous vine native to the Yangtze valley of south and central China. Kiwifruit bears pistillate and staminate flowers separately and requires 700-800 chilling hours below 7°C and mild summer with temperature not exceeding 35°C. Kiwifruit has an excellent table and keeping quality and acclaimed for its nutritive and medicinal values. Approximately, 84 per cent of kiwifruit production is contributed by China, Italy, New Zealand and Chile. In India, the area under this fruit is negligible, however, it can be successfully adapted in areas situated at elevation of 900-1800 m above mean sea level, where the winters are cold and summers are warm and humid and receive well distributed annual rainfall of about 150 cm. A deep friable well drained sandy loam to clay soil coupled with assured irrigation is one of the ideal conditions for growing kiwifruit. The water requirement of kiwifruit plants is high due to their vigorous vegetative growth, larger leaf size, vine habit and high humidity in their natural habitat. Kiwifruit vines are prone to water stress mainly because of their very large leaves and very high rate of water conductivity and transpiration rate. Kiwifruit vines probably die more often from, some type of water stress than any other problems. In Himachal Pradesh, however, kiwifruit cultivation has extended to those areas, where demand for water exceeds that of local resources. The problem of water limitation may prove to be a more critical constraint to temperate fruit productivity in future due to global environmental change. However, some plants may adapt to changing environment more easily than others.

Plant responses to water scarcity are complex, involving adaptive changes and/ or deleterious effects. Plant strategies to cope with drought normally involve a mixture of stress avoidance and tolerance 'strategies' that vary with genotype. In kiwifruit, the potential of cultivar(s) to adapt under water scarcity conditions is not much known therefore, to know the adaptation of cultivars for water stress, this study was undertaken.

MATERIALS AND METHODS

The experiment was conducted at the Department of Fruit Science, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) during 2011 and 2012. The experimental area falls under sub-temperate sub-humid climate. The summers are moderately hot during May-June and winters are severe during Dec-Jan. Twenty five-year-old uniform vines of five different kiwifruit cultivars, *viz.*, Allison, Hayward, Abbott, Monty and Bruno were selected for experiment. These vines were planted at 6 × 4 m and trained on T-bar system. Irrigation was given at two different levels of Field Capacity (FC), *i.e.* irrigation at 80 per cent FC (standard irrigation) and irrigation at 60 per cent FC (deficit irrigation). These treatments were applied from March to October with four replications and each replication with one kiwifruit vine. The experiment was laid in randomized block design (RBD).

*Corresponding author's E-mail: preetepitome@gmail.com

The average canopy temperature of kiwifruit vines under different irrigation treatments, recorded with Infra red thermometer from all the four sides of vine canopy from a distance of five metre from the month of May to August during 2011 and 2012. The temperature was recorded at 12:00 noon from the month of May to Aug. at weekly intervals. The average values were expressed in degree Celsius. Leaf water potential was observed in portable 'Plant Water Status Console' during May and June. Water potential readings were recorded between 10:00 am to 12:00 noon by placing freshly detached leaf in pressure chamber. The chlorophyll content was recorded by taking ten fully grown leaves in morning hours from the current season's growth of each vine during first week of August. The leaves were collected and immediately placed in ice box and then brought to the laboratory. The samples were then kept in the refrigerator below 0°C to avoid degradation of chlorophyll pigments. Chlorophyll was estimated as per method of Hiscox and Israelstam (6). Stomatal resistance, transpiration and photosynthetic rate were recorded when soil moisture content under the respective treatments reached the required tension (*i.e.* 80% FC and 60% FC). Ten mature leaves from each experimental vine were selected randomly from all over the tree periphery. The observations during active growth periods between 9:00 to 11:00 A M with the help of Li-COR 6200 portable photosynthesis system. The results were expressed in S cm^{-1}, m $mol/m^2/s$ and $\mu mol/m^2/s$ for stomatal, resistance, transpiration and photosynthetic rates, respectively.

RESULTS AND DISCUSSION

The canopy temperature increased with the deficit irrigation treatment during both the years of study. During the year 2011-12 (pooled), the average canopy temperature of different cultivars significantly increased from 27.6°C under irrigation at 80 per cent of field capacity to 29.4°C under irrigation at 60 per cent of field capacity (Table 1). The cultivars also showed significant variations in canopy temperature under two different water regimes during both the years of study. The canopy temperature was observed significantly lowest in cultivar Bruno (26.9°C) followed by Allison (27.6°C). The data depicted in Fig. 1 showed that, the per cent increase in canopy temperature due to deficit irrigation was highest in cultivar Hayward (8.63%), and lowest in cultivar Bruno (5.58%) followed by Allison (5.80%). Canopy temperature may be affected by genetic makeup of the species or variety, transpiration rate and internal water regime. Water stress caused a decrease in transpiration, an increase in foliage temperature and closure of stomata (Tan and Buttery, 14). Lakso (8) suggested that the large round leaves of kiwifruit do not exchange heat efficiently with the bulk air unless there is significant air movement and are likely to have significant temperature increases under reduced stomatal conductance (g_s). The relationships between canopy temperature, air temperature and transpiration is not simple, involving atmospheric conditions (vapour pressure deficit, air temperature and wind velocity), soil (mainly available soil moisture) and plant (canopy size, canopy architecture and leaf adjustment to water deficit). However, relatively lower canopy temperature in cultivars Allison, Abbott and Bruno under water stress condition may indicate relatively better capacity for taking up soil moisture and for maintaining a relatively better plant water status by various plant adaptive traits (Blum, 1).

Table 1. Effect of different irrigation levels on canopy temperature, leaf water potential and chlorophyll content in leaves of kiwifruit cultivars.

Cultivar	Pooled (2011-12)					
	Canopy Temperature (°C)		Leaf water potential (-bars)		Chlorophyll content (mg/ FW)	
	SI	DI	SI	DI	SI	DI
Allison	27.6	29.2	8.69	8.85	3.13	2.59
Hayward	27.8	30.2	10.47	12.40	3.07	2.11
Abbott	27.7	29.6	7.85	8.13	3.08	2.15
Monty	27.9	29.7	9.14	9.33	3.09	2.43
Bruno	26.9	28.4	9.58	9.75	3.05	2.58
Mean	27.6	29.4	9.14	9.69	3.08	2.37
$CD_{0.05}$						
I	0.1		0.05		0.02	
C	0.1		0.08		0.03	
I × C	0.2		0.11		0.05	

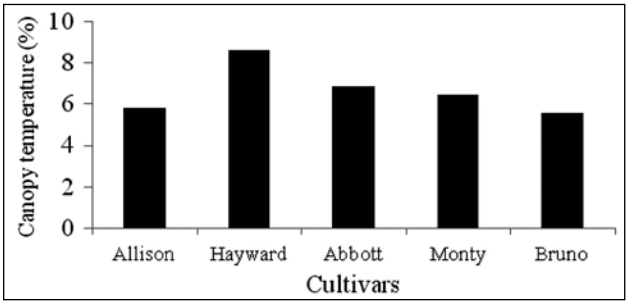


Fig. 1. Per cent increase in canopy temperature of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent FC.

The leaf water potential decreased significantly when kiwifruit vines were subjected to water deficit condition. The leaf water potential was found to be the least negative in cv. Abbott and more negative in cv. Hayward under well irrigated conditions (Table 1). The per cent reduction in the leaf water potential by deficit irrigation treatment (Fig. 2) was more pronounced in cultivar Hayward (15.61% reduction), while the reduction in leaf water potential by applying lesser than normal irrigation was the least in cultivar Bruno (1.75%). The present findings that water stress lead to decline in leaf water potential are in accordance with those of Satisha *et al.* (13) in grape rootstocks. In 'Bruno' and 'Allison' kiwifruit vines, the lesser decline in water potential and consequently maintenance of higher internal water status under deficit irrigation indicated that these cultivars were more capable of performing better at the advent of water stress (Thakur, 15). These findings clearly demonstrated that the leaf water potential may be used as a strong indicator of drought tolerance because of its high sensitivity to irrigation regimes.

The leaf chlorophyll content has been suggested important for leaf colour development as well as for better performance of leaf under drought stress condition (Sanchez, 12). The chlorophyll content in leaves of kiwifruit vines varied significantly among the different cultivars under well irrigated conditions (Table 1). The reduction in chlorophyll content is a typical symptom of oxidative stress and may be the result of chlorophyll degradation or due to deficiency in chlorophyll synthesis together with changes of thylakoid membrane structure. The higher reduction in leaf chlorophyll content of cultivar Hayward under deficit irrigation (Fig. 3), reflect its sensitivity to drought stress (Kadam *et al.*, 7; Romero *et al.*, 11; Miraghaee *et al.,* 10).

The stomatal resistance and transpiration rate were noted higher in cultivars Allison and Hayward, respectively, whereas Bruno recorded lowest stomatal resistance and transpiration rate,

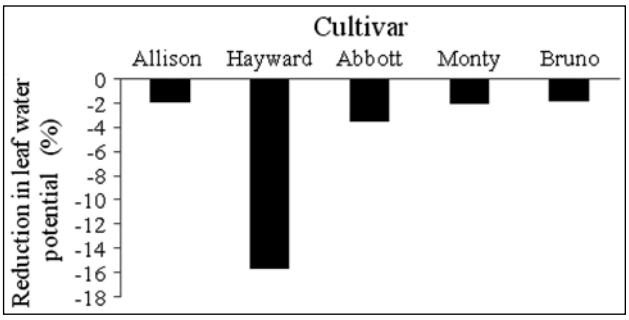


Fig. 2. Per cent reduction in leaf water potential of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent FC.

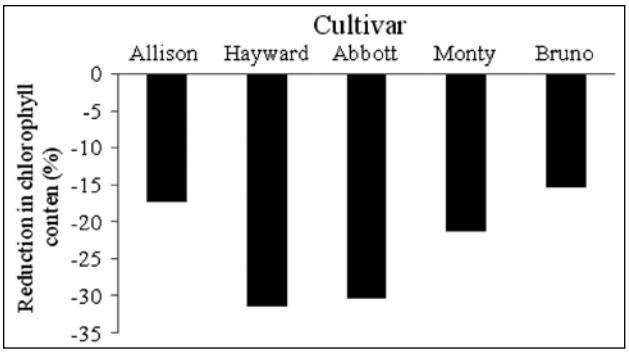


Fig. 3. Per cent reduction in chlorophyll content of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent FC.

in well irrigated vines (Table 2). The deficit irrigation however, increased stomatal resistance, and decreased the transpiration rate. It was observed that the extent of increase in stomatal resistance under water stress was highest in cultivar Bruno and lowest in 'Hayward' (Fig. 4). Conversely, the per cent decrease in the transpiration due to water stress was lowest in 'Hayward' and highest in 'Bruno' cultivar (Fig. 5). In general, the vine of Allison recorded lower transpiration rates at higher value of stomatal resistance. Leaf stomatal resistance and transpiration varied with genotype (Escalona *et al.*, 4), which may also be related with differences in anatomical characters of leaves and water conducting tissues. In this study, higher increase in stomatal resistance and decrease in transpiration rate in

Table 2. Effect of different irrigation levels on stomatal resistance, transpiration and photosynthetic rates in leaves of kiwifruit cultivars.

Cultivar	Pooled (2011-12)					
	Stomatal resistance (S cm^{-1})		Transpiration rate (mmol $m^{-2}s^{-1}$)		Photosynthetic rate (µmol $m^{-2}s^{-1}$)	
	SI	DI	SI	DI	SI	DI
Allison	4.25	4.38	9.80	8.20	19.08	18.59
Hayward	4.01	4.06	11.1	10.8	16.01	15.30
Abbott	4.05	4.11	10.4	9.70	16.18	15.56
Monty	4.11	4.19	10.3	9.10	18.04	17.52
Bruno	3.87	4.12	6.40	4.40	19.80	19.56
Mean	4.06	4.17	9.60	8.50	17.82	17.31
$CD_{0.05}$						
I	0.01		0.1		0.02	
C	0.01		0.1		0.03	
I × C	0.02		0.1		0.04	

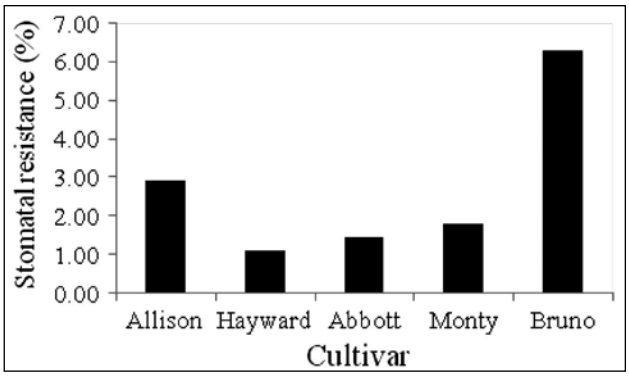


Fig. 4. Per cent increase in stomatal resistance of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent FC.

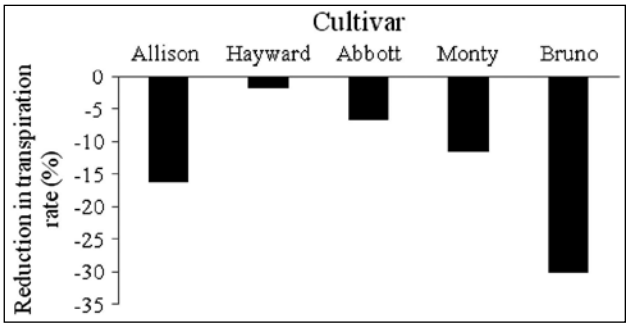


Fig. 5. Per cent reduction in transpiration rate of exposed leaves of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent FC.

cultivar Bruno under water stress can be attributed to higher decrease in stomatal width and xylem vessel development in this cultivar. Buwalda and Smith (2) observed that kiwifruit cultivar Hayward had higher transpiration rate with poor stomatal control. Ghaderi *et al*. (5) also observed that the drought tolerant grape cultivar maintained lower transpiration rate under water deficit conditions. During the course of study, the photosynthetic rate was significantly reduced by deficit irrigation (Table 2), however, the cultivars Hayward and Abbott registered higher per cent decrease, while cultivars Bruno and Allison recorded lower decrease in these attributes (Fig. 6). The reduction in photosynthetic rate in response to water stress may be due to reduction in diffusion of CO_2 to the chloroplast, both by stomatal closure and changes in mesophyll structure, which decreases the conductance to CO_2 diffusion within the leaf as suggested by Ennajeh *et al*. (3).

The vines of cultivars Bruno and Allison under water stress exhibited higher rate of photosynthetic activities than other cultivars probably because of higher relative water content and more efficient in terms of long-distance water transport and smaller

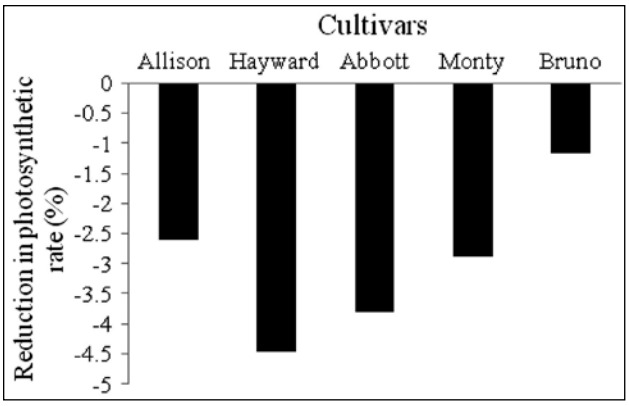


Fig. 6. Per cent reduction in photosynthetic rate of exposed leaves of different kiwifruit cultivars at irrigation at 60 per cent FC over 80 per cent.

diurnal variation in leaf water potential. Therefore, cultivars Bruno and Allison can be rated as water stress tolerant based on maintaining better photosynthetic rate in under deficit irrigation (Ennajeh *et al*., 3). Liu *et al*. (9) also considered the rootstocks, *viz*., *Malus. sieversii*, *M. prunifolia* and *M. toringoides* of apple cv. Gale Gala as more drought tolerant due to smaller decline in relative water content, chlorophyll content and photosynthetic rate.

On the basis of these results, it may be concluded that in response to deficit irrigation the cultivar Bruno exhibited least increase in canopy temperature but highest per cent increase in stomatal resistance whereas the reverse was observed in cultivar Hayward. The per cent reduction in leaf water potential, chlorophyll content and photosynthetic rate was least in Bruno, whereas the per cent reduction in transpiration rate was highest in Bruno and the least in Hayward under deficit irrigation treatment and therefore, the Bruno cultivar has better tolerance to water stress as compared to other cultivars.

REFERENCES

1. Blum, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crop Res.* **112**: 119-23.

2. Buwalda, J.G. and Smith, G.S. 1990. Acquisition and utilization of carbon, mineral nutrients and water by the kiwifruit vine. *Hort. Rev.* **12**: 307-47.

3. Ennajeh, M., Vadel, A.M., Cochard, H. and Khemira, H. 2010. Comparitive impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *J. Hort. Sci. Biotech.* **85**: 289-94.

4. Escalona, J.M., Flexas, J., Bota, J. and Medrano, H. 2003. Distribution of leaf photosynthesis and transpiration within grapevine canopies under different drought conditions. *Vitis*, **42**: 57-64.

5. Ghaderi, N., Siosemardeh, A. and Shahoei, S. 2007. The effect of water stress on some physiological characteristics in Rashe and Khoshnave grape cultivars. *Acta Hort.* **754**: 317-22.

6. Hiscox, J.D. and Isralistan, J.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian J. Biol.* **57**: 332-34.

7. Kadam, J.H., Tambe, T.B. and Kaledhonkar, D.P. 2005. Effect of irrigation regimes on chlorophyll content and chlorophyll stability index in different grape rootstocks. *Indian J. Hort.* **62**: 293-95.

8. Lakso, A.N. 1990. Interactions of physiology with multiple environmental stresses in horticultural crops. *Hort. Sci.* **25**: 1365-69.

9. Liu, B.H., Cheng, L., Liang, D., Zou, Y.J. and Ma, F.W. 2012. Growth, gas exchange, water use efficiency, and carbon isotope composition of Gale Gala apple trees grafted onto 9 wild Chinese rootstocks in response to drought stress. *Photosynthetica*, **50**: 401-10.

10. Miraghaee, Sharam, S., Ali, M. and Kahrizi, D. 2012. Acclimatization related proteins and factors in somaclone lines in kiwifruit (*Actinidia deliciosa*). *J. Food, Agric. Env.* **10**: 198-205.

11. Romero, P., Fernandez, J.I.F. and Cutillas, A.M. 2010. Physiological thresholds for efficient regulated deficit irrigation management in winegrapes grown under semi-arid conditions. *American J. Vitic.* **61**: 300-12.

12. Sanchez, F.J., Manzanares, M., de Andres, E.F., Tenorio, J.L. and Ayerbe, L. 2001. Residual transpiration rate, epicuticular wax load and leaf colour of pea plants in drought conditions influence on harvest index and canopy temperature. *European J. Hortic. Sci.* **15**: 57-70.

13. Satisha, J., Prakash, G.S., Murthi, G.S., Murthi, G.S.R. and Upreti, K.K. 2007. Water stress and rootstocks influences on hormonal status of budded grapevine. *European J. Hortic. Sci.* **72**: 202-05.

14. Tan, C.S. and Buttery, B.R. 1982. The effects of soil moisture stress to various fractions of the root system on transpiration, photosynthesis, and internal water relations of peach seedlings. *J. American Soc. Hort. Sci.* **107**: 845-49.

15. Thakur, A. 2004. Use of easy and less expensive methodology to rapidly screen fruit crops for drought tolerance. *Acta Hort.* **662**: 231-35.

Received : July, 2016; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00101.3

Indian J. Hort. 74(4), December 2017: 520-525

Study of β-carotene enhancing '*Or*' gene effects on yield and contributing traits in mid-season Indian cauliflower (*Brassica oleracea* var. *botrytis* L.)

P. Muthukumar, Pritam Kalia*, Munish Sharma and Sonia Vashisht

Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Nature of gene action of EC 625883, an orange curd colour genotype, on yield and contributing traits in cauliflower was studied by using five generation model (P_1, P_2, F_1, F_2 and F_3) in three cross combinations, *viz*., DC-309 × EC 625883, CC-35 × EC 625883 and DC 18-19 × EC 625883 for ten quantitative traits. The orange curd colour *Or* gene donor homozygous line EC 625883 and three divergent recipient mid season (November-January) maturity white curd lines, namely, DC-309, CC-35, and DC 18-19 were involved in hybridization. Generation mean analysis using scaling test indicated that epistasis gene interaction model fitted well for most of the traits under study in all the three cross combinations. The complementary type of gene interaction was observed for number of days to marketable curd maturity, total plant weight, marketable curd weight and net curd weight. The presence of complementary type of interactions and prevalence of high magnitude of non-additive gene effects suggested exploitation of heterosis breeding for improvement in cauliflower using *Or* gene for enhancing of β-carotene and micronutrient simultaneously.

Key words: β-carotene, generation mean analysis, orange cauliflower, quantitative traits, scaling test.

INTRODUCTION

Cauliflower is the most popular crucifer vegetable grown commercially in India on an area of 0.43 million hectares with a production of 8.57 million tonnes and 19.8 t/ ha productivity (Anon, 1). India ranks second in cauliflower production in the world. Major cauliflower growing states in India are West Bengal, Bihar, Odisha, Haryana, Madhya Pradesh, Gujarat, Jharkhand, Assam, Chhattisgarh and Uttar Pradesh. Cauliflower shares about 4.3% of the total area and 4.7% of the total vegetable production in India. The curd is edible part of cauliflower, which consists of proliferating, arrested inflorescence and floral meristems. It contains an appreciable amount of vitamin B, vitamin C, folate, calcium and protein. Vitamin A is essential for vision, gene transcription, immune function, embryonic development, reproduction, bone metabolism, skin health and antioxidant activity. Cauliflower, however, lacks β-carotones a precursor vitamin A the deficiency of which leads to night blindness, keratomalacia, xerophthalmia and retarded physical growth. The β-carotene is present in a wide variety of yellow-orange coloured fruits and dark green and yellow vegetables such as broccoli, spinach, turnip greens, carrots, squash, sweet potatoes and pumpkin (Farre *et al*., 7). The orange curd colour genotype is a spontaneous novel mutant, a rich source of β-carotene, is governed by single dominant gene (*Or*) with few modifier genes (Crisp *et al*., 4). In view of this, the present study was undertaken to study the effect of *Or* gene on yield and contributing traits in mid-season Indian cauliflower through generation mean analysis.

*Corresponding author's E-mail: pritam.kalia@gmail.com

MATERIALS AND METHODS

The experiment comprised five generations, *viz*., P_1, P_2, F_1, F_2 and F_3 developed from cross combinations of three mid season white curded recipient inbred lines, namely, Pusa Sharad (DC-309), CC-35, DC 18-19 and an *Or* gene homozygous donor line EC 625883. From this, three cross combinations, *viz*., DC-309 × EC 625883, CC-35 × EC 625883 and DC 18-19 × EC 625883 were developed. The experiment was conducted during 2009-2013 in a randomised block design with three replication at the research farm of the Division of Vegetable Science, ICAR-IARI, New Delhi situated at an elevation of about 228 m above mean sea level, 20° 40' N latitude and 77° 13'E longitude. Thirty-day-old seedlings were planted manually at a spacing of 60 × 45 cm between and within rows, respectively. The recommended cultural practices (Singh and Sharma, 16) were followed including recommended rate of 120N-80P-40K kg/ha. The N was from urea, P from single super phosphate, and K from muriate of potash. Half of N and all of P and K fertilizers were applied during land preparation. The remaining N was applied in two splits at 30 and 60 days after planting. The plots were flood irrigated through a furrow system beginning immediately after transplanting and at 12-15 day interval ensuring sufficient moisture. For weed management, the herbicide pendimethalin was

applied 2-3 days prior to transplanting at 3.3 l/ha. One month after transplanting, first earthing up was done and the first split application of nitrogen was applied; after an additional month, the second split application of nitrogen was applied. For managing diseases and insect pests, streptocycline at 0.1 g/ l water + blitox at 2 g/ l water and indoxacarb at 1 ml/ l water were applied 30 days after planting to control black rot disease and *Spodoptera* insect pest, respectively. The observations were recorded on ten plants each in parents and F_1 generations and 20 plants each in F_2 and F_3 generations for ten major traits, such as plant height, stalk length, leaves per plant, leaf length, leaf breadth, days to 50% curd initiation, number of days to marketable curd maturity, total plant weight, marketable curd weight and net curd weight. The quantitative data recorded for each of ten traits were compiled and mean values calculated from the data gathered from three replications for each trait were used for statistical analysis. The genetic effects were estimated using five parameter-model suggested by Hayman (5). The component of gene effects included [m] = mean of F_2 generation, [d´] = additive effect (joint estimates of d and ĵ in 5-parameter model), [h] = dominance effect, [i] = additive × additive effect, [l] = dominance × dominance effect. The scaling tests of Mather (13) were performed to detect non-allelic interactions, and gene effects were estimated according to Jinks and Jones (12).

RESULTS AND DISCUSSION

The mean and standard errors for five generations in respect of ten traits in three crosses have been presented in Tables 1, 2 and 3. The mean effect of all the ten characters studied, *viz.*, plant height, stalk length, leaves per plant, leaf length, leaf breadth, days to 50% curd initiation, number of days to marketable curd maturity, total plant weight, marketable curd weight and net curd weight were positive and significant in all the crosses (DC-309 × EC 625883, CC-35 × EC 625883 and DC 18-19 × EC 625883). The estimates of scaling tests and gene effects on five generation mean in each of the three crosses for all the traits studied are presented in Tables 4 & 5, respectively. In the absence of back cross generation, Hayman (9) and Jinks and Jones (12) prescribed five parameter model for generation mean analysis, which included F_3 as one of the generation in addition to P_1, P_2, F_1 and F_2. However, in the present investigation. C and D scaling test was carried out as suggested by Mather (13) in all the crosses for each trait to examine whether epistatic gene effects exist in the material under study, and its relative importance. Accordingly in interacting crosses, all the five parameters (m. d, h, i & l) were estimated.

Table 1. Generation means ± SE of plant height, stalk length, leaves per plant, leaf length of five generations of three crosses in orange cauliflower.

Trait / Generation	DC-309 × EC 625883	CC-35 × EC 625883	DC 18-19 × EC 625883
Plant height (cm)			
P_1	61.13 ± 2.64	61.20 ± 0.53	71.87 ± 1.80
P_2	62.73 ± 1.00	62.73 ± 1.00	62.73 ± 1.00
F_1	69.53 ± 2.48	68.47 ± 1.53	82.80 ± 0.90
F_2	69.67 ± 0.88	60.13 ± 2.18	65.93 ± 4.58
F_3	61.00 ± 0.58	59.00 ± 2.31	61.73 ± 1.33
Stalk length (cm)			
P_1	2.93 ± 0.44	3.62 ± 0.13	4.42 ± 0.19
P_2	2.53 ± 0.35	2.53 ± 0.35	2.53 ± 0.35
F_1	2.93 ± 0.13	3.31 ± 0.30	7.86 ± 0.27
F_2	3.47 ± 0.17	2.97 ± 0.14	3.19 ± 0.21
F_3	3.25 ± 0.04	2.79 ± 0.11	2.99 ± 0.06
Leaves per plant			
P_1	25.87 ± 0.71	25.07 ± 0.18	22.27 ± 1.55
P_2	23.93 ± 0.13	23.93 ± 0.13	23.93 ± 0.13
F_1	28.13 ± 0.53	25.93 ± 0.68	32.47 ± 1.00
F_2	25.27 ± 0.29	24.67 ± 0.47	25.80 ± 0.64
F_3	25.47 ± 0.18	24.13 ± 0.27	20.87 ± 0.48
Leaf length (cm)			
P_1	43.27 ± 2.66	52.80 ± 1.27	55.07 ± 0.24
P_2	51.40 ± 0.20	51.40 ± 0.20	51.40 ± 0.20
F_1	51.80 ± 2.91	54.80 ± 0.40	61.53 ± 0.85
F_2	57.87 ± 2.56	60.27 ± 2.94	49.53 ± 0.75
F_3	56.67 ± 2.26	58.60 ± 1.59	47.87 ± 0.24

The simple scaling test of Mather was applied to determine the presence of genetic interaction in three cauliflower crosses involving one common donor line carrying beta-carotene enhancing *Or* gene. The perusal of results indicate that C and D were largely significant in all of the crosses for most of the traits studied, suggesting the involvement of either one or both of the epistatic components *i* and *l*. On the basis of simple scaling test for epistasis, the five parameter model was fitted to the observed components of mean in each of the three crosses for all the traits.

In case of plant height, both the scales (C and D) were significant in all the three crosses. Dominance gene effects appeared to play an important role for the inheritance of plant height as it exhibited comparatively higher significant values in all the crosses. All the five components of gene effects (m, d, h, i, l) were significant in cross DC 18-19 × EC 625883 with complementary type of epistasis. In cross CC-35 × EC 625883, two

Table 2. Generation means ± SE of leaf breath, days to 50% curd initiation, number of days to marketable curd maturity of five generations of three crosses in orange cauliflower.

Trait / Generation	DC-309 × EC 625883	CC-35 × EC 625883	DC 18-19 × EC 625883
Leaf breadth (cm)			
P1	16.07 ± 0.27	17.73 ± 0.71	16.13 ± 0.35
P2	17.80 ± 0.12	17.80 ± 0.12	17.80 ± 0.12
F1	19.73 ± 2.32	18.87 ± 1.03	18.67 ± 0.35
F2	16.33 ± 1.19	15.67 ± 1.47	13.13 ± 0.44
F3	14.47 ± 0.37	14.93 ± 0.48	12.17 ± 0.24
Days to 50% curd initiation			
P1	68.80 ± 10.35	91.80 ± 0.31	93.47 ± 0.24
P2	85.40 ± 0.53	85.40 ± 0.53	85.40 ± 0.53
F1	81.87 ± 0.87	94.20 ± 0.31	91.87 ± 0.75
F2	77.00 ± 2.55	72.33 ± 0.24	103.40 ± 0.12
F3	80.93 ± 3.19	73.40 ± 1.29	105.60 ± 0.76
No. of days to marketable curd maturity			
P1	94.20 ± 2.41	108.87 ± 0.18	114.40 ± 0.87
P2	105.33 ± 0.24	105.33 ± 0.24	105.33 ± 0.24
F1	97.13 ± 0.97	113.73 ± 1.04	110.47 ± 0.87
F2	92.80 ± 3.97	86.40 ± 1.60	121.20 ± 0.83
F3	95.00 ± 2.34	91.73 ± 2.29	120.27 ± 0.75

components of genetic effects (m, l) were significant with complementary epistasis and preponderance for additive components, whereas in cross DC-309 × EC 625883, four components of gene effect (m, h, i, l) were significant with duplicate type of epistasis.

In case of stalk length, all the three crosses showed significant values for the scale 'C' only. The dominance component was comparatively higher in all the crosses suggesting its important contribution in inheritance of stalk length. Three components of gene effects (m, h, l) were significant in the cross DC 18-19 × EC 625883 with complementary type of epistasis, which was duplicate in the remaining crosses. In case of leaves per plant, only 'C' scale was significant in two crosses, namely, DC-309 × EC 625883 and DC 18-19 × EC 625883. Dominance component was found to play an important role in inheritance of leaves per plant exhibiting higher values in all the three crosses. Four components of the gene effects (m, h, i, l) in two crosses, namely, DC-309 × EC 625883 and DC 18-19 × EC 625883 were significant with complementary epistasis in the former and duplicate in the latter.

In case of leaf length, all the three crosses showed significant values for the estimates of scaling test C and D except for D in the cross DC 18-19 × EC 625883. Four components of gene effects (m, d, i, l) in cross DC-309 × EC 625883, two (m, l) in CC-35 × EC 625883 and four (m, h, i, l) in DC 18-

Table 3. Generation mean ± SE of total plant weight, marketable curd weight and net curd weight of five generations of three crosses in orange cauliflower.

Trait/ Generation	DC-309 × EC 625883	CC-35 × EC 625883	DC 18-19 × EC 625883
Total plant weight (g)			
P_1	2103.33 ± 171.50	1766.67 ± 52.39	2763.33 ± 38.44
P_2	1926.67 ± 126.67	1926.67 ± 126.67	1926.67 ± 126.67
F_1	3346.67 ± 245.04	3046.67 ± 73.33	4343.33 ± 24.04
F_2	1946.67 ± 238.42	1786.67 ± 150.70	1793.33 ± 173.72
F_3	1778.67 ± 116.17	1600.00 ± 126.62	2156.67 ± 58.40
Marketable curd weight (g)			
P_1	1580.00 ± 240.28	1043.33 ± 8.82	1513.33 ± 44.10
P_2	1314.00 ± 124.13	1314.00 ± 124.13	1314.00 ± 124.13
F_1	2710.00 ± 193.48	2386.67 ± 157.20	3310.00 ± 470.32
F_2	1263.33 ± 162.72	1054.00 ± 49.69	1273.33 ± 184.87
F_3	994.67 ± 90.64	1006.67 ± 66.42	966.00 ± 91.27
Net curd weight (g)			
P_1	1001.33 ± 79.74	653.33 ± 23.33	667.33 ± 15.38
P_2	1060.33 ± 49.77	1060.33 ± 49.77	1060.33 ± 49.77
F_1	2443.33 ± 164.76	1633.33 ± 98.38	3521.33 ± 16.18
F_2	531.33 ± 35.41	479.00 ± 21.22	512.67 ± 61.13
F_3	376.67 ± 44.10	431.33 ± 38.86	330.00 ± 30.55

Table 4. Scaling test for quantitative traits in mid-season orange cauliflower.

Trait	Cross	Simple scaling test	
		C	D
Plant height (cm)	C1	15.73**	-9.33**
	C2	6.71**	2.34**
	C3	2.34**	-3.92**
Stalk length (cm)	C1	2.53**	-0.69
	C2	0.94**	0.38
	C3	2.71**	-1.82
Leaves per plant	C1	-5.00**	-1.60
	C2	1.73	1.07
	C3	-2.88**	-1.49
Leaf length (cm)	C1	33.20**	-15.80**
	C2	12.09**	6.35**
	C3	2.75**	-2.49
Leaf breadth (cm)	C1	-10.33**	-8.43**
	C2	7.67**	3.17**
	C3	-1.35	-2.66**
Days to 50% curd initiation	C1	-22.60**	-3.20**
	C2	14.65**	6.18**
	C3	-0.68	-0.51
No. of days to marketable curd maturity	C1	-22.60**	-3.20**
	C2	16.19**	8.29**
	C3	-1.40	-0.39
Total plant weight (g)	C1	-2936.67**	-1581.33**
	C2	1093.23**	497.94**
	C3	-2.09**	-3.18**
Marketable curd weight (g)	C1	-3260.67**	-966.00**
	C2	804.06**	344.11**
	C3	-4.06**	-2.81**
Net curd weight (g)	C1	-4823.00**	-1317.33**
	C2	370.78**	111.72**
	C3	-13.01**	-11.79**

*,**Significant at 5 and 1% significance levels; Epistasis C1 = DC-309 × EC 625883, C2 = CC-35 × EC 625883, C3 = DC 18-19 × EC 625883

19 × EC 625883 were significant. Leaf length was found to be governed by duplicate type of epistasis in CC-35 × EC 625883 and complementary type in other two crosses.

For leaf breadth, the estimates of scaling test revealed significance for both the scales (C and D) in all the crosses except for scale 'C' in the cross DC 18-19 × EC 625883. Dominance gene effects were significant and predominant in all the crosses. Four components of gene effects (m, h, i, l) were significant in all the crosses except for nonallelic interaction (l) in the cross DC-309 × EC 625883 which also showed duplicate type of epistasis, whereas in others it was complementary type.

In case of days to 50% curd initiation, both the scales (C and D) were significant in two crosses, namely, DC-309 × EC 625883 and CC-35 × EC 625883; however these were non-significant in cross DC 18-19 × EC 625883. Dominance gene effects and dominant × dominant gene interaction 'l' were significant and predominant in all the crosses. Four components of gene effects (m, d, h, l), (m, h, i, l) and (m, d, h, l) in crosses DC-309 × EC 625883, CC-35 × EC 625883 and DC 18-19 × EC 625883, respectively were significant. Duplicate type of gene action was observed in cross DC-309 × EC 625883 and complementary type in the remaining two. For number of days to marketable curd maturity, the estimates of scales C and D were significant in crosses DC-309 × EC 625883 and CC-35 × EC 625883 and non-significant in DC 18-19 × EC 625883. Four components of gene effects (m, d h, i) in the cross C-309 × EC 625883, three (m, h, l) in DC-35 × EC 625883 and five (m, d, h, i, l) in DC 18-19 × EC 625883 were significant. The complementary type of epistasis was found to be predominant in all the crosses.

In case of total plant weight, marketable curd weight and net curd weight significant values were revealed for both the scales (C & D) in all the three crosses, which led to the estimation of all the five type of gene effects (m, d, h, i, l). All the components of the gene effects (allelic and non-allelic) were significant for the three traits in all crosses. Dominance gene effects appeared to play an important role in the inheritance of all the three traits as these revealed comparatively higher significant values as also non-allelic interaction 'l' in all the crosses. Complementary type of epistasis was observed in all the three traits in all the crosses. Since yield being complex polygenic trait resulting from interaction among various inherent traits and environment, it can be further improved through indirect selection on the basis of yield contributing traits (Chandra *et al.*, 2). Sufficient understanding of the inheritance of quantitative traits and information about it is essential to develop breeding strategy. Generation mean analysis is a powerful breeding technique for estimating main gene effects (additive and dominance) and their digenic (additive × additive, additive × dominance and dominance × dominance) interaction responsible for inheritance of quantitative traits. It helps us in understanding the performance of the parents used in the crosses to be used either for heterosis breeding or pedigree selection (Sharma *et al.*, 14). Therefore, the estimates of the relative magnitude of various gene effects including epistasis are of significance, when each cross combination is considered. Since linkage affects the epistatic term in generation mean (Hayman, 9), additive and dominant gene effects cannot be precisely measured in the presence of epistasis (Hayman, 10). Even with these

Table 5. Estimation of gene effects on five generation means for quantitative traits in mid-season orange cauliflower.

Trait	Cross	Gene effect					Type of epistasis
		m	d	h	i	l	
Plant height (cm)	C1	69.67**	-0.80	23.2**	13.82**	-46.58**	D
	C2	60.13**	-0.77	0.71	0.54	16.18**	C
	C3	65.93**	4.57**	22.44**	16.08**	22.58**	C
Stalk length (cm)	C1	3.47**	0.20	0.23	0.43	-2.60	D
	C2	2.97**	0.54	0.71	1.56	-0.05	D
	C3	3.19**	0.94	3.66**	1.17	11.34**	C
Leaves per plant	C1	25.27**	0.97	4.04**	2.74**	3.38**	C
	C2	24.67**	0.57	2.27	1.97	0.53	C
	C3	25.80**	-0.83	17.60**	7.81**	-8.53**	D
Leaf length (cm)	C1	57.87**	-4.07**	-0.84	-13.44**	-22.58**	C
	C2	60.27**	0.70	0.80	-0.50	-23.47**	D
	C3	49.53**	1.83	12.44**	7.81**	23.11**	C
Leaf breadth (cm)	C1	16.33**	-0.87	7.24**	2.71**	-0.89	D
	C2	15.67**	-0.03	4.09**	2.92**	4.62**	C
	C3	13.13**	-0.83	6.27**	2.90**	9.60**	C
Days to 50% curd initiation	C1	77.00**	-8.30**	-7.24**	-28.61	33.96**	D
	C2	72.33**	3.20	11.73**	12.53**	64.00**	C
	C3	103.40**	4.03**	-13.56**	-7.92	-19.02**	C
No. of days to marketable curd maturity	C1	92.80**	-5.57**	-2.98*	-11.48**	-23.29	C
	C2	86.40**	1.77	4.00**	0.90	101.33**	C
	C3	121.20**	4.53**	-4.67**	3.80**	-33.60**	C
Total plant weight (g)	C1	1946.67**	88.33**	1381.33**	226.33**	2837.33**	C
	C2	1786.67**	-80.00**	1337.78**	22.22**	2364.45**	C
	C3	1793.33**	418.33**	731.11**	-430.56**	8737.78**	C
Marketable curd weight (g)	C1	1263.33**	133.00**	1680.89**	683.89**	2424.89**	C
	C2	1054.00**	-135.33**	1014.67**	-464.00**	3301.33**	C
	C3	1273.33**	99.67**	2177.33**	480.33**	3792.00**	C
Net curd weight (g)	C1	531.33**	-29.50**	1687.11**	215.61**	4273.78**	C
	C2	479.00**	203.50**	896.67**	286.83**	2824.00**	C
	C3	512.67**	196.50**	2492.89**	557.61**	7048.89**	C

*, ** Significant at 5 and 1% significance levels; D = Duplicate epistasis C = Complementary epistasis C1 = DC-309 × EC 625883, C2 = CC-35 × EC 625883, C3 = DC 18-19 × EC 625883

limitations, estimates of the several parameters provide indication of the relative importance of various types of gene effects influencing total genetic variation of an attribute (Gamble, 8).

Presence of epistasis / gene interaction varied with the crosses as well as traits, and most of the crosses showed presence of epistasis. The generation mean for most of the traits showed importance of both additive and dominant types of gene effects. However, dominant gene effects were higher than additive gene effects. Several workers have estimated gene effects in cauliflower for different traits (Dixit *et al*., 6; Jindal and Thakur, 11; Singh *et al*., 15; Varalakshmi, 17; Devaraju *et al*., 5; Verma and Kalia, 18) who also reported importance of both additive and dominance components in the control of various traits, however dominance component was preponderant.

In the presence of epistasis, predominance of complementary type of gene action was observed. In such situation, dominance effects tend to be overestimated, while additive component is relatively underestimated. Some crosses showed duplicate type of gene interaction, in such cases intermating or biparental mating between selected plants from early segregating generations could help in improving such traits (Comstock *et al*., 3). Among the epistatic gene effects, dominance × dominance (l) type gene effect was greater in magnitude than additive × additive (i) type. It is evident from the present study that epistasis is important basic mechanism, therefore, while formulating

breeding startegies gene interactions should also be taken into consideration along with main gene effects. In complementary type of gene action, particularly i and l reinforce the effect of dominance component. It is because of this reason that heterosis is expressed with greater magnitude in crosses, where complementary type of interaction is observed (Jinks and Jones, 12), while it may not be observed at all in crosses showing duplicate type of gene action. In the present investigation, complementary type of gene action was exhibited for most of the traits in most of the crosses.

In conclusion, the generation means for most of the characters in the present study showed the importance of both additive and dominance type of gene effects. However, dominance effect, in general, was higher than additive gene effects. Among the epistatic gene effect, dominance × dominance type was greater than additive × additive. In the presence of epistatsis, complementary type of gene interaction was observed in almost all the crosses for most of the traits favouring heterosis breeding.

ACKNOWLEDGEMENTS

This study was a part of project supported by Department of Biotechnology (DBT), Govt. of India funded project. The first author acknowledges the Indian Council of Agricultural Research, New Delhi for financial assistance in the form of ICAR-SRF.

REFERENCES

1. Anonymous 2014. *Indian Horticulture Database*, National Horticulture Board, Ministry of Agriculture and Farmers Welfare, GoI, 85, Institutional Area, Sector 18, Gurgaon 122015, Haryana. http://www.nhb.gov.in

2. Chandra, D., Islam, M.A. and Barman, C.D. 2004. Variability and interrelationship of nine quantitative characters in F_5 bulks of five wheat crosses. *Pakistan J. Biol. Sci.* **7**: 1040-45.

3. Comstock, R.E., Robinson, H.F. and Harvey, P.H. 1949. A breeding procedure designed to make maximum use of both general and specific combining ability. *Agron. J*. **41**: 360-67.

4. Crisp, P., Walkey, D.G., Bellman, E. and Roberts, E. 1975. A mutation affecting curd colour in cauliflower (*Brassica oleracea* L. var. *botrytis* L.). *Euphytica*, **24**: 173-76.

5. Devaraju, Varalakshmi, B. and Savithramma, D.L. 2010. Genetics of yield and its component traits in early cauliflower. *Indian J. Hort.* **67**: 339-42.

6. Dixit, S.K., Singh, B.P. and Ram, H.H. 2004. Heterosis and combining ability in Indian cauliflower (*Brassica oleracea* var. *botrytis* L.). *Veg*. *Sci*. **31**: 164-67.

7. Farre, G., Sanahuja, G., Naqvi, S., Bai, C., Capell, T., Zhu, C. and Christou, P. 2010. Travel advice on the road to carotenoids in plants. *Plant Sci*. **179**: 28-48.

8. Gamble, E.E. 1962. Gene effects in corn (*Zea mays* L.): I. Separation and relative importance of gene effects for yield. *Canadian J. Plant Sci.* **15**: 77-78.

9. Hayman, B.I. 1958. The separation of epistatic from additive and dominance variation in generation means. *Heredity*, **12**: 371-90.

10. Hayman, B.I. 1960. The separation of epistatic from additive and dominance variation in generation means II. *Genetica*, **XXXI**: 133-46.

11. Jindal, S.K. and Thakur, J.C. 2005. Genetic architecture of quantitative characters in November maturity cauliflower. *Crop Improv.* **32**: 92-94.

12. Jinks, J.L. and Jones, R.M. 1958. Estimation of the components of heterosis. *Genetics*, **43**: 223-34.

13. Mather, K. 1949. *Biometrical Genetics*, Methuen and Co. Ltd., London, UK.

14. Sharma, S.N., Sain, R.S. and Sharma, R.K. 2003. The genetic control of flag leaf length in normal and late sown durum wheat. *J. Agric. Sci.* **141**: 323-31.

15. Singh, D., Varalakshmi, B. and Reddy, M.A.N. 2005. Combining ability studies in early cauliflower. (*Brassica oleracea* var. *botrytis* L.). *Indian J. Hort.* **62**: 27-32.

16. Singh, R. and Sharma, S.R. 2003. Cauliflower. **In**: *Textbook of Vegetables*, *Tubercrops and Spices*, S. Thamburaj and Narendra Singh (Eds.), Directorate of Information and Publication of Agriculture, ICAR, New Delhi, pp. 76-97.

17. Varalakshmi, B. 2009. Heterosis and combining ability for yield and its components in early cauliflower. *Indian J. Hort*. **66**: 198-203.

18. Verma, V.K. and Kalia, P. 2011. Combining ability studies in early and mid-maturity CMS based cauliflower lines. *Indian J*. *Hort*. **68**: 503-06.

Received : July, 2015; Revised : September, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00102.5

Indian J. Hort. 74(4), December 2017: 526-532

Genome wide identification of calcium dependent protein kinase and related kinase gene families in *Solanum melongena* L.

Meenu Kumari*, D.P. Wankhede, Manjusha Verma and Pooja Verma**
ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi 110012

ABSTRACT

In plants, Calcium-Dependent Protein Kinases (CDPKs) play multiple roles with a variety of functions in physiological processes, biotic and abiotic stress together with plant hormone signalling. We conducted a genome-wide analysis of CDPK gene family in eggplant. Total 28 CDPKs and 2 CDPK-related kinases (CRKs) genes were identified in eggplant genome. Structural organisation of *Solanum melongena* CDPKs and CRK genes as well as different conserved domains and motifs were studied. Both *SmeCDPKs* and *SmeCRK* proteins harbour STKc_CAMK type protein kinase domain, while only *SmeCDPKs* contained EF-hand type Ca^{2+} binding domain(s). Phylogenetic analysis suggested the conserved basic structure of *SmeCDPKs* and classified into four clades. The amino acid residue patterns of sub-domains including the conserved EF hands were identified for *SmeCDPKs*. An elaborate search of eggplant ESTs available in public domain shows presence of *SmeCDPK* in different tissue specific libraries and most of them were expressed in mixture of flower bud, young fruit, leaf and ovary tissues. *SmeCDPK* 28/18 and 34 were specific to ovary and stamen libraries, respectively. Further, expression of *SmeCDPKs* was checked making use of eggplant transcriptome available in public domain. The information generated will significantly imparts the basic step for further functional study of CDPKs gene family in eggplant.

Key words: Brinjal, Calcium-Dependent Protein Kinases, CD PK-related kinases, eggplant, CRK, ESTs, gene expression.

INTRODUCTION

In eukaryotes; calcium is known to act as universal second messenger that plays key role in signal transduction pathways (Reddy *et al*., 13). It mediates signaling through Ca^{2+} sensors or Ca^{2+} binding proteins and regulates gene expression pattern by reversible phosphorylation. There are three major classes of Ca^{2+} binding proteins have been identified in plants, calcium dependent protein kinase (CDPKs), calmodulins (CaM) and calcineurin B-like proteins (CBL). CDPKs are unique owing as it possesses both CaM-like domain and protein kinase domain. However, other Ca^{2+} sensors characteristically consisted of N-terminus, an auto-inhibitory junction domains followed by the regulatory domain (Klimecka and Muszynska, 8).

The CDPKs are known to play important role in various physiological processes and also in generating response to several biotic or abiotic stresses. CDPKs are activated upon binding of Ca^{2+} to their calmodulin-like domain, which is composed of one to four globular EF-hand motifs (Klimecka and Muszynska, 8). A genome-wide study in several plants demonstrated presence of CDPKs (Reddy *et al*., 13; Asano *et al*., 1; Li *et al*., 10). Among horticultural crops, genome wide CDPKs gene family has been identified in pepper (Cai *et al*., 2) and cucumber (Xu *et al*., 14). Biotic and abiotic stress responsive expressions of CDPKs have been reported in several crop plants (Cai *et al*., 2; Dubrovina *et al*., 3; Li *et al*., 10). Hitherto, there has been limited information about CDPKs at genome wide level in eggplant. Recently, draft eggplant genome sequence has been completed by Hirakawa *et al*. (5). In the present study, the genome-wide identification of *SmeCDPKs* and its characterization is reported.

MATERIALS AND METHODS

The amino acid sequences of 34 *Arabidopsis* CDPKs and its homologous CDPKs were retrieved from the Arabidopsis Information Resource and NCBI GenBank, respectively. Total 176 CDPK sequences were retrieved from 22 divergent plant species including *Arabidopsis thaliana*. Eggplant whole genome protein and CDS sequences were obtained from http://eggplant.kazusa.or.jp/. The 176 CDPK protein sequences were used as query against protein sequences database using standalone BLAST with e values 0.001. Hits with more than 50% identity were retrieved and used for subsequent analysis as reported earlier (Wankhede *et al.,* 12). Briefly, the sequences were manually verified for presence of protein kinase domain and EF-hands. Additionally,

*Corresponding author's present address: ICAR-IIHR, Central Horticultural Experiment Station, Bhubaneswar 751019, Odisha; E-mail: meenu10iari@gmail.com
**ICAR-Central Institute for Cotton Research, Nagpur- 440010.

HMMER3.0 (http://hmmer.org/) was used to search the local protein database (total protein sequences of eggplant) on the basis of the HMM profile. Results from BLAST and HMMER hits were matched and parsed manually. The deduced protein sequences of eggplant were aligned with Clustal Omega. A phylogenetic tree was constructed among CDPKs proteins, by employing the Neighbor-joining (NJ) method and bootstrap test with 1,000 replicates wrapped in MEGA6 software suite. Prediction of myristoylation and palmitoylation sites were done by Myristoylator (http://web.expasy.org/myristoylator/) and CSS-Palm software version 3.0 (http://csspalm.biocuckoo.org/), respectively. The coding sequences corresponding to each predicted gene were analyzed for exon-intron distribution pattern. Multiple sequence alignments of CDPKs proteins were executed by Multalin software (http://bioinfo.genotoul.fr/multalin/multalin).

All the available eggplant ESTs were downloaded from dbEST, NCBI. A standalone blast search was performed using *SmeCDPKs* against total eggplant ESTs. Hits with more than 90% identity were considered significant. Eggplant transcriptome sequence data available in Sequence Read Archives (SRA), NCBI with accession number (SRR1104129) (Yang *et al*., 15) were used for expression study. For expression analysis 'RNAseq protocol' of CLC genomics workbench was followed. A maximum number of two mismatches were allowed for alignment. Unique read counts were normalized by calculating RPKM (Reads per kilobase of transcript per million mapped reads) RPKM values were $\log_2$ transformed showing expression level of SmeCDPKs.

RESULTS AND DISCUSSION

A genome-wide search for CDPK gene family in eggplant identified 28 putative CDPKs and two CDPK-related kinases (Table 1). Orthologous nomenclature was adopted with the basis of *Arabidopsis* CDPKs. It exhibits the useful information pertaining to functional similarities of CDPKs (Mohanta *et al*., 11) with *Arabidopsis* or eudicots. The number of identified 28 *SmeCDPKs* is comparable to other member of Solanaceae family as pepper and tomato have 31 and 29 CDPK genes, respectively (Cai *et al*., 2; Hu *et al*., 8).

The identified *SmeCDPKs* were also studied for biochemical and structural attributes (Table 1). Most of the *SmeCDPKs* showed four EF-hand motifs, however, two *SmeCDPKs* (*SmeCDPK*13-1 and 25*)* were found to have only three EF-hands in the CaM-like domain (Hrabak *et al*., 6). Variations in number of EF-hands in functional domain have been reported in tomato, cucumber (Xu *et al*., 14). The 28 *SmeCDPKs* proteins range in molecular weight from 29.9 to 133.1 kDa, which were equivalent with CDPK genes from other plant species. The length of open reading frame ranged from 808 (*SmeCDPK*12) to 3619 (*SmeCDPK*2-2), which corresponded polypeptides in the range of 268 to 1205 amino acids. *SmeCDPK* had low GC content nucleotide sequences and it varied from 35.5% (*SmeCDPK*34) to 45.2% (*SmeCDPK13-1*). A lipid modification, N-myristoylation motif that tend to localize in the plasma membrane through protein-membrane and protein-protein interactions (Hrabak *et al*., 6) reported to be present in 18 of the *SmeCDPKs* at N-terminus of subset of CDPK proteins. Also palmitoylation, which secure the anchoring between protein and membrane, is seen in all the identified *SmeCDPKs*. These lipid modification shows evidence of *SmeCDPKs* involvement for physiological processes through membrane association and this have been confirmed in other eudicots (Cai *et al*., 2; Xu *et al*., 14). Additionally, in present work, two CDPK-related kinase genes (CRKs) are identified in eggplant genome. In pepper and rice, the number of CRKs are five, whereas in *Arabidopsis* there are eight CRKs reported (Cai *et al*., 2; Asano *et al*., 1; Hrabak *et al*., 6). Molecular weight of SmeCRKs was predicted to be in the range of 36.3 to 38.8 kDa, and their structure is considered to be similar to that of CDPKs except for the EF-hand domains. As observed in earlier studies of *Arabidopsis*, rice, tomato, pepper; *SmeCDPKs* are also categorized into four groups (Fig. 1). Group I and II in eggplant consisted large number of CDPKs, which is near similar to that of tomato and pepper (Cai *et al*., 2; Hu *et al*., 7). The presence of characteristic four groups of CDPKs also has been studied from monocot, dicot and lower eukaryotic plants of different species (Hrabak *et al*., 6; Mohanta *et al*., 11).

The most important implication of genome-wide evolutionary classification based on orthologous clusters is for functional annotation of newly sequenced genomes. Various studies on divergent functions of *AtCDPKs* helped to predict that the orthologs of *AtCPK*1/4 and *AtCPK*11 in eggplant, *SmeCDPK*1/4 and 11 may be associated with defense components, salt, drought and cold stresses (Reddy *et al*., 13). Recently, involvement of *AtCPK*28 for plant immunity through BIK1 phosphorylation has been revealed. The *AtCPK5* orthologous to *SmeCDPK5* could activate defense gene by exhibiting cytoplasmic calcium ion elevations (Knight and Knight, 9). *AtCPK*17 and 34, have been shown to

Table 1. Characteristics of eggplant calcium-dependent protein kinases (CDPKs).

Group	Name	Gene identifier	CDS	Amino acids (No.)	MW (kDa)	pI	GRAVY	GC	N-terminal	N-Myr	N-Pal	EF hands No.	Localization
I	SmeCDPK1	Sme2.5_11161.1_g00001.1	3315	1104	1244	8.21	-0.411	40.7	MGNTCVGP	Y	Y	4	Prx
	SmeCDPK2	Sme2.5_03408.1_g00001.1	1824	607	672	5.53	-0.390	42.7	MGNTCVGP	Y	Y	4	ER
	SmeCDPK2-1	Sme2.5_01206.1_g00007.1	1788	595	671	5.42	-0.410	42.3	MGNNCVHA	Y	Y	4	Chl
	SmeCDPK2-2	Sme2.5_01118.1_g00001.1	3619	1205	1331	6.00	-0.591	42.0	PSEIVESN	N	Y	4	N
	SmeCDPK3	Sme2.5_07017.1_g00004.1	1662	553	619	5.50	-0.370	41.3	MGNTCRGS	Y	Y	4	ER
	SmeCDPK4	Sme2.5_10930.1_g00001.1	1503	500	560	5.09	-0.298	40.2	MDSSKAKT	N	Y	4	ER
	SmeCDPK5	Sme2.5_12109.1_g00002.1	1686	561	625	5.60	-0.262	44.1	MGNACRGS	Y	Y	4	ER
	SmeCDPK11	Sme2.5_03825.1_g00001.1	1491	496	559	5.71	-0.386	40.8	MENSKPSS	N	Y	4	ER
	SmeCDPK12	Sme2.5_29532.1_g00001.1	808	268	299	4.97	-0.469	41.2	AETDNGIF	N	Y	4	N
	SmeCDPK20	Sme2.5_00746.1_g00009.1	1929	642	718	5.77	-0.411	41.9	MGNTCIGP	Y	Y	4	Cyt
	SmeCDPK25	Sme2.5_01484.1_g00010.1	1731	576	637	4.99	-0.318	42.1	MGNNCVGP	N	Y	3	Prx
II	SmeCDPK6	Sme2.5_00013.1_g00035.1	1563	520	584	5.73	-0.453	44.8	MGNCNSLS	Y	Y	4	Prx
	SmeCDPK6-1	Sme2.5_02002.1_g00003.1	1092	363	407	5.04	-0.667	37.1	MGNCCCSR	Y	Y	4	PM
	SmeCDPK17	Sme2.5_00779.1_g00002.1	1566	520	583	5.45	-0.442	38.6	MCDDISFL	N	Y	4	ER
	SmeCDPK19	Sme2.5_00420.1_g00013.1	1383	460	510	6.14	-0.371	42.0	MGICASK	Y	Y	4	ER
	SmeCDPK22	Sme2.5_25256.1_g00001.1	884	293	332	5.25	-0.432	43.2	KVYRDIVG	N	Y	4	ER
	SmeCDPK29	Sme2.5_01233.1_g00004.1	1605	534	601	5.45	-0.410	43.2	MGLCFSKA	Y	Y	4	Cyt
	SmeCDPK33	Sme2.5_00959.1_g00002.1	1431	476	534	6.23	-0.385	40.6	MRPPASPK	N	Y	4	ER
	SmeCDPK34	Sme2.5_00158.1_g00007.1	1503	500	566	5.32	-0.623	35.5	MGSCCSKE	Y	Y	4	N
III	SmeCDPK7	Sme2.5_02438.1_g00002.1	1704	567	634	7.20	-0.350	41.7	MGNCCMMK	Y	Y	4	N
	SmeCDPK8	Sme2.5_00853.1_g00003.1	1605	534	597	6.35	-0.474	42.4	MGNCCGTP	Y	Y	4	N
	SmeCDPK10/30	Sme2.5_04239.1_g00002.1	1299	432	486	5.23	-0.251	41.6	MIGRILFA	N	Y	4	ER
	SmeCDPK13	Sme2.5_04540.1_g00001.1	1446	481	542	6.24	-0.528	44.0	MGNCCRSP	Y	Y	4	Mt
	SmeCDPK13-1	Sme2.5_10488.1_g00001.1	2598	865	981	9.36	-0.260	45.2	MGNFFRYP	Y	Y	3	Plst, Cyt, Nc
	SmeCDPK14	Sme2.5_00011.1_g00046.1	1575	524	593	6.03	-0.510	41.5	MGNCCAVP	Y	Y	4	PM
	SmeCDPK24	Sme2.5_00163.1_g00019.1	1560	519	591	5.62	-0.387	37.6	MKPTPIDQ	N	Y	4	N
IV	SmeCDPK16	Sme2.5_00015.1_g00021.1	1596	531	603	8.99	-0.418	42.8	MGFEMRVL	Y	Y	4	Chl
	SmeCDPK28/18	Sme2.5_00714.1_g00013.1	1728	574	650	9.14	-0.561	40.6	MGSCFSSS	Y	Y	4	Cyt
CRK	SmeCRK1	Sme2.5_30108.1_g00001.1	969	322	363	9.26	-0.386	40.7	MGNCNACI	Y	Y	0	Chl. Cyt. Nc
	SmeCRK2	Sme2.5_01660.1_g00015.1	1032	343	388	6.84	-0.253	42.7	MASSASRT	N	Y	0	Chl, Mt, Nc

N = Nucleus, ER = Endoplasmic reticulum, Prx = Peroxisome, PM = Plasma membrane, Chl = Chloroplast, Mt = Mitochondria, Plst = Plastid, Cyt = cytoplasm

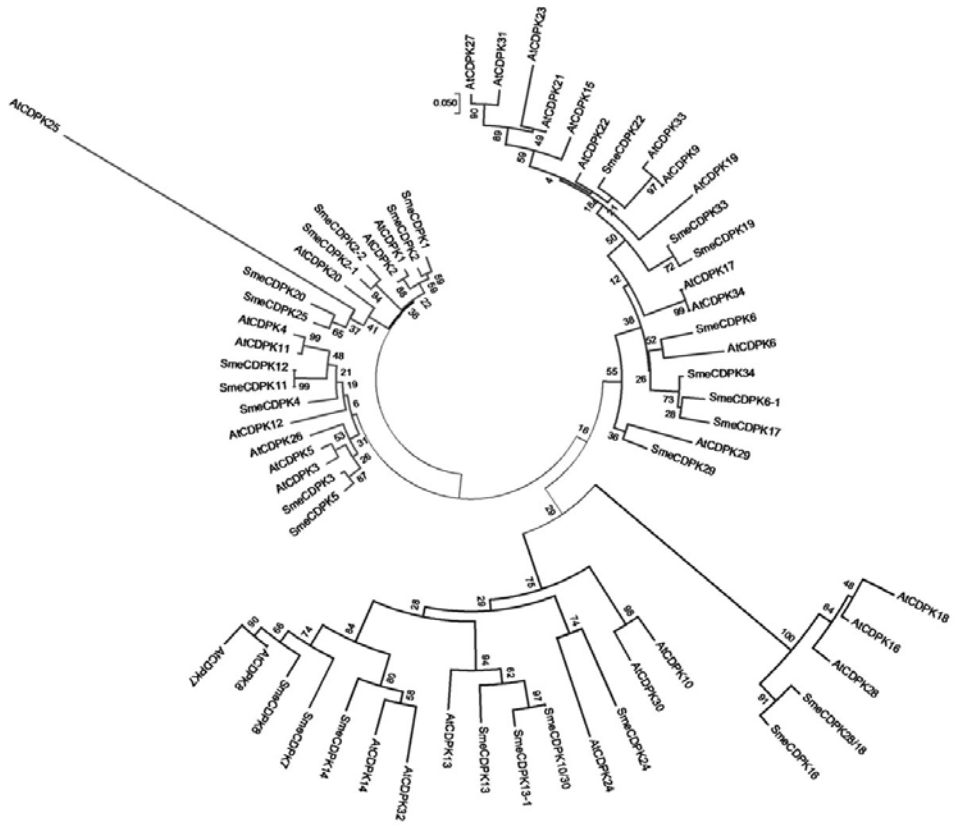


Fig. 1. Evolutionary relationships of *Solanum melongena* CDPK genes.

have role in for pollen tube tip growth (Estruch *et al*., 4), it is interesting to see if its orthologs in eggplant have similar function.

All the eggplant CDPKs were further analyzed for the presence of specific signatures for kinase domains, EF-hand domains and auto-inhibitory domain. Characteristic sequences of kinase domains of CDPKs of dicot plants such as C-x-G-G-E-L-x-D-R-I, H-R-D-L-K-P-E-N-F-L, D-x-V-G-S-x-Y-Y, A-P-E-V-L, D-V/I-W-S, G-V-I-x-Y-I-L-L, G-x-P-P-F-W, P-W-P-x-I-S, A-K-D-L-V and H-P-W (Mohanta *et al*., 11) were also found to be conserved in eggplant CDPKs (Fig. 2). Whereas, EF-hand motifs of CPKs of dicots and monocots share common sequences (E-E-I/x, D-x-D, D/E-E-L, D-Y-x-E-F, F-D-x-D, E-E-L, D-G-x-I and Y-x-E-F-x-x-M-M), which are throughout conserved in nature, were present as signature motif in eggplant. Members of the same group had diverse exon-intron structure (Fig. 3) in contrast to other Solanaceous crop CDPKs, where close relationship obtained (Hu *et al*., 7). Members of sub-family I possessed most complicated pattern, where number of introns ranged from four to eighteen. Clustering the intron-exon structure of *SmeCDPKs* suggests a close relationship between gene organization and evolutionary relationship (Cai *et al*., 2).

In order to get an insight into expression of CDPKs in different tissue and stress conditions, possible ESTs of eggplant were searched. Most of the identified *SmeCDPKs* found significant hits to ESTs from various reproductive tissues and developmental stages such as in fruit, shoot, leaf, mixture of flower bud, sepal, peduncle, callus and other developmental stages (Table 2). It is evident from the analysis that *SmeCDPK*1 and 16 along with others has particularly functional significance for growth and development of vegetative organs (shoot and leaf system) and metabolic function likewise *NtCDPK*1 of *Nicotiana tabacum* (Klimecka and Muszynska, 8). Ovary specific CDPKs (28/18) indicate its unique involvement for germination and placental tissues formation, though *SmeCDPK34* might be related to pollen development similar to maize CDPKs, which was specific for pollen tube growth (Estruch *et al*., 4).

Further, gene expression of *SmeCDPKs* was studied making use of SRA data available in public domain. As shown in Fig. 4, most of the *SmeCDPKs* showed expression except *SmeCDPK*2-1. Since, the

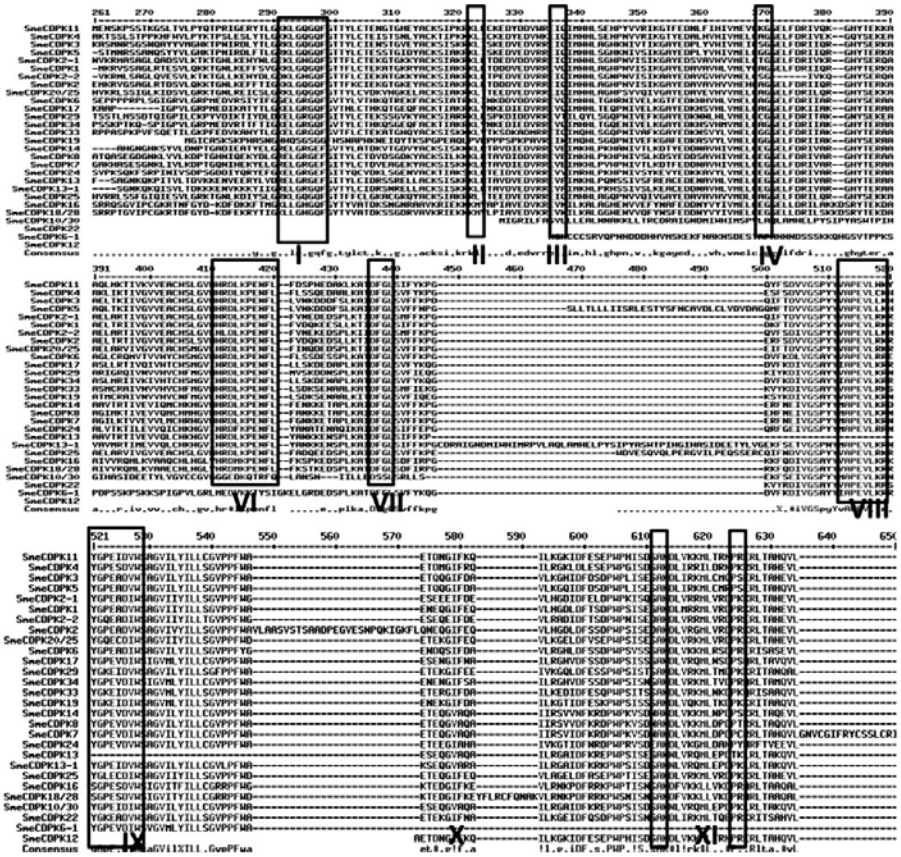


Fig. 2. Alignment of CDPKs family of *Solanum melongena*. The highlighted part shows the conserved motifs from 11 sub-domains of CDPK.

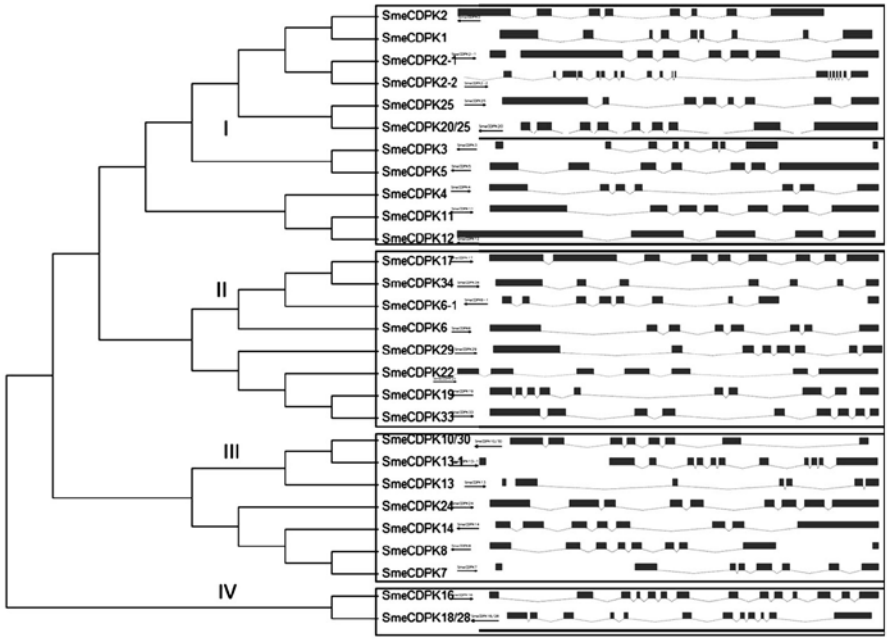


Fig. 3. Exon-intron organization of eggplant CDPK genes. The four sub-groups are marked by square boxes and numbered with roman numerals. The sizes of exons are proportional to their sequence lengths.

Table 2. BLAST hits of *SmeCDPKs* to ESTs (dbEST) from plant tissues at different development stages of eggplant.

Group	Name	Plant tissue
I	*SmeCDPK1*	Shoot
	SmeCDPK2	Shoot, mixture of flower bud leaf and other development stage
	SmeCDPK2-1	Mixture of flower bud leaf and other development stage
	SmeCDPK2-2	Fruit, leaf, mixture of placenta and immature seed, root, flower, fruit and various development stage
	SmeCDPK3	Ovary (anthesis), placenta, mixture of flower bud leaf and other development stage
	SmeCDPK4	mixture of flower bud leaf and other development stage
	SmeCDPK5	Ovary (anthesis), placenta, mixture of flower bud leaf and other development stage
	SmeCDPK11	Fruit, placenta, ovary (2 hr after anthesis), root, mixture of flower bud leaf and other development stage
	SmeCDPK12	Fruit, placenta, ovary (2 hr after anthesis), root, mixture of flower bud leaf and other development stage
	SmeCDPK20	-
	SmeCDPK25	Placenta, root, sepal, leaf, mixture of flower bud leaf and other development stage
II	*SmeCDPK6*	-
	SmeCDPK6-1	mixture of flower bud leaf and other development stage, mixture of petal and stamen
	SmeCDPK17	mixture of flower bud leaf and other development stage
	SmeCDPK19	flower bud leaf and other development stage, callus, placenta
	SmeCDPK22	Pericarp, callus, placenta, leaf, flower bud leaf and other development stage
	SmeCDPK29	-
	SmeCDPK33	Placenta, callus, pericarp, mixture of flower bud leaf and other development stage
	SmeCDPK34	Mixture of petal and stamen
III	*SmeCDPK7*	Mixture of flower bud leaf and other development stage, ovary (5 days pre-anthesis)
	SmeCDPK8	Mixture of flower bud leaf and other development stage, ovary (24 hr after anthesis), ovary
	SmeCDPK10/30	Peduncle, fruit, ovary (5 days pre-anthesis), flower bud leaf and other development stage
	SmeCDPK13	Mixture of flower bud leaf and other development stage, ovary, peduncle, placenta, fruit
	SmeCDPK13-1	Peduncle, fruit, shoot, ovary (5 days pre-anthesis)
	SmeCDPK14	-
	SmeCDPK24	-
IV	*SmeCDPK16*	Leaf
	SmeCDPK28/18	Ovary (5 days post-anthesis)

eggplant transcriptome represented root, stem and young leaves, the expression of *SmeCDPKs* shows from any of the root, stem or leaves tissues. Maximum expression is recorded for *SmeCDPK*14, whereas the lowest expression is recorded for *SmeCDPK*6-1. *SmeCDPK*2-1 showed no mapped reads suggesting no expression in root, leaves and stem at four leaves stage plants under normal condition. It could be possible that *SmeCDPK*2-1 has some role to play in specific tissue type or conditions such as biotic or abiotic stress. These expression results show that the identified CDPKs are genuine and show active gene expression.

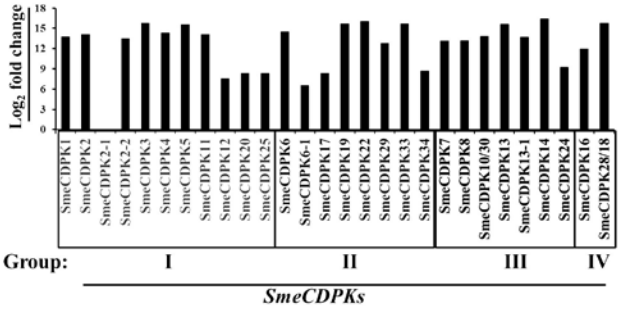


Fig. 4. Expression levels of eggplant CDPKs. Expression of *SmeCDPKs* is represented as $\log_2$ value of RPKM on Y axis.

ACKNOWLEDGEMENTS

The author thanks Director, ICAR-IIHR, Bengaluru and ICAR-NBPGR, New Delhi for providing research facilities and required training. Authors acknowledge use of High Performance Computational facility at ICAR-NBPGR, New Delhi.

REFERENCES

1. Asano, T., Tanaka, N., Yang, G., Hayashi, N. and Komatsu, S. 2005. Genome-wide identification of the rice calcium-dependent protein kinase and its closely related kinase gene families: comprehensive analysis of the CDPKs gene family in rice. *Plant Cell Physiol.* **46**: 356-66.

2. Cai, H., Cheng, J., Yan, Y., Xiao, Z., Li, J., Mou, S. and He, S. 2015. Genome-wide identification and expression analysis of calcium-dependent protein kinase and its closely related kinase genes in *Capsicum annuum*. *Front. Plant Sci.* **6**: 737.

3. Dubrovina, A.S., Kiselev, K.V. and Khristenko V.S. 2013. Expression of calcium-dependent protein kinase (CDPK) genes under abiotic stress conditions in wild-growing grapevine *Vitis amurensis*. *J. Plant Physiol*. **170**: 1491-1500.

4. Estruch, J.J., Kadwell, S., Merlin, E. and Crossland, L. 1994. Cloning and characterization of a maize pollen-specific calcium-dependent calmodulin-independent protein kinase. *Proc. Nat. Acad. Sci. USA*, **91**: 8837-41.

5. Hirakawa, H., Shirasawa, K., Miyatake, K., Nunome, T., Negoro, S., Ohyama, K., Yamaguchi, H., Sato, S., Isobe, S., Tabata, S. and Fukuoka H. 2014. Draft genome sequence of Eggplant (*Solanum melongena* L.): The representative *Solanum* species indigenous to the old world. *DNA Res.* **21**: 649-60.

6. Hrabak, E.M., Chan, C.W.M., Gribskov, M., Harper, J.F., Choi, J.H., Halford, N. and Harmon, A.C. 2003. The *Arabidopsis* CDPK-SnRK superfamily of protein kinases. *Plant Physiol.* **132**: 666-80.

7. Hu, Z., Lv, X., Xia, X., Zhou, J., Shi, K., Yu, J. and Zhou, Y. 2016. Genome-wide identification and expression analysis of calcium-dependent protein kinase in tomato. *Front. Plant Sci.* **7**: 469.

8. Klimecka, M. and Muszyńska, G. 2007. Structure and functions of plant calcium-dependent protein kinases. *Acta Biochim. Pol.* **54**: 219-33.

9. Knight, H. and Knight, M.R. 2001. Abiotic stress signalling pathways: specificity and crosstalk. *Trends Plant Sci*. **6**: 262-67.

10. Li, A.L., Zhu, Y.F., Tan, X.M., Wang, X., Wei, B., Guo, H.Z., Zhang, Z.L., Chen, X.B., Zhao, G.Y., Kong, X.Y., Jia, J.Z. and Mao, L. 2008. Evolutionary and functional study of the CDPK gene family in wheat (*Triticum aestivum* L.). *Plant Mol. Biol*. **66**: 429-43.

11. Mohanta, T.K., Mohanta, N., Mohanta, Y.K. and Bae, H. 2015. Genome-Wide Identification of Calcium Dependent Protein Kinase gene family in plant lineage shows presence of novel D-x-D and D-E-L motifs in EF-hand domain. *Front. Plant Sci.* **6**: 1146.

12. Wankhede, D.P., Kumari, Meenu, Aravind, J. and Rajkumar, S. 2017. Genome wide identification andcharacterization of Calcium Dependent Protein Kinase gene family in *Cajanus cajan*. *J. Env. Biol.* **38**: 167-77.

13. Reddy, A.S., Ali, G.S., Celesnik, H. and Day, I.S. 2011. Coping with stresses: roles of calcium-and calcium/ calmodulin-regulated gene expression. *Plant Cell*, **23**: 2010-32.

14. Xu, X., Liu, M., Lu, L., He, M., Qu, W., Xu, Q., Qi, X. and Chen, X. 2015. Genome-wide analysis and expression of the calcium-dependent protein kinase gene family in cucumber. *Mol. Genet. Genom.* **290**: 1403-14.

15. Yang, X., Cheng, Y.F., Deng, C., Ma, Y., Wang, Z.W., Chen, X.H. and Xue, L.B. 2014. Comparative transcriptome analysis of eggplant (*Solanum melongena* L.) and Turkey berry (*Solanum torvum* Sw.): Phylogenomics and disease resistance analysis. *BMC Genomics*, **15**: 412.

Received : March, 2016; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00103.7

Indian J. Hort. 74(4), December 2017: 533-540

Forecasting impact of climate change on potato productivity in West Bengal and adaptation strategies

V.K. Dua* **and Jagdev Sharma**

ICAR-Central Potato Research Institute, Shimla 171 001, Himachal Pradesh

ABSTRACT

The WOFOST crop growth simulation model was used to study the impact of climate change on potential potato productivity in West Bengal and also to devise management strategy to minimize the impact of climate change through selection of suitable variety and shifting the date of planting. The study was carried out at 13 locations in West Bengal. The simulation was done for baseline scenario and future climate scenario of 2020 and 2055 for three potato cultivars, *viz.*, Kufri Badshah (long duration), Kufri Jyoti (medium duration) and Kufri Pukhraj (short duration), for A1FI high emission scenario of temperature and CO_2. Simulation revealed that although the increase in temperature is likely to reduce the yield by 8.8 to 10.1% in 2020 and 23.7 to 28.8% in 2055, a corresponding increase in CO_2 may increase the yields by 4.5 to 4.7% in 2020 and 19.2 to 20.5% in 2055. However, under the combined effect of CO_2 and temperature, the highest decline of 6.1% in productivity of Kufri Pukhraj is expected followed by 5.9% in Kufri Jyoti and 5.1% Kufri Badshah in 2020, with corresponding figures of 12.0, 10.5 and 8.8% in 2055. Results further revealed that the negative effect of climate change on potato productivity can be counter-balanced to some extent through change in date of planting and/ or selection of suitable varieties, as it may bring down the reduction in yield from 5.7 to 1.5% in 2020 and 9.7 to 7.1% in 2055.

Key words: Adaptation, climate change, potato, West Bengal, yield.

INTRODUCTION

In India, potato is largely grown during winter season and is mainly confined to Indo-Gangetic plains. The autumn/winter planted crop in northern plains of India comprising the states of Uttar Pradesh, West Bengal, Bihar, Punjab and Haryana contributes 90% to total potato production in India. However, in the future changing climates, the productivity of potato in this region is likely to decrease as the availability of its suitable growing period is likely to be impacted seriously. As per the IPCC 4th Assessment Report, an increase in temperature ranging from 0.78ºC during September, October, November to 1.17ºC during December, January, February is expected under A1FI scenario by 2020, in South Asia. These changes are expected to aggravate and range from 1.71ºC during June, July, August to 3.16ºC during December, January, February, *i.e.* the main potato growing season in 2055. Thus, in 2020 the potato season is likely to be warmer by 0.78º to 1.18ºC and in 2055, by 2.41º to 3.16ºC under A1FI scenario. During the same period, the CO_2 calculation is likely to increase from present 400 to 415 ppm in 2020 and 590 ppm in 2055 (IPCC, 7). The increase in CO_2 is expected to bring on increase in productivity of potato as reported by many workers. However, Increase in temperature and atmospheric CO_2, both are interlinked and occur simultaneously and the CO_2 enrichment does not appear to compensate for the detrimental effects of higher temperature on tuber yield. Thus, here is an urgent need to study the impact of likely changes in temperature and CO_2 on regional vulnerability of potato productivity in future, in order to direct our research efforts to meet the challenges and devise adaptation strategy to minimize the likely impact of climate change. For this purpose, crop growth models are very useful and are used widely to simulate crop growth and yield of annual and perennial crops under diverse situations. Keeping this in view a study was undertaken to study the impact of climate change on potential potato productivity West Bengal, which is the second largest producer of potato in India and to select the suitable variety and date of planting to minimize the impact of climate change.

MATERIALS AND METHODS

WOFOST (WOrld FOod STudies), a mechanistic model developed at Wageningen University, the Netherlands, simulates the growth of a crop based upon eco-physiological processes. This model was used in the present study for impact assessment of climate change on potato productivity and scheduling planting date and selection of suitable cultivar to minimize climatic impact in West Bengal. The model is widely used to assess the effect of climate change

*Corresponding author's present address : Head, Division of Crop Production, ICAR-Central Potato Research Institute, Shimla 171 001; E-mail : vkdua65@yahoo.co.in

on the growth and yield of many crops including potato throughout the world (Wolf *et al*., 15) and is one of the three most widely used crop growth models for climate change studies (Tubiello and Ewert, 14). The major processes in this model are phenological development, CO_2 assimilation, transpiration, respiration, partitioning of assimilates to the various organs, and dry matter production (Boogaard *et al*., 2).

Further, WOFOST model has been calibrated for Indian potato cultivars using the time course data on potato growth and development for Indo-Gangetic region. The potato cultivars for which the model has been calibrated are Kufri Badshah, Kufri Jyoti, Kufri Bahar and Kufri Pukhraj (Dua *et al*., 3). Comparison of actual and simulated values revealed a difference of two days in emergence of Kufri Pukhraj, while no difference in case of Kufri Badshah and Kufri Pukhraj (Table 1). The difference in time taken for initiation of tubers from planting varied from + 1 (Kufri Badshah) to + 7 (Kufri Pukhraj) days for the three (Dua *et al*., 3). The simulated total dry matter yield ranged from 0.5 to 5.6% and the simulated total tuber dry matter yield varied from 0.6 to 7.5%, thus the calibration was satisfactory (Table 1). Fortnightly mean minimum and maximum temperatures (ºC) of some locations under study in West Bengal during baseline scenario (2000) are given in Table 2. For the present study, the model was run for 13 locations spread across the state (Table 3).

Three potato cultivars, belonging to late (Kufri Badshah), medium (Kufri Bahar) and early (Kufri Pukhraj) maturity group were selected for simulation studies. The latter two cultivars alone account for about 88% of total potato acreage in West Bengal (Annual Report, CPRI, 1). Since the normal date of planting in West Bengal is second week of October, the model was run for 10th of October. The simulation study was carried out to estimate potential yields of potato cultivars for baseline and both the future scenarios.

Indian Meteorological Department (IMD) district normal of 1971-2000 of 13 districts of West Bengal (Table 2) were used for baseline scenario (year

Table 1. Comparison of measured (actual) and simulated values of some important parameters of potato cultivars.

Parameter	Kufri Badshah			Kufri Jyoti			Kufri Pukhraj		
	Mea.	Sim.	Diff.	Mea.	Sim.	Diff.	Mea.	Sim.	Diff.
Emergence (days)	17	15	2	15	15	0	15	15	0
Tuber initiation (days)	37	36	1	32	35	3	29	36	7
Total DM yield (t/ha)	14.6	15.5	5.6%	12.3	12.6	2.5%	13.1	13.1	0.5%
Tuber DM yield (t/ha)	8.7	8.9	2.1%	7.3	7.9	7.5%	8.3	8.3	0.6%

Mea. = measured; sim. = simulated; diff. = difference

Table 2. Fortnightly mean minimum and maximum temperatures (ºC) of some locations under study in West Bengal during baseline scenario (2000).

Month	Fortnight	Bankura		Hoogly		Jalpaiguri		Malda		Medinipur	
		Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
October	I	23.7	32.9	24.4	32.9	21.4	31.7	24.5	32.3	24.6	33.9
	II	21.7	32.1	22.2	32.2	18.4	31.2	22.3	31.8	22.5	33.7
November	I	19.2	30.5	19.5	31.1	16.0	30.0	19.8	30.5	20.1	32.9
	II	16.7	29.3	16.7	29.8	13.9	28.6	17.1	29.1	17.4	31.5
December	I	13.5	26.9	13.5	27.8	11.5	27.0	14.6	27.3	14.8	29.7
	II	11.6	25.9	12.3	26.7	9.7	25.5	12.9	25.7	13.7	28.4
January	I	11.3	25.6	11.9	25.6	9.0	24.8	12.0	24.4	13.3	28.0
	II	12.5	26.8	12.6	26.5	9.6	24.8	12.3	25.2	14.2	28.2
February	I	14.0	27.6	14.4	27.8	10.5	25.4	13.6	26.9	16.5	29.2
	II	16.3	30.3	16.3	30.0	12.1	27.5	15.5	29.5	18.4	31.7
March	I	17.9	32.2	18.6	32.0	14.1	29.2	17.3	32.1	20.5	33.7
	II	20.9	36.0	21.3	34.4	16.7	31.4	19.9	34.9	22.9	35.8

Table 3. Geographical area of West Bengal (%) under different yield classes in 2000 (baseline scenario).

Potential productivity (t/ha)	Kufri Badshah	Kufri Jyoti	Kufri Pukhraj
24.0-30.0	0.0	8.0	4.6
30.1-36.0	29.0	27.8	23.5
36.1-42.0	50.1	48.6	42.3
42.1-48.0	20.9	15.6	29.7

2000). Hargreaves-Samani equation, which uses the maximum and minimum temperature to estimate solar radiation was employed for working out total solar radiation and is reported to be the best suited for Indian conditions (Samani, 11). After converting weather data into WOFOST weather file format the simulation studies were carried out for A1FI high emission scenario. For generation of scenario for 2020 and 2055, projected changes in surface air temperature for sub-regions of the Asia under SRES A1FI pathway based on the Fourth Assessment Report (AR4) Atmosphere-Ocean General Circulation Models (AOGCMs) were added to the baseline data (IPCC, 8). Based on the Bern-CC model for A1FI scenario projected atmospheric CO_2 concentration was used for incorporating the effect of change in CO_2 concentration in WOFOST model (IPCC, 8). For atmospheric CO_2 concentration 367 ppm (for baseline), 415 ppm (for 2020) and 590 ppm (for 2055) were used in the present study.

A 25-40% (mean 32.5%) increase in yield in C_3 plants due to doubling of CO_2 from 355-710 ppm has been reported (Wolf *et al.*, 15) hence for incorporating the impact of CO_2, the changes were made in the WOFOST model for parameters, *viz.* light-use efficiency of single leaf and maximum leaf CO_2 assimilation rate. In the earlier studies, changes have been made in initial angle (+11%) and in maximum of the CO_2 assimilation light response curve and response curve (+60%) parameters of WOFOST model for doubling CO_2 concentration from 355 to 710 ppm. Under the experimental conditions with non-limiting supply of water and nutrients, and where temperatures are kept near the optimum for crop growth, the yield increase for C_3 crops with a doubling of CO_2 has been estimated at 30% by various workers (Fuhrer, 6). Therefore, assuming linear relationship between the CO_2 increase and the growth processes we have taken these figures for yield as +10% (30/32.5 × 11) and + 55% (30/32.5 × 60) for doubling CO_2 concentration for potato. Accordingly, these parameters were changed for 2020 and 2055 for A1FI scenario as follows:

Change	2020	2055
Light-use efficiency of single leaf	+10% × (415-367)/ 355 = +1.4%	+10% × (590-367)/ 355 = + 6.28%
Maximum leaf CO_2 assimilation rate	+55% × (415-367)/ 355 = +7.4%	+55% × (590-367)/ 355 = +34.5%

An image of 500 m pixel size of West Bengal was used for generation of GIS maps using 'Geomatica' software for creation of maps of the base line productivity and change in productivity of different potato cultivars under future climate scenarios. Surface layers of the attributes data of each district containing productivity and change in productivity under different future climate scenarios were geo-statistically interpolated using kriging technique were produced. For estimation of the area falling under different class of attributes and modeling for % change in productivity was done in EASIPACE.

RESULTS AND DISCUSSION

The potato productivity varied largely within the state under baseline scenario ranging from 31.2 to 46.9, 29.0 to 45.4 and 29.6 to 48.2, for Kufri Badshah, Kufri Jyoti and Kufri Pukhraj, respectively. The mean productivity for respective cultivars was 38.9, 37.2 and 39.2 t/ha (Table 3) when the point data of 13 locations was extrapolated over entire state using kriging. In general a decline in average productivity was observed on moving from northern to southern part of the state to coastal parts. The northern districts, namely, Jalpaiguri, Dinajpur and Bardhaman had the higher baseline productivities, whereas the

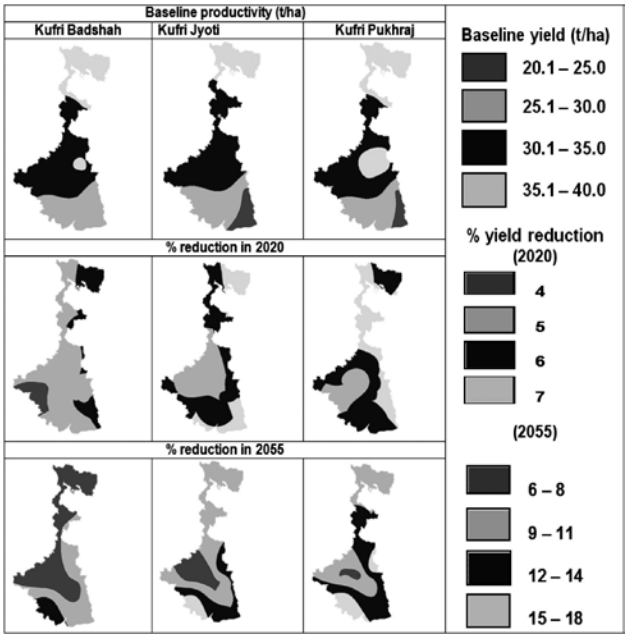


Fig. 1. Productivity decline in different potato growing areas of West Bengal.

southern districts Medinipur, North Parganas and South Parganas had lowest productivity for all the three potato cultivars for which the model was run (Fig. 1).

With the combined effect of temperature and CO_2, it is likely that mean productivity of Kufri Pukhraj will experience greatest decline (6.1%) followed by Kufri Jyoti (5.9%) and Kufri Badshah (5.1%) under 2020 climatic scenario. The similar trends are expected in 2055 with the corresponding figures of 12.0, 10.5 and 8.8%. In 2020, the maximum area of West Bengal (73%) is likely to experience about 5% yield reduction under Kufri Badshah; while about 88.7% geographical area is likely to experience 5 to 6% reduction under Kufri Jyoti. However, >80% area under Kufri Pukhraj is likely to experience 6-7% reduction in yield. The overall reduction under these varieties in 2020 is likely to range from 5.1% (Kufri Badshah) to 6.1% (Kufri Pukhraj) in 2020, when the whole state is taken under consideration.

The mean decline in productivity in 2055, when the temperature is likely to be 3.16ºC higher over baseline and the CO_2 concentration to be 590 ppm, is expected to 8.8% for Kufri Badshah, 10.5% of Kufri Jyoti and the maximum 12.0% for Kufri Pukhraj. About 92% of geographical area under Kufri Badshah is likely to experience 6 to 11% decline in 2055, while about 94% area is expected to show a decline ranging to 6-14% in Kufri Jyoti yield. However, in case of Kufri Pukhraj, much higher yield reduction is expected, *i.e.* 9-18% in about 97% area of West Bengal (Table 5).

In general, in 2020, the much reduction in Kufri Badshah productivity is expected in southern (coastal) parts of West Bengal under the future climate scenario. With the rise in temperature alone, model results have shown an average decline in the productivity of Kufri Badshah, Kufri Jyoti and Kufri Pukhraj to the tune of 8.8, 9.8 and 10.1% in 2020 and 23.7, 26.8 and 28.8%, respectively in 2055, averaged over 13 locations over baseline scenario. Increase in temperature has adverse effects on potato growth. High temperature is reported to reduce tuber number and size. Tuberization as well as gross photosynthetic rate is inhibited by even moderately high temperatures, ultimately affecting the total biomass production and tuber yield and these losses will be compensated to a greater extent by increase in CO_2 content. The corresponding expected increase in productivity of Kufri Badshah, Kufri Jyoti and Kufri Pukhraj due to rise in CO_2 is likely to be 4.5, 4.7, and 4.6% in 2020 and 19.2, 20.5 and 20.1% in 2055. The CO_2 concentration and assimilation are positively correlated and a 10% increase in tuber yield is estimated for every 100 ppm increase in CO_2 concentration (Miglietta *et al.,* 10). These positive effects are attributed to increased photosynthesis by 10 to 40% (Schapendonk *et al*., 12; Katny *et al*., 9). However, the increase in temperature and CO_2 go hand in hand and the increase in CO_2 does not seem to compensate the yield losses caused by increase in temperature at any location and under any scenario (Singh *et al*., 13). However, it offsets the losses to some extent. Thus, the net decline in the productivities of Kufri Badshah, Kufri Jyoti and Kufri Pukhraj as result of combined effect of rise in temperature and CO_2 will be 4.6, 5.4 and 5.9% in 2020, respectively. However, in 2055 more yield declines are predicted. The corresponding figures of productivity decline for the same cultivars are 8.1, 9.8 and 11.3%. Amongst three cultivars the highest yield loss (6.9%) will be in Malda district in case of Kufri Pukhraj followed by Kufri Jyoti (6.4%) in North Nadia South Parganas district and 5.3% in Kufri Badshah in Malda district under 2020 scenario. Amongst three cultivars, the highest yield loss (18.4%) will be in case of Kufri Pukhraj followed by Kufri Jyoti (17.15) and Kufri Badshah (13.9%) In case of Kufri Pukhraj highest loss of yield (18.4%) under 2055 scenario. However, for the same cultivar Nadia district will have more yield decline (5.2%) in 2020. In case of Kufri Jyoti again the highest loss of yield (17.1%) is predicted in 2055 and for the same cultivar Nadia district will have more yield decline (5.2%) in 2020.

The baseline productivity of all the three cultivars studied was highest in Jalpaiguri district. Rise in temperature is likely to decrease the productivity of Kufri Jyoti by 8 to 10.7%, while increased CO_2 will increase the productivity ranging from 4.6 to 5.2%. However, under combined effect of increase in temperature and CO_2, it is likely to decline productivity by 4.4 to 6.4% with an average reduction of 5.4% across the locations in 2020 scenario. However, in 2055 scenario, rise in temperature on Kufri Badshah is likely to decrease the productivity by 22.9 to 38.3%, and increased CO_2 will increase the productivity ranging from 19.5 to 22.9%. However, combined effect of increase in temperature and CO_2 would reduce the productivity by 6.1 to 17.1% with an average reduction of 9.8% across the locations in 2055. The reduction will be highest in Medinipur (17.1%) followed by Nadia (12.6%) district.

Increase in temperature by 1.18°C in Kufri Pukhraj (early) is likely to decrease the productivity by 8.7 to 10.8%, while increased CO_2 has shown to increase the productivity ranging from 4.4 (in Malda, Dinajpur and South Parganas districts) to 5.2% in Nadia district. However, combined effect of increase in temperature and CO_2 will reduce the

productivity by 4.4 (Bardhaman district) to 6.9% in Malda district with an average reduction of 5.9% across the locations in 2020 scenario. However, in 2050 scenario rise in temperature in Kufri Badshah (late) is likely to decrease the productivity by 23.5 in Bardhaman to 43.8% in Medinipur district, and increased CO_2 will increase the productivity ranging from 19.1 (Malda) to 22.7% (Nadia). However, combined effect of increase in temperature and CO_2 will reduce the productivity by 7.3 to 18.4% with an average reduction of 11.3% across the locations in 2050 scenario. The reduction will be highest in Medinipur (18.4%) followed by Nadia (16.0%) district. Under 2020 scenario productivity of the three cultivars will be decreased most in Medinipur followed Nadia by the combined effect of increased temperature and CO_2, whereas in Bardhaman and Bankura districts productivity of all the three cultivars will be least affected. Amongst three cultivars, Kufri Pukhraj on an average will experience maximum reduction in yield (5.9%) followed by Kufri Jyoti (5.4%) and Kufri Badshah (4.6%) across the districts. Under 2055 scenario also the similar trend was observed. Productivity of the three cultivars will be decreased most in Medinipur followed Nadia under the combined effect of increased temperature and CO_2, whereas in Bardhaman and Bankura districts productivity of all the three cultivars will be least affected. Amongst three cultivars, Kufri Pukhraj on an average will experience maximum reduction in yield (11.3%) followed by Kufri Jyoti (9.8%) and Kufri Badshah (8.1%) across the districts. Similar effects on potato productivity were observed in Punjab and Uttar Pradesh by Dua *et al*. (5, 6).

WOFOST model was run for nine dates at ten days interval starting from 11 October for adaptation strategies for all the three varieties under study. The model was run for five locations, *viz*. Bankura, Hoogly, Jalpaiguri, Malda and Medinipur, which are the representative of different potato growing areas spread across the state. The model results for three different cultivars and for three different climate scenario, one present and two future, are presented in Fig. 2. The productivity of all the three cultivars declined in the future climate scenarios, irrespective of date of planting. However, degree of decline varied at different locations. At Medinipur, Bankura and Malda, greater decline was simulated in Kufri Pukhraj and Kufri Jyoti at earlier planting dates, whereas, at Jalpaiguri, the decline in the yield of all the three cultivars was more at earliest planting and the gap reduced with delay in planting. The result further revealed that after reaching the peak in terms of productivity, during 31st October to 10th November period, a less decline in the productivity of Kufri

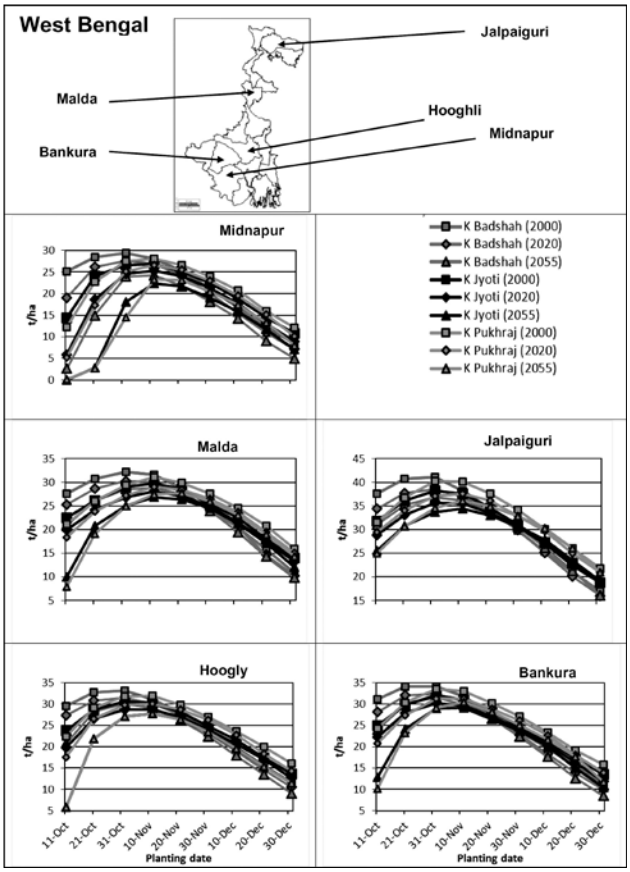


Fig. 2. Simulated productivity of different potato cultivars in different parts of West Bengal as affected by date of planting under baseline and future climate scenario.

Pukhraj was observed compared to remaining two cultivars, as a result, Kufri Pukhraj was found to be much suitable for late planting as compared to Kufri Badshah and Kufri Jyoti (Fig. 2 and Table 6). The analysis of change in planting date has revealed that shifting the date of planting to 10 days later at all the locations gave the highest yield of Kufri Badshah in 2020 scenario. This could result in a mean reduction of only 1.5% averaged over 5 districts compared to 5.1% under normal date of planting. Similarly, a zero to 10 day delay in planting may bring down the yield reduction in the same variety from 8.8 to 7.6% in 2055. However, change in date of planting was not found to be a suitable option for Kufri Badshah in 2055 for northern districts of Malda and Medinipur as advancing or delaying planting, both, resulted in decline in productivity of Kufri Badshah there, whereas in Bankura, Hoogly and Jalpaiguri, advancing planting by 10 days from the normal 10th November, gave the highest productivity as simulated by WOFOST for 2055.

Table 4. Productivity of potato cultivars in baseline year and changes thereof in future climates (interpolated results for total geographical area of West Bengal).

Particulars	Kufri Badshah	Kufri Jyoti	Kufri Pukhraj
Baseline yield (t/ha)	38.9	37.2	39.2
Overall reduction In yield (%)			
2020	5.1	5.9	6.1
2055	8.8	10.5	12.0
Yield reduction classes (%) in 2020	% geographical area of W. Bengal		
4	8.9	0.0	0.0
5	73.0	33.8	19.6
6	18.2	44.9	50.4
7	0.0	21.3	30.0
Yield reduction (%) classes in 2055			
6-8	54.6	17.2	2.8
9-11	37.0	56.3	44.1
12-14	8.4	20.1	40.4
15-18	0.0	6.4	12.7

A perusal of dates presented in Table 6 showed that in case of Kufri Badshah, advancing potato planting by 10 days from normal date of planting *i.e.* 10th of November, is likely to give the best yield at all the five locations in the future climate of 2020 and this could result in the lesser decline (-1.5%) in potential productivity as compared to productivity at normal dates of planting (-5.1%) (Table 4). It shows that just by manipulating the date of planting, the decline in the productivity of Kufri Badshah can be reduced by around 70%. In the year 2055, the change in date of planting is recommended for northern districts of Malda and Medinipur as advancing or delaying planting, both, resulted in decline in productivity of Kufri Badshah there, whereas in Bankura, Hoogly and Jalpaiguri, advancing planting by 10 days from the normal 10th November, gave the highest productivity as simulated by WOFOST for 2055. Planting Kufri Jyoti 10 days earlier than the normal date of planting (10th November) resulted in maximum simulated yield at Bankura, Hoogly and Jalpaiguri only in future climate scenario of 2020. However, the change in date of planting is not a suitable option for Kufri Jyoti under scenario of 2055. The model has shown that for Kufri Jyoti (2055 scenario) and for Kufri Pukhraj (both 2020 and 2055 scenarios) 10th November would be the best planting date.

From the simulation studies, it appears that Kufri Badshah has greater resilience against climate

Table 5. WOFOST simulated potential productivity of potato cultivars under baseline (2000) and future climate scenarios at different locations in West Bengal.

Station	Kufri Badshah							Kufri Jyoti							Kufri Pukhraj						
	Baseline yield (t/ha)	Change over baseline yield (%)						Baseline yield (t/ha)	Change over baseline yield (%)						Baseline yield (t/ha)	Change over baseline yield (%)					
		2020			2055				2020			2055				2020			2055		
		Due to temp.	Due to CO_2	Temp.+ CO_2	Due to temp.	Due to CO_2	Temp.+ CO_2		Due to temp.	Due to CO_2	Temp.+ CO_2	Due to temp.	Due to CO_2	Temp.+ CO_2		Due to temp.	Due to CO_2	Temp.+ CO_2	Due to temp.	Due to CO_2	Temp.+ CO_2
Bankura	38.4	-8.4	4.5	-4.2	-22.1	19.3	-6.4	37.3	-8.8	4.7	-4.4	-22.9	20.5	-6.1	39.7	-9.5	4.6	-5.3	-23.9	20	-7.8
Bardhaman	41.4	-7.8	4.5	-3.6	-21.5	19.3	-5.7	40.6	-9.1	4.7	-4.6	-23.3	20.5	-6.4	43.3	-8.7	4.6	-4.4	-23.5	20	-7.3
Dinajpur	42.5	-9	4.3	-4.9	-22.7	18.6	-7.6	40.7	-10.1	4.6	-5.9	-25.5	19.8	-9.4	42.7	-9.9	4.4	-5.7	-26.4	19.3	-11
Hawrah	36.5	-8.9	4.5	-4.8	-22.7	19.1	-7	34.8	-9.5	4.7	-5.2	-24.8	20.3	-8.3	36.6	-10.3	4.5	-6.2	-26	19.8	-9.9
Hoogly	37.9	-8.6	4.5	-4.5	-22.2	19.3	-6.4	36.2	-9.3	4.8	-5	-24.7	20.5	-8	38.3	-10.2	4.6	-6	-26	20.1	-9.5
Jalpaiguri	46.9	-8.8	4.4	-4.7	-22.7	18.8	-7.1	45.4	-9.9	4.7	-5.6	-25.1	20	-8.9	48.2	-10.5	4.5	-6.3	-26.1	19.6	-10.3
Kolkata	35.0	-9.1	4.4	-5	-22.7	19	-7.2	33.6	-10	4.6	-5.6	-25.6	20.3	-9.2	35.0	-10.6	4.5	-6.5	-26.9	19.9	-10
Malda	38.0	-9.2	4.3	-5.3	-23.2	18.5	-8.4	35.8	-9.7	4.5	-5.5	-25.7	19.5	-9.8	37.3	-10.9	4.4	-6.9	-28.2	19.1	-12.5
Medinipur	33.8	-8.5	4.6	-4.2	-29.9	20.1	-13.9	32.2	-10.2	5	-5.6	-38.3	21.7	-17.1	33.7	-9.7	4.8	-5.2	-43.8	21.3	-18.4
Nadia	42.7	-9.5	4.9	-5.1	-27.4	21.4	-10.6	40.7	-10	5.2	-5.3	-30.7	22.9	-12.6	43.8	-10.8	5.2	-6.1	-34.5	22.7	-16
North Parganas	31.2	-9.1	4.4	-5.1	-24.2	18.8	-9.2	29.0	-10.7	4.7	-6.4	-28.7	20.4	-11.4	29.6	-10.4	4.6	-6.2	-31.7	20	-12.4
South Parganas	31.6	-9.1	4.4	-5.1	-24.2	18.9	-9.1	29.4	-10.7	4.7	-6.4	-28.6	20.5	-11.4	30.0	-10.4	4.6	-6.2	-31.5	20	-12.2
Purulia	39.0	-8	4.4	-3.9	-22.3	18.8	-6.9	37.7	-9.6	4.6	-5.3	-24.6	19.9	-8.5	39.6	-9.4	4.4	-5.3	-25.4	19.3	-9.9
Mean	38.0	-8.8	4.5	-4.6	-23.7	19.2	-8.1	36.4	-9.8	4.7	-5.4	-26.8	20.5	-9.8	38.3	-10.1	4.6	-5.9	-28.8	20.1	-11.3

Table 6. Potential productivity at normal date of planting in baseline year (2000) and change in date of planting in future climates.

Location / Change over baseline	2000			2020				2055		
	Normal DOP*	Productivity (t/ha)	Best DOP	Productivity (t/ha)	Best DOP (days)	Productivity (%)	Best DOP	Productivity (t/ha)	Best DOP (days)	Productivity (%)
Kufri Badshah										
Bankura	10 Nov.	38.4	31-Oct	38.9	-10	1.3	31-Oct	37.8	-10	-1.6
Hoogly	10 Nov.	37.9	31-Oct	37.7	-10	-0.5	31-Oct	36	-10	-5.0
Jalpaiguri	10 Nov.	46.9	31-Oct	46.2	-10	-1.5	31-Oct	44.2	-10	-5.8
Malda	10 Nov.	38.0	31-Oct	36.3	-10	-4.5	10 Nov.	34.7	0	-8.7
Medinipur	10 Nov.	33.8	31-Oct	33.1	-10	-2.1	10 Nov.	28.1	0	-16.9
Mean		39.0		38.4		-1.5		36.2		-7.6
Kufri Jyoti										
Bankura	10 Nov.	37.3	31-Oct	36.1	-10	-3.2	10 Nov.	35	0	-6.2
Hoogly	10 Nov.	36.2	31-Oct	34.5	-10	-4.7	10 Nov.	33.3	0	-8.0
Jalpaiguri	10 Nov.	45.4	31-Oct	42.8	-10	-5.7	10 Nov.	41.3	0	-9.0
Malda	10 Nov.	35.8	10 Nov.	33.7	0	-5.9	10 Nov.	32.3	0	-9.8
Medinipur	10 Nov.	32.2	10 Nov.	30.3	0	-5.9	10 Nov.	30.3	0	-5.9
Mean		37.4		35.5		-5.1		34.4		-7.8
Kufri Pukhraj										
Bankura	10 Nov.	39.7	31 Oct and 10 Nov.	37.5	0 or -10	-5.5	10 Nov.	36.6	0	-7.8
Hoogly	10 Nov.	38.3	10 Nov.	36.0	0	-6.0	10 Nov.	34.7	0	-9.4
Jalpaiguri	10 Nov.	48.2	10 Nov.	45.2	0	-6.2	10 Nov.	43.3	0	-10.2
Malda	10 Nov.	37.3	10 Nov.	34.8	0	-6.7	10 Nov.	33.0	0	-11.5
Medinipur	10 Nov.	33.7	10 Nov.	32.0	0	-5.0	10 Nov.	27.9	0	-17.2
Mean		39.4		37.1		-5.9		35.1		-11.2

*DOP = Date of planting

change and could be more suitable cultivar in the future climates than Kufri Jyoti and Kufri Pukhraj at all the locations. The productivity of Kufri Badshah was 4.3% higher than Kufri Jyoti in the baseline scenario but in future scenario it increased to 5.2% (2055) and 8.2% (2020). Similarly, Kufri Badshah, which gave the lesser yield than Kufri Pukhraj in the baseline year (2000), is likely to yield more than 3% higher in future climates. Hence, with and advancement in 10 days in planting and by replacing the other two cultivars with Kufri Badshah at all the five locations representing the state can bring down the reduction in yield from 5.7% in 2020 (without adaptation) to +1.3 to 4.4% (mean -1.5%) and in 2055 from 10.4 to -7.1%.

REFERENCES

1. Anonymous. *Annual Report 2014-15*. ICAR-Central Potato Research Institute, Shimla.

2. Boogaard, H.L., Van Diepen, C.A., Rötter, R.P., Cabrera, J.M.C.A. and Van Laar, H.H. 1998. WOFOST 7.1; User's guide for the WOFOST 7.1 crop growth simulation model and WOFOST Control Center 1.5. Wageningen (Netherlands), DLO Win and Staring Centre, Technical Document.

3. Dua, V.K., Govindakrishnan, P.M. and Singh, B.P. 2014. Calibration of WOFOST model for potato in India. *Potato J*. **41**: 105-12.

4. Dua, V.K., Singh, B.P., Sushil Kumar and Lal, S.S. 2015. Impact of climate change on potato productivity in Uttar Pradesh and adaptation strategies – A simulation study. *Potato J*. **42**: 95-110.

5. Dua, V.K., Singh, B.P., Govindakrishnan, P.M., Sushil Kumar and Lal, S.S. 2013. Impact of climate change on potato productivity in Punjab - A simulation study. *Curr. Sci.* **105**: 787-94.

6. Fuhrer, J. 2003. Agro-ecosystem responses to combinations of elevated CO_2, ozone, and global climate change. *Agric. Ecosyst. Env.* **97**: 1-20.

7. IPCC, Climate Change 2001: *The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*, Houghton, J.T. *et al*. (Eds.), Cambridge University Press, Cambridge, UK, 881 p.

8. IPCC, Climate Change. 2007. *Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* Parry, M.L. *et al*. (Eds.), Cambridge University Press, Cambridge, UK, 2007, 976 p.

9. Katny, M.A.C., Hoffmann, G.T., Schrier, A.A., Fangmeier, A., Jager, H.J. and Bel van, A.E.J. 2005. Increase of photosynthesis and starch in potato under elevated CO_2 is dependent on leaf age. *J. Plant Physiol*. **162**: 429-38.

10. Miglietta, F., Magliulo, V., Bindi, M., Cerio, L., Vaccari, F. P., Loduca, V. and Peressotti, A.1998. Free air CO_2 enrichment of potato (*Solanum tuberosum* L.): Development, growth and yield. *Global Change Biol*. **4**: 163-72.

11. Samani, Z. 2000. Estimating solar radiation and evapotranspiration using minimum climatological data. *J. Irrig. Drain. Engg*. **126**: 265-67.

12. Schapendonk, A.H.C.M., Oijen van, N., Dijkstra, P., Pot, C.S., Jordi, W.J.R.M. and Stoopen, G.M. 2000. Effects of elevated CO_2 concentration on photosynthetic acclimation and productivity of two potato cultivars grown in open-top chambers. *Australian J. Plant Physiol*. **27**: 1119-30.

13. Singh, J.P., Lal, S.S., Govindakrishnan, P.M., Dua, V.K. and Pandey, S.K. 2010. Impact of climate change on potato in India. **In**: *Challenges of Climate Change: Indian Horticulture,* Singh, H.P., Singh, J.P. and Lal, S.S. (Eds.), Westville Publishers, New Delhi, pp. 90-99.

14. Tubiello, F.N. and Ewert, F. 2002. Simulating the effects of elevated CO_2 on crops: Approaches and applications for climate change. *European J. Agron.***18**: 57-74.

15. Wolf, J., Mandryk, M., Kanellopoulos, A., Oort, P.V., Schaap, B., Reidsma, P. and Ittersum, M.V. 2010. *Methodologies for Analyzing Future Farming Systems and Climate Change Impacts in Flevoland as Applied within the Agri Adapt Project*, Wageningen University and Research Centre, Wageningen, 108 p.

Received : May, 2016; Revised : September, 2017; Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00104.9

Indian J. Hort. 74(4), December 2017: 541-545

Effect of integrated nutrient management on growth and yield of tomato in Begusarai district of Bihar

Sanjay Kumar*, R.N. Singh, V.P. Choudhary and Neeraj
Krishi Vigyan Kendra, Khodawandpur, Begusarai 848202, Bihar

ABSTRACT

A field experiment consisting of nine treatments including control was conducted to find out the most appropriate integrated nutrient management system for sustainable tomato production during 2013-15. It was found that the application of 50% RDF + vermicompost @ 2 tonnes/ha + soil and seedling treatment with *Azotobacter* and PSB appeared as the most suited combination for providing maximum plant height and number of primary branches/ plant, earliest days to flowering and picking of first fruit, maximum number of fruits/ plant, most superior fruit weight, highest yield / plant and highest TSS. The maximum yield/ ha (784 q/ ha), net return (Rs. 850,300) and B:C ratio (9.39) were also recorded with application of 50% RDF + vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB. The application of 50% RDF + vermicompost @ 2 tonne/ ha proved as the next better treatment followed by 100% RDF.

Key words: Integrated nutrient management, growth, yield, tomato.

INTRODUCTION

Tomato *Lycopersicon esculentum* Mill.) is the most popular and nutritious vegetable crop, widely grown around the world and second ranked after potato. It is the most important 'protective foods' of Bihar grown in area of 47,690 ha with a production of 106,177 MT annually (Anon, 1). Tomato requires large quantity of both organic and inorganic fertilizers. It has been realized worldwide that chemical fertilizers, while increasing crop yield may have adverse effect on soil health and its fertility in case of imbalanced use (Kumar *et al.*, 4). Hence, an alternate technology, i.e. use of organic manures and biofertilizers in conjunction with inorganic fertilizers, which still sustain high yield over years and environmental safety. The beneficial use of *Azotobacter* and phosphorus solubilizing bacteria as a supplementary source of plant nutrition on agricultural crop is well documented (Shukla *et al.*, 12). These non-conventional sources of fertilizers are not only cost effective but simultaneously boost the productivity of soil and crop. No attempt has been reported on the effect of organic manure, biofertilizers and inorganic fertilizers on tomato, especially in Begusarai (Bihar) conditions. Therefore, the present study was undertaken to assess the effect of application of biofertilizer, organic and inorganic sources of nutrition on growth and yield of tomato at farmer's field.

MATERIALS AND METHODS

A field experiment at four locations was carried out at farmer's field of Barauni, Begusarai, Gadhpura and Bakhri blocks of Begusarai district during 2013-15 under irrigated conditions. The soil is sandy loam at all the locations and laid out in randomized block design with three replications. The soil samples of all the locations before the transplanting in main field were analyzed for essential nutrients, organic carbon, EC and pH (Jackson, 3). The details of soil value is given in Table 1, which shows the soils to be low in available nitrogen at Barauni, Bakhri and Gardhpura though available phosphorus was low at all the locations. Available potash was low in Bakhri and Gadhpura, while organic carbon was medium at all the locations and available nitrogen was medium in Begusarai, whereas available potash was also medium in Begusarai and Barauni. Half dose of nitrogen and full dose of phosphorus with potassium were applied as basal dose. The rest amount of nitrogen was applied in two split doses after transplantation in the main field. The experiment was conducted with nine treatments and each treatment in three replications. The details of experiment are given in Table 2. The seed of tomato hybrid VL-642 (Seminis Co.) was sown in nursery beds containing mixture of vermicompost and soil in 1:1 ratio. One-month-old seedlings were transplanted at 90 × 75 cm. The plot size was 4 × 2.5 m and 12 plants were accommodated in each plot. Cultural activities with irrigation were done whenever required. Picking was done as per maturity of fruits from each plant and yield data was recorded at all the locations, whereas yield attributes were recorded only from the fields of Begusarai block. Total soluble solids (TSS) was determined with the help of Erma hand refracto-meter

*Corresponding author's E-mail: sanjay4b1967@gmail.com

Table 1. Nutrient content (%) of vermicompost and chemical properties of the soils of experimental field at different locations.

Parameter	Vermi-compost		Location							
			Barauni		Begusarai		Gadhpura		Bakhari	
	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15	2013-14	2014-15
pH (1:2)	7.2	7.3	7.8	7.8	7.9	7.8	7.9	7.9	8.1	8.00
EC (1.2) dS m^{-1}	-	-	0.62	0.59	0.54	0.53	0.69	0.68	0.72	0.72
Organic C (%)	-	-	0.67	0.69	0.72	0.74	0.59	0.59	0.51	0.50
N (kg/ ha)	1.53	1.41	194	201	282	287	206	213	201	200.00
P_2O_5 (kg/ ha)	1.62	1.39	40	43	32	29	31	27	30	30.00
K_2O (kg/ ha)	0.87	0.93	167	161	172	172	92	102	89	86.00

Table 2. Details of the treatments.

Treatment	Code	Details
Control	T1	No fertilizer / Vermicompost
100% recommended dose of fertilizer (RDF)	T2	N : P : K : : 120 : 80 : 80
50% RDF	T3	N : P : K : : 60 : 40 : 40
Vermicompost (2 tonnes/ ha)	T4	2 kg Vermicompost per plot
Azotobacter (3 kg/ ha) + PSB (3 kg/ ha)	T5	3 kg each of *Azotobacter* and PSB per plot were thoroughly mixed in soil before transplanting and seedling inoculation was done one hour before transplanting with culture of *Azotobacter* and PSB
50% RDF + vermicompost (2 tonnes/ ha)	T6	T3 + T4
50% RDF + *Azotobacter* (3 kg/ha) + PSB (3 kg/ ha)	T7	T3 + T5
Vermicompost (2 tonnes/ha) + *Azotobacter* (3 kg/ ha) + PSB (3 kg/ ha)	T8	T4 + T5
50% RDF + Vermicompost (2 tonnes/ ha) + *Azotobacter* (3 kg/ ha) + PSB (3 kg/ ha)	T9	T3 + T4 + T5

and expressed as percent TSS. Data obtained from tomato crops for two consecutive years were pooled and statistically analyzed as procedure given by Panse and Sukhatme (8).

RESULTS AND DISCUSSION

The experimental data (Table 3) clearly revealed that the maximum plant height (126 cm) was recorded with T_9 (50% RDF + vermicompost + soil and seedling treatment with *Azotobacter* and PSB) and found at par with treatment T_6 (123.2 cm), where integrated application of 50% RDF with vermicompost @ 2 tonnes/ha was applied. The next better treatment was T_2 (122.6 cm) at recommended dose of chemical fertilizer application and found statistically similar with the treatments T_7 and T_3 but significantly superior over the treatments T_4, T_5 and T_8. The shortest plant height (90.4 cm) was recorded with control. Similarly, maximum number of primary branches/ plant (8.50/ plant) were also recorded with T_9 (50% RDF + vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB) and found statistically identical with T_6 (50% RDF + vermicompost @ 2 tonnes/ ha) and T_2 (100% RDF). The least number of branches were recorded with control (6.42/ plant). The increase in height and number of branches/ plant due to application of organic and inorganic fertilizers might be due to better inorganic nitrogen utilization in the presence of biofertilizer, enhanced biological fixation and better development of root system with possible higher synthesis of plant growth hormones (Kumaraswami and Madalageri, 6; Pandey and Kumar, 7). The results also revealed that the initiation of flowering in tomato was influenced by the nutrient and biofertilizer application. The earliest flowering (37 days) was attained by the integrated application of 50% recommended dose of fertilizer and vermicompost @ 2 tonnes/ ha alongwith soil and

Table 3. Tomato growth, yield attributing characters and yield as influenced by inorganic and organic fertilizers in Begusarai Block.

Treatment	Plant ht. (cm)	No. of main br.	Days to first flowering	Days to first picking	No. of fruits per plant	Av. fruits per plant	Yield per plant (g)	TSS (%)
T1	90.40	6.42	42.00	77.00	47.00	76.00	3576.00	4.69
T6	122.60	8.00	39.00	71.20	60.00	87.00	5542.00	5.42
T3	117.30	7.50	40.30	72.00	56.00	83.00	4651.00	5.59
T4	112.00	7.08	41.20	74.60	51.00	82.00	4194.00	5.22
T5	109.40	6.92	41.60	75.00	48.00	78.00	3747.00	5.07
T6	123.20	8.17	38.00	71.00	61.00	101.00	6167.00	5.72
T7	120.60	7.83	40.00	71.60	57.00	92.00	5246.00	5.60
T8	114.80	7.17	41.00	71.80	54.00	89.00	4856.00	5.86
T9	126.00	8.50	37.00	70.00	62.00	105.00	6535.00	6.09
CD (p = 0.05)	9.8	0.58	4.4	6.96	8.22	22.06	724.14	0.41

seedling treatment with *Azotobacter* & PSB, which was statistically identical with all the treatment except control where days to first flowering was more (42 days) than all the eight treatments. Similarly, days to first picking of fruit was also recorded least (70 days) in T_9 and statistically at par with all the treatments except control in which this value was maximum (77 days). All treatments showed earliness in flowering over control for days to first harvesting. Earliness of flowering and fruiting is an important trait in tomato crop and in these cases; it could be attributed to the faster enhancement of vegetative growth and storing sufficient reserve food material for differentiation of buds into flower buds. The results thus indicate that balanced application of all the essential elements is essential to get early flowering in tomato. Earlier, Renuka and Shankar (10) also reported earliness in flowering and fruiting in tomato when FYM was used with biogas slurry.

The results (Table 3) indicated that the treatment T_9 (50% RDF + vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB) produced maximum number of fruits per plant (62). The next best treatment in this regard was T_6 (50% RDF + vermicompost @ 2 tonnes/ ha) with 61 fruits/ plant and followed by T_2 (100% RDF) with 60 fruits/ plant. All the treatments were found statistically at par to each other except T_4, T_5 and control in respect of bearing the number of fruits/ plant. The treatment T_9 (50% RDF + vermicompost @ 2 tonnes/ha + soil and seedling treatment with *Azotobacter* and PSB) produced fruits with superior fruit weight (105 g/ fruit) but statistically at par with all the treatment except T_4, T_5 and control. The results of present studies are in accordance with Kumaran *et al.* (5) who reported an integrated application of organic and inorganic sources of nutrients gave more mean fruit number and fruit weight in tomato.

The treatment T_9 (50% RDF + vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB) emerged as most superior in production of more yield per plant (6535 g/ plant) and recorded similarly identical with production of tomato fruit 6167 g/plant in T_6 (50% RDF + vermicompost @ 2 tonnes/ ha). The next better treatment in this regard were T_2 (100% RDF), T_7 (50% RDF + soil and seedling treatment with *Azotobacter* and PSB), T_8 (vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB), T_3 (50% RDF), T_4 (vermicompost @ 2 tonnes/ ha) and T_5 (soil and seedling treatment with *Azotobacter* & PSB) in decreasing order. The lowest yield/ plant was obtained from treatment T_1 (control). It is a well known fact that nitrogen and phosphorus are essential constituents of protein and chlorophyll alongwith their movement in many other compounds of physiological importance in plant metabolism. Hence, increase in yield due to application of organic manure, fertilizer and biofertilizer together might be responsible for synthesis of plant growth hormones, development of good root system, therefore better nutrient utilization by better the crop plants. The results of the study are also in agreement with the findings of Sepat *et al.* (11). The total soluble solids (TSS) in fruit was significantly affected by the treatments. The highest TSS (6.09%) was recorded with an integrated application of 50% RDF + vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotabacte*r and PSB but found at par with the application of vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacter* and PSB (5.86%) and integrated application of 50% RDF + vermicompost

@ 2 tonnes/ ha (5.72%). Yadav *et al.* (13) reported that TSS increased when plants were supplied either with organic sources alone or in combination with inorganic components. Shukla *et al.* (12) also reported the fertilizer from different sources in combination resulted in higher yield of quality fruits.

It is revealed (Table 4) that the combined application of 50% RDF+ vermicompost @ 2 tonnes/ ha + soil and seedling treatment with *Azotobacte*r and PSB resulted in the highest yield at all the four locations but found statistically identical with the integrated application of 50% RDF + vermicompost @ 2 tonnes/ha and 100% RDF. The lowest yield was recorded with control treatment. It is clear that the integrated application of organic and inorganic sources of nutrition with biofertilizer and combined application of organic and inorganic sources of nutrition proved superiority over the recommended dose of fertilizers. These results are is close agreement with the findings of Gajbhiye *et al.* (2) and Patil *et al.* (9). This might be due to the availability of plant nutrients by improving soil physical conditions and solubilizing the nutrients in soil by applying organic sources of nutrition with biofertilizers.

Regarding the economics of tomato cultivation (Table 5) clearly indicate that though the cost of cultivation was higher with application of 50% RDF + vermicompost treatment alongwith soil and seedling treatment with *Azotobacter* and PSB (T_9) but it gave higher yield and net profit (Rs. 8,50,300/ ha) over control (Rs. 4,40,800/ ha). This treatment also gave highest B:C ratio (9.39) compared to control. Other two treatments (T_2 and T_6) gave almost equal B:C ratio. Sole use of organic and biofertilizer (T_4 and T_5) brought lower B:C ratio (6.06), while least in control (5.95). Application of organic, biofertilizer and inorganic sources of nutrition together showed superiority over inorganic (50% RDF) alone. Thus, it was inferred that application of 50% RDF + vermicompost (2 tonnes/ ha) + soil and seedling treatment with biofertilizer could be the best option for getting sustainable yields of tomato in Begusarai district of Bihar.

Table 4. Effect of vermi-compost, biofertilizer and chemical fertilizer on fruit yield at different locations of tomato.

Treatment	Yield (q/ha)			
	Barauni	Begusarai	Gadhpura	Bakhari
T_1	362	429	359	292
T_2	601	665	564	464
T_3	524	558	502	432
T_4	494	503	498	426
T_5	421	450	402	394
T_6	626	740	584	479
T_7	578	629	542	459
T_8	560	583	521	443
T_9	652	784	603	512
CD (p = 0.05)	66.4	124.5	56.4	50.2

Table 5. Effect of benefit:cost ratio on tomato cultivation in Begusari Block.

Treatment	Yield (q/ha)	Cost of cultivation (Rs/ha)	Gross return (Rs/ha)	Net return (Rs/ha)	B:C ratio
T_1	429	74000	514800	440800	5.95
T_2	665	80500	798000	717500	8.91
T_3	558	79500	669600	590100	7.42
T_4	503	85500	603600	518100	6.06
T_5	450	76500	540000	463500	6.06
T_6	740	89500	888000	798500	8.92
T_7	629	83500	754800	671300	8.03
T_8	583	86500	699600	613100	7.09
T_9	784	90500	940800	850300	9.39

Tomato price = Rs. 12/kg

REFERENCES

1. Anonymous. 2015. *Economic Survery* 2014-15, Finance Department, Govt. of Bihar.
2. Gajbhiye, R.P., Sharma, R.R. and Tewari, R.N. 2003. Effect of biofertilizers on growth and yield of tomato. *Indian J. Hort*. **60**: 368-71.
3. Jackson, M.L. 1967. *Soil Chemical Analysis*, Prentice Hall, of India, Pvt. Ltd. New Delhi.
4. Kumar, R., Batra, V.K. and Singh, R.K. 2012. Effect of organic and inorganic nutrition on growth, yield nutrient uptake and fruit quality of tomato. *Haryana J. Hort. Sci.* **41**: 164-67.
5. Kumaran, S., Natarajan, S. and Thamuraj, S. 1998. Effect of organic and inorganic fertilizers on growth, yield and quality of tomato. *South Indian Hort*. **46**: 203-05.
6. Kumarswamy, D. and Madalageri, B.B. 1990. Effect of *Azotobacter* on tomato. *South Indian Hort.* **38**: 345-46
7. Pandey, A. and Kumar, S. 1989. Potential of *Azotobacter* and *Azospirillum* as bio-fertilizers for upland agriculture - A review. *J. Sci. Indust. Res*. **48**: 134-44.

8. Panse, V.G. and Sukhatme, P.V. 1978. *Statistical methods for Agricultural Workers*, ICAR, New Delhi.

9. Patil, M.B., Mohammed, R.G. and Ghadge, P.M. 2004. Effect of organic and inorganic fertilizers on growth, yield and quality of tomato. *J. Maharashtra Agric. Univ.* **29**: 124-27.

10. Renuka, B. and Shankar, R.C. 2001. Effect of organic fertilizers on growth and yield of tomato. *South Indian Hort.* **49**: 216-17

11. Sepat, N.K., Kumar, A., Yadav, J. and Srivastava, R.B. 2012. Effect of integrated nutrient management on growth, yield and quality of tomato in trans Himalayan. *Ann. Pl. Soil Res*. **14**: 120-23.

12. Shukla, Y.R., Thakur, A.K. and Joshi, A. 2009. Effect of inorganic and bio-fertilizers on yield and horticultural traits in tomato. *Indian J. Hort.* **66**: 285-87.

13. Yadav, B.D., Singh, B. and Sharma, Y.K. 2004. Production of tomato under organic condition. *Haryana J. Hort. Sci.* **33**: 306-07.

Received : April, 2016; Revised : September, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00105.0

Indian J. Hort. 74(4), December 2017: 546-551

Effect of bentonite on arsenic uptake by beet leaf cultivar Pusa Bharti grown on contaminated soil

Raj Mukhopadhyay[**], K.M. Manjaiah[*], S.C. Datta and R.K. Yadav[***]

Division of Soil Science and Agricultural Chemistry, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Greenhouse experiments were conducted to assess the effect of bentonite amendment on arsenic uptake by beet leaf (*Beta vulgaris* L. bar. *bengalensis* Roxb.) cultivar Pusa Bharti. Application of bentonite to the contaminated soil increased biomass yield from 0.24 (control) to 0.44 g pot^{-1} @ 0.25% bentonite amended soil (T_3) at first harvest and 0.65 g pot^{-1} @ 0.5% bentonite amended soil (T_4) after second harvest over the two years. The bio-accumulation factor (%) of arsenic varied from 13.60 in control (T_1) to 3.77 (T_3) in soil amended with 0.25% bentonite at first harvest and reduced by 62.4% in the second harvest. The hazard quotient was significantly reduced below 0.50 because of 0.25 and 0.50% bentonite application after first and second harvest. The arsenic concentration in plants was reduced to 0.49 from 1.77 mg kg^{-1} in first harvest, while the values were 0.50 and 1.39 mg kg^{-1} in 0.5% amended and control soil, respectively after second harvest. Importantly, clay amendments effectively reduced the labile arsenic content up to 54.8 and 58.5% in soil during first and second harvest, respectively. Soil pH was raised significantly only in 0.5% clay amended soil (7.89) as compared to control (6.70) after complete crop harvest. The effect of bentonite application @ 0.25 and 0.5% was statistically at par in most of the parameters. Hence, it may be recommended that application of bentonite @ 0.25% may be useful to reduce the arsenic uptake by beet leaf as well as its immobilization in polluted soil.

Key words: Arsenic, bentonite, hazard quotient, immobilization, beet leaf.

INTRODUCTION

Vegetable cultivation has become important to ensure the nutritional security of ever growing population in the world. At present, it is very common in the marginal lands of developing countries of the world. On the other hand, worldwide 170 million people are exposed to arsenic (As) contamination. The contamination of water occurs due to the dissolution of minerals like arsenopyrites from parent materials, geochemical reactions, and biological activities or from anthropogenic sources such as the leaching of manmade arsenic compounds from smelting of metal ores, and wood preservatives (Shevade and Ford, 11). Arsenic contamination of groundwater and soil caused by those materials pose a great threat to vegetable cultivation by application of contaminated irrigation water. Consumption of arsenic contaminated drinking water may cause kidney, urinary tract, liver, skin and rectum cancers in humans. Non-carcinogenic diseases related to arsenic exposure are hypertension, diabetes mellitus, cerebrovascular and cardiovascular systems, and dysfunction of respiratory system (Thomas *et al*., 13). Thus, arsenic contaminated soils affect the food quality and safety by risk of biomagnifications and bioaccumulation in human food chain because it is poorly biodegradable (Mihaltan *et al*., 7).

Therefore, a strong immobilization technique is required to reduce the transfer of arsenic from soil to crop and crop to human food chain. Clay minerals are one of the adsorbents of arsenic, which act as good amendment due to its ease availability, non-toxic, cost effective and large specific surface area (McBride and Martinez, 5; Peremolov *et al*., 9). The probable mechanism of arsenic immobilization in soil is through adsorption. Application of such clay minerals to soil for arsenic alleviation, health risk assessment of arsenic intake through vegetables grown on contaminated soil is an underexplored area of research. In view of that, bentonite clay mineral was selected as immobilizing agent for remediation of arsenic contaminated soil.

MATERIALS AND METHODS

Smectite clay mineral in the form of bentonite was evaluated to check the bioavailability of arsenic from soil to crop and arsenic uptake by beet leaf. Bentonite clays were purchased from S D Fine-Chem Ltd., Mumbai, India. The arsenic contaminated soil used for pot experiment was collected (0-15 cm depth, order: Inceptisols) from Mitrapur, West Bengal, India. The soil is slightly acidic to neutral (pH = 6.49) having electrical conductivity (EC) 0.26 dS m^{-1}, organic

*Corresponding author's E-mail: manjaiah@iari.res.in

**Division of Soil and Crop Management, ICAR-Central Soil Salinity Research Institute, Karnal 132001, Haryana

***Division of Vegetable Science, ICAR-IARI, New Delhi 110012

carbon (OC) 4.50 g kg^{-1}, total soil arsenic content 14.10 mg kg^{-1} and available arsenic content 3.60 mg kg^{-1}. Pot culture experiments were conducted during winter 2016 and 2017 with beet leaf cultivar Pusa Bharti as a test crop and to study the residual effect of clay amendment, under greenhouse conditions. Air-dried, grounded, 2 mm sieved 4 kg of contaminated soil was used in each pot. Bentonite clay mineral was applied to the soil at 4 levels (T_1 = control (0%) where no bentonite was applied, T_2 = 0.125%, T_3 = 0.25%, and T_4 = 0.50%) with three replications. Recommended dose of fertilizer 240:120:120 mg pot^{-1} NPK was added to soil. Half dose of nitrogen and full dose of phosphorus and potassium was applied as basal and remaining nitrogen was added 30 days after sowing. The plants were thinned to 4 seedlings per pot after germination. 60% water holding capacity of the soil was maintained during the course of study. Two cuttings were taken at 30 day interval and concentration of arsenic in the digested samples was determined with Inductively Coupled Plasma Mass spectroscopy (ICP-MS). Available soil arsenic was extracted by Olsen's reagent (0.5 *M* $NaHCO_3$, pH = 8.5) and total soil arsenic was determined by HF (Hydrofluoric acid) digestion method (McLaren *et al.*, 6) and arsenic in the filtrate was analyzed by ICP-MS. The non-carcinogenic risk of consumption of beet leaf grown on arsenic contaminated soil amended with bentonite was characterized by hazard quotient (HQ). Hazard quotient is defined as the ratio of the average daily dose (ADD; mg^{-1} kg^{-1} day^{-1}) of As to their reference dose (RfD; mg^{-1} kg^{-1} day^{-1}) (Kumararaja *et al.*, 3). For arsenic, RfD is 0.0003 mg kg^{-1} body weight day^{-1} (Ramirez-Andreotta *et al.*, 10). Statistical analysis of data over the two years was done using pool analysis.

RESULTS AND DISCUSSION

Beet leaf has high nutritional value as a leafy vegetable. The leaf and shoot biomass dry weight usually show plants' capability to resist the adverse environmental impact like temperature stress, moisture stress, heavy metal contamination *etc*. In the present study, the amount of clay mineral had a significant contribution to the high biomass yield of beet leaf grown in an arsenic contaminated soil. Plant dry biomass yield increased with increasing the amount of clay application. After first harvest at 30 DAS (days after sowing), highest yield was obtained under T_3 (@ 0.25% bentonite ameneded soil) and T_4 treatments (@ 0.5% bentonite amended soil) after second harvest (30 DAS) over the two years as compared to the control T_1 (contaminated soil). The biomass yield was found to be increased from 0.24 in T_1 (control) to 0.44 g pot^{-1} in T_3 at first harvest over the two years (Table 1). However, at second harvest, the highest biomass yield was increased by 62.5% by application of bentonite @ 0.5% compared to the unamended soil over the two years. However, there was no significant difference in yield was observed between the two experimental years. The higher biomass yield indicated that application of bentonite improved the plant growth by arsenic adsorption/ immobilization in soil. The probable mechanism might be due to increase in alkalinity, which helped to adsorb arsenic through physical adsorption on Si-O and Al-O groups on the edges of clay particles (Su *et al*., 12; Mar *et al*., 4).

Bioaccumulation factor is the ratio of metal concentration in plant to that in the soil or the effectiveness of a plant in concentrating the pollutant into aerial part. This parameter assessed the efficiency of bentonite to immobilize arsenic in soil (Table 2). The bioaccumulation factor (%) of arsenic varied from 13.60 in control (T_1) to 6.41 (T_2), 3.77 (T_3), and 4.60 (T_4) in soil ameneded with 0.125, 0.25 and 0.5 % bentonite, respectively at first harvest over the two years. At second harvest, bioaccumulation factor reduced by 34.01% (T_2), 56.7% (T_3), and 62.4% (T_4) over the control (T_1) by application of 0.125, 0.25, and 0.5% bentonite, respectively, pooled over the two years. But there was no significant difference observed in bioaccumulation factor between the two experimental years during both the harvesting times. The results clearly indicated that bioaccumulation factor decreased with increasing the amount of clay addition to the arsenic contaminated soil. Very small bio-accumulation factor (%) of beet leaf had been shown in control as well as bentonite treated plot because the factor was calculated in terms of dry weight basis, which is far more less than the fresh weight. Similar report was also obtained by Gaw *et al*. (1) when they used

Table 1. Effect of different levels of bentonite on beet leaf biomass yield (g pot^{-1}) after 1st and 2nd harvest pooled over two years (2016 and 2017).

Year	Biomass yield 1st harvest	Year	Biomass yield 2nd harvest
1st	0.34	1st	0.50
2nd	0.37	2nd	0.56
$CD_{0.05}$	0.08	$CD_{0.05}$	0.14
Treatment		Treatment	
T_1	0.24	T_1	0.40
T_2	0.35	T_2	0.44
T_3	0.44	T_3	0.61
T_4	0.38	T_4	0.65
$CD_{0.05}$	0.12	$CD_{0.05}$	0.19

Table 2. Effect of different levels of bentonite on bioaccumulation factor (%), hazard quotient and arsenic concentration (mg kg^{-1} dry weight) in beet leaf after 1st and 2nd harvest pooled over two years (2016 and 2017).

Year	Bioaccumulation factor		Hazard quotient		Arsenic concentration	
	1st harvest	2nd harvest	1st harvest	2nd harvest	1st harvest	2nd harvest
1st	7.23	5.68	0.74	0.57	0.92	0.73
2nd	6.96	8.29	0.91	0.62	0.88	0.95
$CD_{0.05}$	3.06	4.82	0.44	0.08	0.35	0.49
Treatment						
T_1	13.60	11.32	1.64	0.98	1.77	1.39
T_2	6.41	7.47	0.75	0.60	0.78	0.90
T_3	3.77	4.90	0.49	0.43	0.49	0.57
T_4	4.60	4.26	0.41	0.39	0.55	0.50
$CD_{0.05}$	1.76	3.05	0.30	0.23	0.22	0.40

lettuce and radish to decontaminate the horticultural soils from sigmaDDT, arsenic and heavy metals like cadmium, lead, and copper. Hence, beet leaf showed good arsenic uptake capacity as a test crop grown in arsenic contaminated soil.

Consumption of arsenic contaminated food materials is one of the important pathways to affect the human being. Along with food intake, it is also possible that incidental ingestion and inhalation of dust containing arsenic may be a significant pathway of exposure (Huq *et al*., 2). To assess the arsenic immobilization efficiency of bentonite clay mineral, health risk assessment on vegetable consumption from clay amended soil and contaminated soils, hazard quotient was calculated using standard protocol (Kumararaja *et al*., 3). The results suggested that (Table 2) hazard quotient was significantly reduced from 1.64 in control (T_1) to 0.75 (T_2), 0.49 (T_3), 0.41 (T_4) in clay amended soil when bentonite was applied @ 0.125, 0.25 and 0.5%, respectively, after first harvest over the two years. At second harvest, the hazard quotient was reduced by 36.7, 54.1 and 58.2% over the control when bentonite applied @ 0.125, 0.25 and 0.5%, respectively, over the two years. Although, there was no significant difference in HQ values during the two experimental years at both the harvesting times, however it was clear that HQ values were lower in all the treatments during second harvest. The reduction in hazard quotient on application of bentonite might be due to reduced metal uptake by the beet leaf due to its adsorption/ immobilization. Values of HQ equal to or more than 1 indicates that consumption of food materials may be hazardous to human being due to intake of arsenic (Ramirez-Andreotta *et al.*, 10). Consumption of leafy vegetables constitutes only food materials, which contribute to arsenic uptake in human food chain. If other sources of contamination like drinking water, groundwater for irrigation is taken into account, then HQ of 0.50 may be considered as safe limit in risk assessment of contaminated soil. Therefore, according to this limit, the unamended soil as well as 0.125% bentonite treated soil may still be hazardous to human being after first and second harvest. Moreover, the control soil having hazard quotient of 1.64 and 0.98 after first and second harvest, respectively, might be unsafe for growing any vegetable on this soils and need application of amendments for safe and sustainable crop production.

Bioavailability of arsenic depends on its uptake by plants and forms of arsenic. The concentration of arsenic in above ground portion of beet leaf was significantly reduced by bentonite application to the contaminated soil (Table 2). Application of bentonite reduced the arsenic concentration in beet leaf to 0.78 mg kg^{-1} (T_2), 0.49 mg kg^{-1} (T_3) and 0.55 mg kg^{-1} (T_4) from 1.77 mg kg^{-1} (T_1) in control soil at first harvest and the values for second harvest were found to be 1.39 mg kg^{-1} (T_1) in control soil and 0.90 mg kg^{-1} (T_2), 0.57 mg kg^{-1} (T_3), 0.50 mg kg^{-1} (T_4) when bentonite was applied @ 0.125, 0.25 and 0.5%, respectively to experimental soil. However, arsenic concentration in plants in the soils of T_3 and T_4 was statistically at par during both the harvesting time over the two experimental years. Hence, Application of bentonite @ 0.25% to the soil might have served the purpose in practical sense. The bioavailability of arsenic depends on its concentration in soil solution and its rate of release from soil solids. The arsenic concentration in the leafy portion of beet leaf decreased considerably due to immobilization of arsenic in soil after the application of clay amendment to soil.

The labile fraction (available form) of arsenic were significantly reduced over the two years by

Table 3. Effect of different levels of bentonite on available and total arsenic in soil (mg kg^{-1}) after 1st and 2nd harvest, and soil pH (1: 2.5 soil: water; measured after completion of crop cycle) pooled over two years.

Year	Available arsenic in soil after		Total arsenic in soil after		Soil pH after crop cycle
	1st harvest	2nd harvest	1st harvest	2nd harvest	
1st	2.83	2.50	12.63	12.69	7.22
2nd	2.98	2.44	12.47	11.53	7.31
$CD_{0.05}$	0.85	0.67	0.91	2.72	0.26
Treatment					
T_1	4.05	3.67	13.48	12.47	6.70
T_2	3.66	3.14	12.20	12.11	7.23
T_3	2.08	1.62	12.74	12.23	7.18
T_4	1.83	1.52	11.78	11.64	7.89
$CD_{0.05}$	1.32	1.19	1.88	2.03	0.80

bentonite application to the soil after each harvest (Table 3), respectively. Similarly, the labile fraction of arsenic also reduced by 9.6 , 48.6 and 54.8% in T_2, T_3, and T_4, respectively over the control (T_1) after first harvest and the corresponding values are 14.4, 55.8 and 58.5%, respectively over the control after second harvest over the two years. Most importantly, after both the harvesting time, total arsenic content in soil did not change significantly over the two years with bentonite application as compared to control soil (Table 3). This is might be due to the effectiveness of bentonite clays to immobilize arsenic in the solution phase. Similarly, no significant difference in labile and total arsenic content was found in between the two experimental years. Application of bentonite clay minerals reduced the labile fraction of arsenic to a great extent, which might be due to larger surface area of clay minerals and their high adsorption capacity in the solution phase.

Application of bentonite clay minerals also raised the soil pH from 6.70 in control to 7.23, 7.18, and 7.89 in T_2, T_3, and T_4, respectively after the final harvesting of beet leaf but the increase was not statistically significant between all the treatments as compared to control except T_4 (Table 3). Only application of bentonite @ 0.5% could significantly raise the soil pH as compared to control over the two years. Smectite dominant clay minerals like bentonite adsorb maximum arsenic in the pH range of 6 to 8 (Mohapatra *et al.*, 8; Mar *et al.*, 4). The raise in pH may be one of the important mechanism of reduced availability and strong adsorption of arsenic in bentonite amended soil.

Arsenic is held in soil through their parent materials, chemical adsorption on clay, organic matter or may be through biological adsorption. Addition of bentonite increased the chemical sorption of arsenic and reduced its mobility and rate of release in soil as a result of strong adsorption with clay particles. The results showed that the effect of bentonite application @ 0.25 and 0.5% was statistically at par in most of the cases for arsenic immobilization (Table 4). Hence, it may be recommended that application of bentonite @ 0.25% may be useful instead of applying @ 0.50% to the contaminated soil. The method described enables application of bentonite to soil reduced the bioavailability of arsenic and thereby reduced the risk of vegetable consumption grown on contaminated soil.

ACKNOWLEDGEMENTS

The first author is thankful to Post Graduate School, IARI, New Delhi for providing IARI-merit Fellowship. The authors are also thankful to the Head, Division of Soil Science and Agricultural Chemistry, IARI, New Delhi for facilities.

REFERENCES

1. Gaw, S.K., Kim, N.D., Northcott, G.L., Wilkins, A.L. and Robinson, G. 2008. Uptake of sigmaDDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. *J. Agric. Food Chem.* **56**: 6584-93.

2. Huq, S.M.I., Joardar, J.C., Parvin, S., Correll, R. and Naidu, R. 2006. Arsenic contamination in food chain: Transfer of arsenic into food materials through groundwater irrigation. *J. Health Popln. Nutr.* **24**: 305-16.

3. Kumaraja, P., Ahammed Shabeer, T.P. and Manjaiah, K.M. 2016. Effect of bentonite on heavy metal uptake by amaranth (*Amaranthus blitum*

Table 4. Statistical ANOVA of pool analysis of different parameters of beet leaf during the year 2016 and 2017.

Parameter	BAF 1st harvest	BAF 2nd harvest	HQ 1st harvest	HQ 2nd harvest	As in plant 1st harvest	As in plant 2nd harvest	Biomass yield 1st harvest	Biomass yield 2nd harvest	As (t) in soil 1st harvest	As (t) in soil 2nd harvest	Available As in soil 1st harvest	Available As in soil 2nd harvest	Soil pH
Year df	1	1	1	1	1	1	1	1	1	1	1	1	1
Treatment df	3	3	3	3	3	3	3	3	3	3	3	3	3
Year × Treatment df	3	3	3	3	3	3	3	3	3	3	3	3	3
Error (a) df	2	2	2	2	2	2	2	2	2	2	2	2	2
Error (b) df	9	9	9	9	9	9	9	9	9	9	9	9	9
Year ss	0.49	40.97	0.16	0.002	0.009	0.284	0.0004	0.006	0.14	8.16	0.131	0.006	0.03
Treatment ss	360.50	185.04	5.72	1.29	6.67	2.98	0.12	0.27	9.75	2.17	22.38	21.1	4.30
Year × Treatment ss	14.09	16.58	0.13	0.01	0.212	0.148	0.004	0.001	3.97	1.22	0.50	0.117	0.47
Error (a) ss	6.07	15.08	0.13	0.004	0.078	0.156	0.004	0.012	0.536	4.791	0.465	0.289	0.043
Error (b) ss	16.27	49.22	0.49	0.27	0.27	0.860	0.076	0.190	18.60	21.76	9.25	7.41	3.37
Year Ms	0.45	40.98	0.16	0.002	0.01	0.28	0.0004	0.006	0.14	8.16	0.13	0.01	0.03
Treatment Ms	120.17	61.68	1.91	0.43	2.22	0.99	0.04	0.09	3.25	0.72	7.46	7.03	1.43
Year × Treatment Ms	4.70	5.53	0.04	0.003	0.07	0.05	0.0013	0.001	1.32	0.41	0.17	0.04	0.16
Error (a) Ms	3.04	7.54	0.06	0.002	0.04	0.08	0.002	0.006	0.27	2.40	0.23	0.14	0.02
Error (b) Ms	1.81	5.47	0.05	0.03	0.03	0.10	0.01	0.02	2.07	2.42	1.03	0.82	0.37
Year 'F' cal	0.15	5.43	2.55	1.21	0.24	3.63	0.20	0.93	0.52	3.40	0.56	0.04	1.45
Treatment 'F' cal	66.5	11.3	35.4	14.3	75.15	10.18	4.75	4.29	1.57	0.30	7.26	8.54	3.83
Year × Treatment 'F' cal	2.60	1.01	0.80	0.11	2.39	0.52	0.17	0.01	0.64	0.17	0.16	0.05	0.42
Year 'F' tab	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Treatment 'F' tab	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86
Year × Treatment 'F' tab	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86	3.86

No. of replications taken = 3; df = degrees of freedom; ss = Sum of square; Ms = Mean square; cal = calculated value; tab = tabulated value; BAF = Bioaccumulation factor; HQ = Hazard quotient; Arsenic = As; As (t) = Total arsenic in soil; Soil pH (Soil = solution = 1:2.5) measured after completion of crop cycle; CD ($P_{0.05}$) values for each parameters are given in each table.

cv. Pusa Kirti) grown on metal contaminated soil. *Indian J. Hort*. **73**: 224-28.

4. Mar, K.K., Karnawati, D., Sarto., Putra, D.P.E., Igarashi, T. and Tabelin, C.B. 2013. Comparison of arsenic adsorption on lignite, bentonite, shale and iron sand from Indonesia. **In**: *Intl. Symp. Earth Sci. Tech*. *CINEST 2012*. *Proc. Earth. Planet. Sci*. **6**: 242-50.
5. McBride, M.B. and Martinez, C.E. 2000. Copper phytotoxicity in a contaminated soil: Remediation test with adsorptive materials. *Env. Sci. Tech.* **34**: 4386-91.
6. McLaren, R.G., Naidu, R., Smith, J. and Tiller, K.G. 1998. Fractionation and distribution of arsenic in soils contaminated by cattle dip. *J. Env. Qual.* **27**: 348-54.
7. Mihaltan, A.I., Frentiu, T., Ponta, M., Petreus, D., Frentiu, M., Darvasi, E. and Marutoiu, C. 2013. Arsenic and antimony determination in non- and biodegradable materials by hydride generation capacitively coupled plasma microtorch optical emission spectrometry. *Talanta*. **109**: 84-90.
8. Mohapatra, D., Mishra, D., Roy Chaudhury, G. and Das, R.P. 2007. Arsenic adsorption mechanism on clay minerals and its dependence on temperature. *Korean J. Chem. Engg.* **24**: 426-30.
9. Perelomov, L., Sarkar, B., Rahman, M.M., Goryacheva, A. and Naidu, R. 2016. Uptake of lead by Na-exchanged and Al-pillared bentonite in the presence of organic acids with different functional groups. *Appl. Clay Sci*. **119**: 417-23.
10. Ramirez-Andreotta, M.D., Brusseau, M.L., Beamer, P. and Maier, R.M. 2013. Home gardening near a mining site in an arsenic-endemic region of Arizona: Assessing arsenic exposure dose and risk *via* ingestion of home garden vegetables, soils, and water. *Sci. Total Env.* **454-55**: 373-382.
11. Shevade, S. and Ford, R.G. 2004. Use of synthetic zeolites for arsenate removal from pollutant water. *Water Res.* **38**: 3197-3204.
12. Su, J., Huang, H.G., Jin, X., Lu, X. and Chen, Z. 2011. Synthesis, characterization and kinetic of a surfactant-modified bentonite used to remove As (III) and As (V) from aqueous solution. *J. Hazard. Mater.* **185**: 63-70.
13. Thomas, D.J., Styblo, M. and Lin, S. 2001. The cellular metabolism and systemic toxicity of arsenic. *Toxicol. Appl. Pharm*. **176**: 127-44.

Received : May, 2017; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00106.2

Indian J. Hort. 74(4), December 2017: 552-556

Improvement of bio-efficacy of bacterial antagonists by using bleaching powder and resistant cultivars to control bacterial wilt of tomato

Dinesh Singh*, D.K. Yadav and Shweta Sinha

Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

In present investigation, two bacterial antagonists *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were tested for their bio-efficacy against *Ralstonia solanacearum* causing bacterial wilt of tomato in combination with chemicals, *viz*., bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate under *in vitro* conditions. Sodium carbonate (0.1%) with *P. fluorescens* inhibited the maximum growth of *R. solanacearum* (10.4 cm² inhibition zone) followed by sodium bicarbonate with *B. subtilis* (7.23 cm² inhibition zone). Bleaching powder and calcium chloride in combination with the bioagents significantly increased growth inhibition of *R. solanacearum* as compared to bioagents alone. Bleaching powder along with resistant cultivar Arka Abha and susceptible cultivar Pusa Ruby were taken under this investigation to improve the bio-efficacy of bacterial antagonists under glasshouse conditions. Six treatments, *viz*., bleaching powder (0.01%) *B. subtilis* DTBS-5, *P. fluorescens* DTPF-3, bleaching powder (0.01%) + *B. subtilis* DTBS-5, bleaching powder (0.01%) + *P. fluorescens* DTPF-3 and control (only *R. solanacearum*) without chemical and bioagents were taken. Minimum wilt disease incidence 19.0 and 29.6% was found in combination of bleaching powder (0.01%) + *B. subtilis* DTBS-5 followed by 19.6 and 31.6% in bleaching powder + *P. fluorescens* DTPF-3 after 30 days of inoculation of *R. solanacearum* in Arka Abha and Pusa Ruby tomato, respectively. Integration of antagonistic bacteria, bleaching powder (0.01%) and Arka Abha reduces bacterial wilt incidence and improved bio-efficacy under glasshouse conditions.

Key words: *Bacillus subtilis,* bacterial wilt, *Pseudomonas fluorescens*, *Ralstonia* sp., rhizobacteria, tomato.

INTRODUCTION

Bacterial wilt in tomato caused by *Ralstonia solanacearum* (Smith) Yabuuchi is a serious problem in coastal, hilly and foot hill areas including Goa, Karnataka, Kerala, Maharashtra, Odisha, Jharkhand, West Bengal and states of north-eastern hills like Himachal Pradesh, Jammu & Kashmir, Uttarakhand (Singh *et al.*, 13) and causes heavy loss to the tomato crop, which vary from 2- 90% under different climates and seasons (Singh *et al.*, 13). Since, the disease is soil-borne and has wide host range (450 species and 54 families) including tomato, potato, eggplant, pepper, groundnut, tobacco, weeds and also roots of non-host plants (Allen, 1), hence it is very difficult to control the disease. There are no antibiotics or other group of chemicals available to effectively control the bacterial plant diseases particularly bacterial wilt of solanaceous crops. However, some chemicals like bleaching powder and calcium chloride were used for controlling the bacterial wilt disease of tomato and other solanaceous crops (Sharma *et al.*, 12; Singh *et al.*, 14); but these chemicals are not much effective and also phytotoxic to the plants at higher doses particularly bleaching powder (Singh *et al.*, 14). Moreover, these chemicals may cause soil and water pollution. Hence, alternative approaches of non-chemical methods such as cultural methods, resistant cultivars and biocontrol with antagonistic bacterial agents have been used to manage bacterial diseases of plants successfully (Almoneafy *et al.*, 2). In bio-control method, various fungal and bacterial antagonists are used to control plant diseases. Among these, bacterial antagonist has become good candidate as an agent of biocontrol plant growth promoting bacteria (Chung *et al.*, 4; Tan *et al.*, 16).

Biological control of bacterial wilt solanaceous crops particularly tomato has been done through the antagonistic bacteria, which reduces the incidence of bacterial wilt disease in great extent (Almoneafy *et al.*, 2; Aiye *et al.*, 3; Tan *et al.*, 16). The rhizobacteria are found quite effective to suppress the bacterial pathogen as antagonists and promote plant growth (Rajendran *et al.*, 10; Singh *et al.*, 15) due to well-developed secretary system and produces structurally diverse secondary metabolites with a wide spectrum of antibiotic activity and their fast growth ability. However, biocontrol agents have their own limitations to control the plant diseases and it is difficult task to manage such deadly soil-borne diseases by using only microbes. Hence, it is a good option to integrate

*Corresponding author's E-mail: dinesh_iari@rediffmail.com

other control methods like cultural practices, resistant or tolerant varieties and chemicals at lower doses to improve bio-efficacy of antagonistic bacteria to control bacterial wilt disease of tomato.

MATERIAL AND METHODS

Ralstonia solanacearum UTT-25 was obtained from the Division of Plant Pathology, ICAR-IARI, New Delhi, which was isolated from wilted tomato plants from farmer's field at Haldwani (Village: Chorgaliya), Nainital, Uttarakhand. The culture of bacteria was maintained in 20% glycerol and stored at -80°C for further study. Bacterial antagonist *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were obtained from Plant Bacteriology Laboratory, Division of Plant Pathology, ICAR-IARI, New Delhi. Bacterial cultures were maintained on the respective slants and stored at 4°C for further use.

Dual culture method was used for the screening of antagonistic properties of bacteria against *R. solanacearum*. Bacterial antagonist *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were selected for this study based on preliminary experiments *in vivo*. Four chemicals such as bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate were taken and concentration was decided based on earlier studied reported by Singh *et al.* (14). Chemical @ 0.1% concentration was added in CPG agar medium and poured into the petriplates. 100 µl of 48-h-old culture of *R. solanacearum* UTT - 25 (10^{10} cfu/ ml) was spread onto the petri plates to make a lawn of the bacteria. Three wells of 0.5 cm diameter in each petri plate were made and 40 µl of 48-h-old culture of both the antagonistic bacteria *B. subtilis* DTBS-5 and *P. fluorescens* DTPF-3 at inoculum load of 10^8, 10^9 and 10^{10} cfu/ ml was poured in each well separately. The plates were incubated at 28 ± 1°C for 48 h with three replications. Inhibition zone formed by bacteria was recorded after 24 h of inoculation and converted into the area of inhibition zone using πr^2 formula.

The tomato cultivars Pusa Ruby (susceptible) and Arka Abha (resistant) were grown in nursery tray at National Phytotran Facility, ICAR-IARI, New Delhi. Seedlings (25 days) of both the cultivars were transplanted in autoclaved pots (6 inch dia.) having 1 kg of soil mixture. Six treatments, *viz.*,1. Bleaching powder (0.01%), 2. *B. subtilis* DTBS-5, 3. *P. fluorescens* DTPF-3, 4. Bleaching powder (0.01%) + *B. subtilis* DTBS-5, 5. Bleaching powder (0.01%) + *P. fluorescens* DTPF-3, 6. control (only *R. solanacearum*) without chemical and bioagent. The *Bacillus* was grown on TSA medium, *Pseudomonas* on King's B medium and *R. solanacearum* on CPG medium and after 48 h, bacterial growth was harvested and inoculum load of *R. solanacearum* was maintained 0.1 OD at 600 nm by using spectrophotometer (Schaad *et al.*, 13). Before transplanting, 0.01% concentration of bleaching powder was mixed in 1 kg of sterilized soil and then 50 ml of 48-h-old biocontrol agents *B. subtilis* DTBS-5 and *P. fluorescens* DTPF-3 was added as per treatment and thoroughly mixed into the soil. Then 25 ml of 48-h-old culture of *R. solanacearum* UTT-25 containing (10^{11} cfu/ ml) poured in each pot without making injury to the tomato plants. Bacterial wilt incidence was recorded at 30 days of inoculation. Biological control efficacy (BCE) was calculated by using formula BCE = $[(Dc-D_T)/Dc \times 100$ as given by Guo *et al.* (6). Where Dc is disease of control and D_T is disease of the treatment group. Population of *R. solanacearum* was recorded at initial stage and after 30 days of inoculation into the soil and converted into log value as described earlier (Schaad *et al.*, 11). The analysis of variance for antagonistic ability was performed by using factorial CRD as standard procedure (Gomez and Gomez, 5). Mean comparisons were conducted using a least significant difference (LSD) test (P = 0.05). Standard error and a LSD result were recorded.

RESULTS AND DISCUSSION

To improve the efficacy of antagonistic bacteria bleaching powder, calcium chloride, sodium carbonate and sodium bicarbonate @ 0.1% concentration with bioagents was tested to improve their bio-efficacy against *R. solanacearum in vitro*. In case of *B. subtilis*, maximum inhibition zone (7.23 cm^2) was formed by sodium carbonate (0.1%) and *B. subtilis* (10^{10}cfu/ ml population) followed by sodium bicarbonate (Table 1). In case of *P. fluorescens*, maximum inhibition zone (10.4 cm^2) was formed by sodium carbonate with *P. fluorescens* (10^{10} cfu/ml) followed by sodium carbonate and *P. fluorescens* (Table 2). The bio-efficacy of both the bioagents was reduced significantly by decreasing the population 10^{10} cfu/ ml to 10^8 cfu/ml from 7.08 to 4.90 cm^2 and from 4.65 to 2.77 cm^2 against *R. solanacearum* in *P. fluorescens* DTPF-3 and *B. subtilis* DTBS-5, respectively. Bio-efficacy of both the bioagents was enhanced by combining with the chemicals. Improvement of bio-efficacy of both the bioagents *B. subtilis* and *P. fluorescens* might be due to antibacterial property of these chemicals, which reduces the inoculum of *R. solanacearum*, thus bioagents were able to form more inhibition zone (Sharma *et al.*, 12).

Minimum disease incidence 19.0 and 29.6% was found in bleaching powder (0.01%) + *B. subtilis* followed by 19.6 and 31.6% in bleaching powder + *P. fluorescens* after 30 days of inoculation in Arka Abha and Pusa Ruby cultivars, respectively. Biological

Table 1. Improvement of bio-efficacy of *P. fluorescens* against *R. solanacearum in vitro.*

Chemical (0.1% conc.)	Inhibition zone (area cm^2)			Mean
	10^{10} (cfu/ ml) of *B. subtilis* DTBS-5	10^{9} (cfu/ ml) of *B. subtilis* DTBS-5	10^{8} (cfu/ ml) of *B. subtilis* DTBS-5	
Bleaching powder	4.90	4.52	3.25	4.22
Calcium chloride	6.90	5.31	3.91	5.38
Sodium carbonate	9.25	8.05	7.39	8.23
Sodium bicarbonate	10.40	9.31	7.22	8.99
Control	3.96	3.14	2.74	3.28
Mean	7.08	6.07	4.90	
$CD_{0.05}$			Treatment = 0.46	
			Population of bacteria = 0.36	
			Treatment × Population of bacteria = 0.80	

Table 2. Improvement of bio-efficacy of *B. subtilis* against *R. solanacearum in vitro.*

Chemical	Inhibition zone (cm^2)			Mean
	10^{10} (cfu/ ml) of *P. fluorescens* DTPF-3	10^{9} (cfu/ ml) of *P. fluorescens* DTPF-3	10^{8} (cfu/ ml) of *P. fluorescens* DTPF-3	
Bleaching powder (0.1%)	3.96	2.93	2.52	3.13
Calcium chloride (0.1%)	4.16	3.14	2.05	3.12
Sodium carbonate (0.1%)	7.23	6.31	5.18	6.24
Sodium bicarbonate (0.1%)	5.45	2.18	2.04	3.23
Control	3.14	2.45	1.83	2.48
Mean	4.65	3.33	2.77	
$CD_{0.05}$			Treatment = 0.45	
			Population of bacterium = 0.37	
			Treatment × Population of bacteria = 0.81	

control efficacy of both the biocontrol agents was found comparatively better and it was maximum in bleaching powder + *B. subtilis* 37.29 and 36.06% followed by bleaching powder + *P. fluorescens* 35.31 and 31.75% in Arka Abha and Pusa Ruby cultivars, respectively. Bleaching powder ($CaOCl_2$) also reduced the wilt incidence in both the cultivars with the biological control efficacy of 7.59% in Arka Abha and 29.92% in Pusa Ruby. The reduction of bacterial wilt incidence by using bleaching powder may be due to reduction in *R. solanacearum* population by releasing chlorine, which acted as bactericide and also Ca accumulation in leaf tissue of tomato plant and soil, which reduces the rate of bacterial development (Sharma *et al.*, 12). Although, bleaching powder (0.01%) along with bioagents performed better than the applied separately. Resistant cultivar Arka Abha has lower wilt incidence as compared to susceptible cultivar in all the treatments (Table 3). It might be due to resistant gene found in Arka Abha against *R. solanacearum.* Yamazati *et al.* (17) reported that bacterial population was negatively correlated with Ca concentration and increased Ca content in plant tissues induced resistance to some disease by inhibition of polygalacturonase, increase resistance in cell walls (Padmaja and Jayaram, 8) and inhibition of ethylene production.

Minimum population of *R. solanacearum* was found in bleaching powder (0.01%) + *B. subtilis* DTBS-5 in the soil rhizospheric of Pusa Ruby (4.2 log value / g soil) and Arka Abha (4.3 log value/ soil) followed by after 30 day of inoculation. It was also noticed that population of *R. solanacearum* was significantly declined from initial level in due course of time, which was significantly lower in treated soil either bioagents or in combination with bleaching powder as compared to control (Table 4). Similar reports of bacterial pathogen decline in the soil due to antagonistic, and bacterial wilt reduction have been reported by Ran *et al*. (9), Lamessa and Zeller (7) and

Table 3. Effect of bleaching powder and antagonists on bacterial wilt incidence in tomato cultivars after 30 days of inoculation.

Treatment	Wilt incidence (%)		Biological control efficacy (%)	
	Arka Abha	Pusa Ruby	Arka Abha	Pusa Ruby
Bleaching powder (0.01%)	28.0^{ab}	34.3^{ab}	7.59	28.92
P. fluorescens DTPF-3	21.0^{bc}	32.0^{ab}	30.69	31.75
B. subtilis DTBS-5	25.0^{abc}	37.3^{b}	17.67	19.40
Bleaching powder (0.01%) + *P. fluorescens* DTPF-3	19.6 ^{c}	31.6^{bc}	35.31	31.75
Bleaching powder (0.01%) + *B. subtilis* DTBS-5	18.6^{c}	29.6^{c}	37.29	36.06
Control	30.3^{a}	46.3^{a}	-	-

Values are means of three replications. Data followed by the same letter(s) in a column are not significantly different from each other according to DMRT at P = 0.05.

Table 4. Effect of bleaching powder and biocontrol agents on population of *R. solanacearum* and antagonistic bacteria after 30 days in tomato cultivars.

Treatment	Initial population of *R. solanacearum* (log value/ g of soil)	Population of *R. solanacearum* (Log value/ g of soil) after 30 days of inoculation	
		Arka Abha	Pusa Ruby
Bleaching powder (0.01%)	5.6^{b}	4.4^{ab}	4.6^{b}
P. fluorescens DTPF-3	6.0^{ab}	4.5^{a}	4.6^{bc}
B. subtilis DTBS-5	6.0^{ab}	4.2^{ab}	4.4^{d}
Bleaching powder (0.01%) + *P. fluorescens* DTPF-3	6.1^{a}	4.3^{ab}	4.4^{cd}
Bleaching powder (0.01%) + *B. subtilis* DTBS-5	6.1^{a}	4.3^{ab}	4.2^{c}
Control	6.2^{a}	5.2^{b}	5.9^{a}

Values are means of three replications. Data followed by the same letter in a column are not significantly different from each other according to DMRT at p = 0.05.

Singh *et al.* (15). However, no significant variation in declining the population of *R. solanacearum* in soil was noted either treated with the bio-control agent or bleaching powder alone or in combination. But, resistant cultivar (Arka Abha) and susceptible cultivar (Pusa Ruby) showed variation in their rhizospheric population of *R. solanacearum.*

Integration with bleaching powder, biocontrol agent and resistant variety reduced wilt incidence significantly and these combination has potential to control bacterial wilt disease in tomato under the field conditions.

ACKNOWLEDGEMENTS

We acknowledge the financial support from the Indian Council of Agricultural Research, New Delhi through project (PhytoFura). We are also thankful to the Director, ICAR-Indian Institute of Spices Research, Calicut and the Head, Division of Plant Pathology, ICAR-IARI, New Delhi for facilities.

REFERENCES

1. Allen, C., Prior, P. and Hayward, A.C. 2005. Bacterial wilt disease and the *Ralstonia solanacearum* species complex, APS Press, St. Paul, MN, USA.

2. Almoneafy, A.A., Xie, G.L., Tian, W.X., Xu, L.H., Zhang, G.Q. and Ibrahim, M. 2012. Characterization and evaluation of *Bacillus* isolates for their potential plant growth and biocontrol activities against tomato bacterial wilt. *African J. Biotech.* **11**: 7193-201.

3. Aiye, N., Chemeda, F. and Hiskias, Y. 2008. Evaluation of rhizosphere bacterial antagonists their potential to bio-protect potato (*Solanum tuberosum*) against bacterial wilt (*Ralstonia solanacearum*). *Biol. Cont*. **47**: 282-88.

4. Chung, S., Kong, H., Buyer, J.S., Lakshman, D.K., Lydon, J., Kim, S.D. and Roberts, D.P.

2008. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soil borne pathogens of cucumber and pepper. *Appl. Microbiol. Biotech.* **80**: 115-23.

5. Gomez, K.M. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*, Wiley-Interscience, 680 p.

6. Guo, J.H., Qi, H.Y., Guo, Y.H., Ge, H.L., Gong, L.Y., Zhang, L.X., Sun, P.H. 2004. Biocontrol of tomato wilt by growth-promoting rhizobacteria. *Biol. Cont.* **29**: 66-72.

7. Lemessa, F. and Zeller, W. 2007. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Boil. Cont*. **42**: 336-44.

8. Padmaja, N. and Jayaram, J. 2000. Effect of salicylic acid and *Pseudomonas fluorescens* on *Xanthomonas campestris* pv. *malvacearum* in susceptible cotton. *Ann. Plant Protect*. *Sci.* **11**: 397-99.

9. Ran, L.X., Liu, C.Y., VanLoon, L.C. and Bakker, P.A.H.M. 2005. Suppression of bacterial wilt in *Eucalyptus urophylla* by fluorescent *Pseudomonas* spp. in China. *Biol. Cont*. **32**: 111-20.

10. Rajendran, L., Ramjegathesh, R., Shanthiyaa, V., Raghuchander, T., Kartheikeyan, G. and Samiyappan, R. 2012. Biocontrol potential and mode of action of the strains EPC 5 and EPC 8 of endophytic bacterium, *Bacillus subtilis*. *Indian Phytopath*. **65**: 122-27.

11. Schaad, N.W., Jones, J.B. and Chun, W. 2001. *Laboratory Guide for the Identification of Plant Pathogenic Bacteria* (3rd Edn.), Amer. Phytopathol. Soc., St Paul, USA, 373 p.

12. Sharma, J.P. and Kumar, S. 2009. Management of *Ralstonia* wilt of tomato through microbes, plant extract and combination of cake and chemicals. *Indian Phytopath.* **62**: 417-23.

13. Singh, D., Sinha, S., Yadav, D.K., Sharma, J.P., Srivastava, D.K., Lal, H.C., Mondal, K.K. and Jaiswal, R.K. 2010. Characterization of biovar/ races of *Ralstonia solanacearum,* the incidence of bacterial wilt in solanaceous crops. *Indian Phytopath.* **63**: 261-65.

14. Singh, D., Yadav, D.K., Sinha, S. and Singh, H. 2012a. Effect of safe chemicals and bleaching powder on bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 bv. 3. *Ann. Plant. Protec. Sci.* **20**: 426-29.

15. Singh, D., Yadav, D.K., Sinha, S. and Upadhyay, B.K. 2012b. Utilization of plant growth promoting *Bacillus subtilis* isolates for the management of bacterial wilt incidence in tomato caused by *Ralstonia solanacearum* race 1 biovar3. *Indian Phytopath*. **65**:18-24.

16. Tan, S., Jiang, Y., Song. S., Huang, J., Ling, N., Xu, Y. and Shen, Q. 2013. Two *Bacillus amyloliquefaciens* strains isolated using the competitive tomato root enrichment method and their effects on suppressing *Ralstonia solanacearum* and promoting tomato plant growth. *Crop Protec.* **43**: 134-40.

17. Yamazati, H., Kikuchi, S., Hoshima, T. and Kimera, T. 1995. Effect of compost application on bacterial wilt (*Ralstonia solanacearum*) development of calcium uptake of tomato seedling. *Japanese J. Soil Plant Nutr.* **69**: 78-79.

Received : October, 2015; Revised : September, 2017; Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00107.4

Indian J. Hort. 74(4), December 2017: 557-561

Performance of cassava brown streak disease-tolerant varieties in Zanzibar, Tanzania

Edward Kanju, Veronica Nwakaego E. Uzokwe*, Haji Saleh, Shaali Mohamed**, Esther Masumba***, Silver Tumwegamire and Kiddo Mtunda*****

Agricultural Research Institute (ARI), Kizimbani, Zanzibar, International Institute of Tropical Agriculture (IITA), Dar es Salaam, Tanzania

ABSTRACT

Cassava is an important staple food in subtropical regions; however, its production is adversely affected by cassava brown streak disease and poor soil fertility. Five improved and two local cassava varieties were evaluated for three seasons across two sites in Kizimbani, Zanzibar. Highly significant differences were detected among varieties, sites and years for fresh shoot yield, and fresh root yield. For cassava brown streak disease-associated root necrosis, highly significant differences were detected only between varieties but not sites or years. On average, the site that had a slightly higher soil nitrogen level recorded ~126% higher fresh root yield. Two improved varieties, 'Kizimbani' and 'Machui', produced significantly higher fresh root yields than the best local variety, 'Mwari'. However, the local variety 'Boma' is preferred by farmers in Zanzibar because it has better fresh consumption qualities than 'Mwari'. 'Boma' is highly susceptible to cassava brown streak disease and produces a poor yield. The four released varieties, 'Kama', 'Kizimbani', 'Mahonda' and 'Machui' were superior to 'Boma' in cassava brown streak disease resistance and yield. Further, soil fertility improvement and production system intensification are needed to enhance productivity.

Key words: Cassava, cassava brown streak disease, soil fertility, Tanzania.

INTRODUCTION

Cassava is grown throughout the state of Zanzibar and is the second most important food staple, after rice. Although cassava is a very hardy crop that grows well under marginal conditions in which few other crops could survive, to achieve its full potential, productivity must be improved (Ceballos, 2; Dixon and Ssemakula, 4; FAO, 7). Cassava is grown on 12,480 ha in Zanzibar, with an estimated production of 187,213 tonnes/ year (FAO, 6). This implies that the average yield is ~15 t/ha. However, cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and low soil fertility are the key limiting factors for its production (Spittel and Van Huis, 14).

CBSD is presently the most important cause of food insecurity in the coastal and lake zones of eastern Africa (Mohammed *et al*., 9). CBSD has been endemic in the coastal lowlands of eastern Africa since the 1940s, but no serious outbreaks had been reported in Zanzibar, despite the occurrence of foliar incidences (Nichols, 11; Thresh and Mbwana, 15). For reasons not yet known, CBSD devastated cassava crops in Zanzibar during the early 2000s. Farmers in Zanzibar had only two popular local varieties, 'Boma' and 'Kibiriti', which have very good cooking qualities when fresh. Unfortunately, these varieties are highly susceptible to CBSD. By 2003, most farmers were abandoning the cultivation of these varieties and desperately looking for resistant varieties. One of the few promising local varieties was 'Mwari', which, although inferior to 'Boma' and 'Kibiriti' in cooking qualities, produced a higher yield. However, it was not widely available.

The Ministry of Agriculture in Zanzibar officially released four new high-yield, CBSD-tolerant and CMD-resistant varieties in 2007. These varieties have been under evaluation, both on-station and on-farm, since then.

MATERIALS AND METHODS

Five promising breeding lines that were in the pipeline for official release were evaluated on-station at the Agricultural Research Institute (ARI), Kizimbani, Zanzibar, at two sites that differed in soil fertility (Table 1). The sites, Michikichini (06.08617 S; 039.26360 E, 59 m above sea level) and Mibunini (06.08845 S, 039.2646 E, 62 m above sea level) were less than 1 km apart within the research station. The lines being evaluated were KBH 2002/477, KBH 2002/482, KBH 2002/494, KBH 2002/517 and KBH 2002/344. The first four were officially released in 2007 under the names 'Kama', 'Kizimbani', 'Mahonda' and 'Machui', respectively. Two popular local varieties, 'Boma' and 'Mwari', were also included.

*Corresponding author's E-mail: v.uzokwe@cgiar.org
**Agricultural Research Institute (ARI), Kizimbani, Zanzibar
***Agricultural Research Institute (ARI) Kibaha, Kibaha, Tanzania

Table 1. Soil chemical properties at the two sites, Michikichini and Mibunini, in ARI, Kizimbani, Zanzibar.

Parameter	Michikichini		Mibunini	
	0–20 cm	20–40 cm	0–20 cm	20–40 cm
pH	4.95	5.10	6.05	5.95
Organic C (%)	0.95 (low*)	0.55 (very low)	0.95 (low)	0.75 (low)
Total N (%)	0.129 (low)	0.116 (low)	0.175 (medium)	0.140 (low)
Available P (ppm)	13.5 (medium)	4.0 (low)	7.5 (low)	5.5 (low)
Exchangeable K (meK/ 100 g soil)	0.11(very low)	0.16 (very low)	0.27 (low)	0.125 (very low)
Exchangeable Zn (ppm)	<0.1	<0.1	<0.1	<0.1

*Interpretation as per Hazelton and Murphy (2007).

The first trial, performed in randomized complete block design with two replications for two consecutive years. The plot size was 10 m × 4 m. Planting was performed in March, which is the start of the long rainy season. The experiment was conducted for two consecutive years in three replications. A spacing of 1 m × 1 m was used between ridges and between plants within a ridge. The plots were kept weed-free by hand weeding. Plants were harvested 12 months after planting. At harvest, the following data were recorded: Number of plants harvested from the net plot area, number of roots harvested, weight of roots harvested, weight of shoots (stems with their leaves plus stumps), and plant height. Root weight and plant height are known to be important characters in cassava yield (Padma *et al.*, 13). Roots were sliced transversally into three pieces that were scored for root necrosis on a scale of 1-5, where 1 indicated no visible symptoms and 5 indicated very severe symptoms (Hillocks and Jennings, 8). Data were subjected to an analysis of variance (after being checked for normality) using the General Randomized Block Treatment Structure of the GenStat Discovery Edition 3 software program. Soil samples were taken from the trial fields before planting, and analyzed at the ARI Kizimbani soil laboratory.

RESULTS AND DISCUSSION

Zanzibar has a bi-modal rainfall system and normally receives more than 1,000 mm of rain per year. The long rainy season starts in March and ends in June. The short rainy season starts in October and ends in December. However, throughout the year some rainfall, although small in amount, is expected. This rainfall distribution is very suitable for cassava production (Table 2). The greatest amount of total rainfall was received in 2006 (1,719.1 mm) when the trial started. The least amount of rainfall was recorded in 2008 (1,311.6 mm). This implied that rainfall was not a constraint on cassava crop production during the whole trial period.

Highly significant differences in fresh shoot weight were detected between varieties, sites, and years. The site × year interaction was also highly significant (Table 3) and contributed the highest value of total sum of squares (29%), followed by year (18%). 'Kizimbani' and 'Machui' had significantly higher FSY values than the rest of the lines (Table 4). The Mibunini site produced, on average, significantly higher FSYs than Michikichini (9.2 and 6.5 t/ha, respectively). The higher FSY at Mibunini could be attributed to the slightly higher levels of nitrogen at this site. This is in agreement with findings from other researchers on other root and tuber crops. Research on sweet potato indicated that plants that received 20 t/ha of "sunshine organic fertilizer" [composted poultry manure + sorted city refuse at 1:2 (nitrogen: phosphorus: potassium at 2.58%:1.10%:0.68%)] produced significantly longer vines, more leaves, more branches and greater tuber weights than the control (Adekoya *et al.*, 1).

Table 2. Rainfall (mm) received each month in the three seasons at the research station at Kizimbam.

Month	Rainfall amount (mm)		
	2006	2007	2008
January	65.7	32.6	52.5
February	12.1	9.2	34.8
March	135.1	229.4	150.8
April	486.2	313.7	526.6
May	162.8	402.3	93.3
June	169.5	78.3	64.8
July	39.6	22.3	47.9
August	30.7	79.4	41.3
September	33.3	24.6	43.0
October	112.4	136.7	51.7
November	246.0	112.0	143.6
December	225.7	67.2	61.3
Total	1,719.1	1,507.7	1,311.6

Table 3. Analyses of seven cassava varieties grown for three seasons at two locations in Zanzibar.

Source	DF	Mean square	% of total sum of squares
Fresh shoot yield			
Variety	6	37.19***	9.4
Site	1	810.01***	34.1
Year	2	185.32***	15.6
Variety × Site	6	11.28	2.8
Variety × Year	12	8.98	4.5
Site × Year	2	93.38***	7.9
Variety × Site × Year	12	4.57	2.3
Residual	68	7.85	22.5
Plant height			
Variety	6	4560.9***	40.5
Site	1	8211.4***	12.2
Year	6	482.9	1.4
Variety × Site	2	387.3	3.4
Variety × Year	12	672.3**	11.9
Site × Year	2	522.5	1.5
Variety × Site × Year	12	312.5	5.5
Residual	68	229.6	23.1
Fresh root yield			
Variety	6	349.49***	17.3
Site	1	5004.12***	41.3
Year	6	571.94***	9.4
Variety × Site	2	126.58***	6.3
Variety × Year	12	38.58	3.8
Site × Year	2	163.56**	2.7
Variety × Site × Year	12	47.00*	4.7
Residual	68	24.46	13.7
CBSD-associated root necrosis			
Variety	6	9.56***	60.9
Site	1	0.04	0.0
Year	6	1.16**	2.5
Variety × Site	2	0.51**	3.3
Variety × Year	12	0.47**	6.0
Site × Year	2	1.01**	2.1
Variety × Site × Year	12	1.05***	13.4
Residual	68	0.15	11.1

***,**,* = Significant at p = 0.001, 0.01 and 0.05 levels

Table 4. Mean fresh shoot yield (t/ ha) among cassava varieties evaluated across two sites and three seasons.

Variety	Site		Mean
	Mibunini	Michikichini	
Boma	8.31 (5)	3.83 (6)	6.07
Kama	9.72 (4)	5.00 (2)	7.36
KBH 2002/344	8.09 (6)	3.95 (5)	6.02
Kizimbani	13.00 (2)	6.38 (1)	9.69
Machui	13.12 (1)	4.50 (4)	8.81
Mahonda	10.06 (3)	4.99 (3)	7.53
Mwari	7.63 (7)	3.66 (7)	4.64
Mean	9.99	4.61	7.30
CV (%)	34.5	47.5	38.4
$LSD_{0.05}$	3.50	2.23	1.98

*No. in brackets indicates the ranking of the variety within the site.

Highly significant differences were detected between varieties, sites, and years for FRY. Highly significant differences were detected for variety × site interactions. In addition, variety × year and variety × site × year interactions were significant (Table 3). Sites and varieties contributed the highest percentages to the total sum of squares (41 and 17%, respectively). Similar results have been reported by other researchers, underscoring that cassava, as a crop, is widely adaptable to a variety of environmental conditions, but that the usual ranges of most varieties are narrow and have large genotype by environment (G × E) interaction effects (Dixon *et al.*, 3; Ngeve, 10; Ntawuruhunga *et al*., 12). 'Kizimbani' had, on average, the highest FRY (24.6 t/ha) among the varieties (Table 5). The FRY of the local variety 'Boma' was significantly lower, at 9.5 t/ha, than those of the other varieties. Mibunini was a significantly a higher yielding site (24.0 t/ha) than Michikichini (10.6 t/ha), producing a yield increase equivalent to ~126%. The same argument regarding the higher level of nitrogen that was made for the higher FSY applies here. This difference in the soil fertility within such a short distance highlights that the traditional farming systems producing cassava are characterized by highly variable edaphic and biological conditions. The large G × E interaction makes it difficult for breeders to identify cultivars suitable for such farming systems (Eyzaguirre and Iwanaga, 5). Extensive trials reviewed by the Food and Agriculture Organization of the United Nations have shown that many cassava varieties respond very well to fertilization. The ability of cassava to produce on low-fertility soils has given rise to the misconception that cassava does not require, nor even respond to, the application of mineral fertilizers (FAO, 7). The preliminary results

Table 5. Mean fresh root yield (t/ ha) among cassava varieties evaluated across two sites and three seasons.

Variety	Site		Mean
	Mibunini	Michikichini	
Kizimbani	36.36 (1)*	12.76 (1)	24.56
Machui	28.73 (2)	10.97 (4)	19.85
Kama	24.44 (4)	11.89 (3)	18.17
Mahonda	25.27 (3)	12.04 (2)	18.66
Boma	13.25 (7)	5.86 (7)	9.55
KBH 2002/344	19.49 (6)	10.15 (6)	14.82
Mwari	20.40 (5)	10.69 (5)	15.54
Mean	23.99	10.62	17.31
CV (%)	23.00	41.20	28.6
$LSD_{0.05}$	5.62	4.45	3.49

*No. in brackets indicate the ranking of the variety within the site.

Table 6. Mean plant height (cm) among cassava varieties evaluated across two sites and three seasons.

Variety	Site		Mean
	Mibunini	Michikichini	
Boma	151.2 (6)	128.9 (6)	140.1
Kama	171.9 (3)	159.8 (3)	165.8
KBH 2002/344	136.9 (7)	127.4 (7)	132.1
Kizimbani	185.4 (2)	165.0 (1)	175.2
Machui	193.9 (1)	160.1(2)	177.0
Mahonda	168.5 (4)	150.4 (5)	159.4
Mwari	160.0 (5)	156.4 (4)	149.7
Mean	166.8	149.7	158.3
CV (%)	7.3	11.5	9.6
$LSD_{0.05}$	12.4	17.5	10.69

*No. in brackets indicates the ranking of the variety within the site.

from this study highlight the need to investigate and improve natural resource management options to increase cassava yields. Although the Michikichini site had slightly higher levels of phosphorus, the root yields were poor. This corroborates reports that cassava is highly tolerant of soils with low phosphorus levels and can generally grow even without phosphorus-fertilizer applications because cassava forms a mutually beneficial association with a group of soil fungi called vesicular-arbuscular mycorrhizae (FAO, 7).

Highly significant differences were detected between varieties and sites for plant height. The variety × year interaction was also significant (Table 3). The highest contribution to the total sum of squares was attributed to variety (40.0%) followed by site (12.2%) and variety × year (12.0%). 'Machui' had, on average, the tallest plants (177.0 cm), which were significantly taller than the rest of the varieties, except 'Kizimbani' (Table 6). The Mibunini site had, on average, taller plants (166.8 cm) than Michikichini (149.7 cm), arguably for the same reason it had higher FSY and FRY values.

The analysis of variance revealed highly significant differences among varieties. Significant differences were detected between years. The variety × site, variety × year and site × year interactions were significant. The variety × site × year interaction was highly significant (Table 3). The highest percentage of the total sum of squares was attributed to variety (61%), followed by the variety × site × year interaction (13%). Only the local variety 'Boma', which is susceptible to CBSD, had a significantly higher mean severity score (3.1) than the improved varieties and the other local variety 'Mwari' (Table 7). No differences between sites were detected for root necrosis. It is commonly assumed that plants grown in fertilized soils are healthier than those on poor soils. It has been reported that increasing the organic matter content of the soil lowered CMD severity in experiments carried out in Zanzibar (Spittel and Van Huis, 14). This does not appear to apply to CBSD because the Mibunini site has a slightly higher nitrogen level than Michikichini (Table 2).

Two improved varieties, 'Kizimbani' and 'Machui', produced significantly higher FRYs than the best local variety, 'Mwari'. However, the local variety 'Boma' is preferred by farmers in Zanzibar because it has better fresh consumption qualities than 'Mwari'. 'Boma' which are is highly susceptible to CBSD and farmers had almost abandoned its cultivation by the time this

Table 7. Mean CBSD-associated root necrosis values among cassava varieties evaluated across two sites and three seasons.

Variety	Site		Mean
	Mibunini	Michikichini	
Boma	2.70 (1)	3.5 (1)	3.12
Kama	1.00 (3)	1.12 (2)	1.06
KBH 2002/344	1.25 (2)	1.00 (3)	1.12
Kizimbani	1.25 (2)	1.00 (3)	1.12
Machui	1.25 (2)	1.00 (3)	1.12
Mahonda	1.00 (3)	1.12 (2)	1.06
Mwari	1.00 (3)	1.00 (3)	1.00
Mean	1.36	1.39	1.37
CV (%)	36.90	16.00	28.5
$LSD_{0.05}$	0.51	0.23	0.28

*No. in brackets indicate the ranking of the variety within the site.

study was conducted. It also produces a poor yield. The four released varieties, 'Kama', 'Kizimbani', 'Mahonda', and 'Machui' were superior to 'Boma' in CBSD resistance and yield, and should therefore be promoted to replace it.

ACKNOWLEDGEMENTS

The help and collaboration of farmers in Zanzibar in the selection and final official release of these varieties are highly appreciated. We are very grateful to the Field Technicians at ARI Kibaha (Mr. Stanslaus Tollano) and ARI Kizimbani (Ms. Fatma Haji Khamis, Mr. Shaali A. Shaali, Mr. Ali Hamad Ali, and Mr. Hamad Omar Talib) for data collection. Mr. Juma Yabeja is gratefully acknowledged for his assistance and advice on the data analysis. This work was done with funding from the Rockefeller Foundation through grants numbers RF 2001 FS 177 and RF 2006 FS 026.

REFERENCES

1. Adekoya, I.M., Aiyelaagbe, I.O.O., Bodunde, J.G., Lawal, O.I. and Sanni, L.O. 2012. Growth, yield and tuber quality of sweet potato (*Ipomoea batatas*, L.) in response to organic and mineral fertilizer. **In**: *Tropical Roots and Tuber Crops and the Challenges of Globalization and Climate Changes. Proc. 11th ISTRC-AB Symp.* 4-6 Oct, 2010, Okechuwu, R.U. and Ntawuruhunga, P. (Eds.), Kinshasa, Democratic Republic of Congo.

2. Ceballos, H., Iglesias, C.A., Perez, J.C. and Dixon, A. 2004. Cassava breeding opportunities and challenges. *Plant Mol Biol.* **56**: 503-16.

3. Dixon, A.G.O., Asiedu, R. and Hahn, S.K. 1994. Genotypic stability and adaptability: analytical methods and implications for cassava breeding for low input agriculture. **In**: *Tropical Root Crops in a Developing Economy. Proc. of the 9th Symp. of the Int'l Society for Tropical Root Crops.* 20-26 Oct, 1991, F. Ofori and S.K. Hahn (Eds.), Accra, Ghana.

4. Dixon, A.G.O. and Ssemakula, G. 2008. Prospects for cassava breeding in Sub-Saharan Africa in the next decade. *J. Food Agric. Env.* **6**: 132-38.

5. Eyzaguirre, P. and Iwanaga, M. 1996. Farmers' contribution to maintaining genetic diversity in crops, and its role within the total genetic resources system. **In**: *Participatory Plant Breeding. Proc. Workshop on Participatory Plant Breeding*, 26-29, July, 1995, Wageningen, the Netherlands, P. Euzagirre and M. Iwanaga (Eds.), IPGRI, Rome, Italy.

6. Food and Agriculture Organization of the United Nations (FAO). 2012. *Assessment of Post-harvest Losses of Major Food Crops and Fish in Zanzibar*, Revolutionary Government of Zanzibar, Ministry of Agriculture, Livestock and Natural Resources.

7. Food and Agriculture Organization of the United Nations (FAO). 2013. *Save and Grow: Cassava - a Guide to Sustainable Production Intensification*, Rome, Italy.

8. Gillocks, R.J. and Jennings, D.L. 2003. Cassava brown streak disease: a review of present knowledge and research needs. *Int. J. Pest Manage.* **49**: 225-34.

9. Mohammed, I.U., Abarshi, M.M., Muli, B., Hillocks, R.J. and Maruthi, M.N. 2012. The symptom and genetic diversity of cassava brown streak viruses infecting cassava in East Africa. *Adv. Virol.* 2012: 1-10. http://dx.doi.org/10.1155/2012/795697

10. Ngeve, J.M. 1994. Yield stability parameters for comparing cassava varieties. **In**: *Tropical Root Crops in a Developing Economy. Proc. 9th Symp. Int'l Soc. Trop. Root Crops*, 20-26 Oct, 1991, F. Ofori and S.K. Hahn (Eds.), Accra, Ghana.

11. Nichols, R.F.W. 1950. The brown streak disease of cassava: distribution, climatic effects and diagnostic symptoms. *East African Agric. J.* **15**: 154-60.

12. Ntawuruhunga, P., Rubaihayo, P., Whyte, J.B.A., Dixon, A.G.O. and Osiru, D.S.O. 2001. Additive main effects and multiplicative interaction analysis for storage root yield of cassava genotypes evaluated in Uganda. *African Crop Sci. J.* **9**: 591-98.

13. Padma, S.S.V., Kameswari, P.L. and Ramana, K.T.V. 2009. Correlation studies and path analysis in edible cassava germplasm in the tribal zone of Andhra Pradesh. *Indian J. Hort.* **66**: 319-22.

14. Spittel, M. and Van Huis, A. 2000. Effect of cassava mosaic disease, soil fertility, plant spacing and their interactions on cassava yields in Zanzibar. *Int. J. Pest Manage.* **46**: 187-93.

15. Thresh, J.M. and Mbwana, M.W. 1998. Cassava mosaic and cassava brown streak in Zanzibar. *Roots,* **5**: 6-9.

Received : July, 2016; Revised : September, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00108.6

Indian J. Hort. 74(4), December 2017: 562-567

Genetic divergence studies in tulip (*Tulipa gesneriana* L.)

Reeta Bhatia*, S.S. Dey and Raj Kumar

ICAR-Indian Agricultural Research Institute Regional Station, Katrain 175 129, Himachal Pradesh

ABSTRACT

Twenty one tulip genotypes were assessed for their genetic divergence based on 16 agro-morphological traits following Mahalanobis D^2-statistic. On the basis of D^2 values, the 21 genotypes were grouped in to five clusters, of which cluster II accommodated 6 genotypes, while cluster IV had single genotype. The high magnitude of D^2 cluster means indicated that there is a considerable diversity in the population studied. The D^2 and inter-cluster coverage divergence were utilized for the choice of parents to decide the cross combination to produce heterotic effect. The highest inter cluster D^2 value was recorded between clusters III and V (11005.75) indicating that crosses may be attempted between the genotypes of cluster III (Character, Christian Dream, Hamilton and Horizon) and cluster V (Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb) to obtain new desirable recombinants in tulip. The study of cluster mean value of 5 clusters indicated high range of variation for days to sprout, days to flower, wrapper leaf area, percent sprouting & flowering, plant height and scape length among the different clusters. The cluster V includes genotypes with earliness and exhibited longest duration of flowering, highest wrapper leaf area and bulb weight. Genotypes of this cluster also possessed desirable floral traits (scape length, floral size and scape thickness) and bulb traits (number of bulbs per plant and bulb weight). Hence, genotypes from this cluster could serve as valuable parents to develop superior cultivars. Out of 16 principal components first six accounted 91.88% of total variability. The first principal component accounted for 57.25% of variability, while, the second and third accounted for 12.37 and 7.54% of total variability, respectively. Hierarchical cluster analysis (HCA) was performed for getting more clear idea among the genotypes. Based on HCA one dendrogram was constructed with two major clusters. These clusters could be divided into 8 minor sub-clusters.

Key words: Tulip, D^2 analysis, genetic diversity.

INTRODUCTION

Tulip (*Tulipa gesneriana* L.) is one of the most important ornamental crops in the world. It is ranked third among the top ten flowers sold worldwide (Podwyszynska and Sochacki, 12), being extremely popular for landscaping, and also as garden plant and cut flower. Tulips are highly valued for their attractive, coloured, upright flowers, mainly produced in springs. In, India tulip has been recently introduced in the Kashmir valley. It is gaining great popularity there over the last few years. There is tremendous scope for its commercial cultivation in Himachal Pradesh, Jammu & Kashmir, Uttarakhand and similar other hilly terrains of India (Jhon *et al*., 4). However, this crop has never been opted as commercial crop in India due to lack of adaptive genotypes, agro-techniques and planting material (Bhatia *et al*., 2).

In tulip, most of the varieties have been imported from Holland, and the performance of these varieties depends upon climatic conditions of the region under which they are grown. As a result, cultivars which perform well in one region may not perform same in other regions of varying climatic conditions (Kamble *et al*., 5). It is also important to study the performance of existing cultivars for their superior desirable characters (Archana *et al*., 1). The extent of genetic variability is of paramount importance for the improvement of a crop as greater is the genetic variability in the existing germplasm better would be the chances of selecting superior genotypes. Improvement through selection depends upon the variability existing in the available cultivars, which may be due to the difference either in genetic constitution of cultivars or in the environments in which they grow (Sestra *et al.*, 14). A breeding strategy becomes purposeful and effective when it is based on genetic diversity present in particular species (Patil *et al*., 11). Genetic diversity is being used as source of genes in crop improvement for production of high yielding varieties, hybrids and to effect ecologically sustainable economic and social development (Kameshwari *et al*., 6). The present study was an attempt to investigate the extent of divergence among various genotypes of tulip using D^2 analysis.

MATERIALS AND METHODS

The present study was carried out at the research farm of Indian Agricultural Research Institute, Regional Station, Katrain, Kullu, Himachal Pradesh

*Corresponding author's E-mail: reetaiari@yahoo.com

(32°12N'; 77°13'E; 1,560 m asl) during 2012-14. The Field received 950-1,000 mm annual rainfall and 1,000-1,100 mm snowfall annually. The experimental material comprised 21 tulip genotypes introduced from Holland and collection from various parts of Jammu & Kashmir. The morphological characteristics of these tulip genotypes have been given in Table 1. Healthy and uniform sized bulbs weighing about 10-15 g were planted 8-10 cm deep at a distance of 20 cm × 15 cm. Eighteen bulbs were planted per treatment per replication in randomized block design with three replications. After planting of bulbs the crop was mulched with grass in order to maintain the soil temperature and moisture. The recommended package of practices along with plant protection measures was followed to raise the successful crop. The data were recorded on five randomly selected plants per replication. The observations were recorded for 16 quantitative traits, namely days to sprouting, percentage sprouting, number of leaves/ plant, wrapper leaf area, days to flower, plant height, scape length, scape thickness, flower diameter, percentage flowering, duration of flowering, number of bulbs per plant, bulb weight (g), bulb size (cm), number of bulblets per plant and bulblets weight. The D^2 statistic was used for assessing the genetic divergence among the populations as suggested by Mahalanobis (9). Based on the D^2 values thus obtained, the entire germplasm was classified into distinct clusters, grouping together the less divergent

Table 1. Morphological characterization of 21 tulip genotypes.

Genotype	Cultivar group	Flowering time	Morphological description
Ali bi	Triumph Tulip	Mid-late spring	Medium cup shaped, lavender pink flowers
American Dream	Darwin Hybrid	Mid spring	flower is pale yellow, edged in glowing red with a golden apricot-sheen
Apeldoorn	Darwin Hybrid	Mid spring	Bowl shaped, cherry red flowers with signal red margins. Inside signal red with contrasting black center encircled with yellow border
Apeldoorn Elite	Darwin Hybrid	Mid spring	Crimson flowers with a contrasting yellow edge, black anthers and black basal marks inside
Blushing Apeldoorn	Darwin Hybrid	Mid spring	Cup shaped, sunny yellow with delicate red outline and tangerine blush,
Cassini	Triumph Tulip	Mid- late spring	Dark red, goblet shape, fragrant
Character	Darwin Hybrid	Mid Spring	Bowl shaped, golden yellow flowers with black anthers.
Christmas Dream	Single Early Tulip	Early spring	Pink to rose colored, cup shaped blooms
Ganders Rhapsody	Triumph Tulip	Late spring	Cup shaped, deep pink flowers with white stripes
Golden Apeldoorn	Darwin Hybrid	Mid Spring	Cup shaped golden yellow flowers with black anthers, contrasting black center with bronze green border
Golden Melody	Triumph Tulip	Late spring	
Golden Oxford	Darwin Hybrid	Mid Spring	Bowl shaped, golden yellow flowers with black anthers.
Hamilton	Fringed Tulip	Late Spring	Buttercup yellow flowers with dark yellow fringes
Horizon	Double Late Tulip	Late spring	Peony flowered
Leen Vander Mark	Triumph Tulip	Mid spring	Vibrant red with white blooms
Lle De France	Triumph Tulip	Mid-late spring	Cardinal red flowers with dark bronze-green basal marks and yellowish brown margins
Monte Carlo	Double Early tulip	Mid spring	Double, sulphur yellow flowers with small red feathers
Oxford Wonder	Darwin hybrid		Large, golden yellow with an orange-red flame.
Pretty Women	Lily flowered Tulip	Mid-late	Cardinal red flowers with pointed and slightly reflexed petals
Strong Gold	Triumph Tulip	Late spring	Cup shaped, primrose yellow exterior with faint orange flames, canary yellow interior
Tulip Hb	Darwin Hybrid	Mid spring	Blood red flowers with signal red flames and buttercup yellow bases with greenish yellow margins

genotypes (Rao, 13). Principal Component Analysis was conducted by SPSS 16. Mean values registered for each variable were used for statistical analysis. The Principal Components (PC) for the dataset, eigen values (variance) for the PCs loadings (correlation of each variables with PCs) and PC score for each genotypes under the concerned PCs were used for interpretation of the analysis. PCs showing eigen values lesser than one was considered non-significant. PC loadings greater than selection criterion (SC) were considered significant. The SC value was calculated as 0.5/ √PC eigen value (Ovalles and Collins, 10). A dendrogram was constructed based on hierarchical cluster analysis using SPSS 16.0 statistical package.

RESULTS AND DISCUSSION

There was highly significant genotypic differences for all the traits studied revealing the existence of substantial amount of variation among the genotypes. Based on D^2 analysis the 21 genotypes were classified into five clusters (Table 2). The cluster II had the maximum (6) genotypes and cluster IV had only one genotype. The cluster I and cluster V had 5 genotypes in them. The cluster III had four genotypes. The pattern of distribution of genotypes from different eco-geographical regions into different clusters with different divergence values was at random, supporting that geographical diversity is not related to genetic diversity. The main forces other than geographical origin responsible for this genetic diversity may be natural/ artificial selection, exchange of breeding material, genetic drift and environmental variation. Similar conclusions were drawn by Kavitha and Anburani (7) in African marigold and Kumar *et al*. (8) in Snapdragon.

There was considerable amount of genetic divergence in the present collection as evident from inter- and intra-cluster distances among five clusters (Table 3). Intra-cluster distance was highest (2067.0) in cluster III with four genotypes and lowest (0.0) in cluster IV as represented by only one line (Ganders Rhapsody). This indicated that the genotypes in cluster III were highly diverse. Highest inter-cluster distance was between cluster III and V (11005.8) followed by I and III (7395.9); III and IV (6882.3), and II and III (5542.4). The lowest inter-cluster distance was between cluster I and V (1641.1). From D_2 analysis it was evident that crosses may be attempted between the genotypes of cluster III (Character, Christian Dream, Hamilton, Horizon) and cluster V (Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb) to obtain new desirable recombinants in tulip. Since all kinds of gene actions and interactions are possible in the expression of quantitative traits it is advisable to make crosses between genotypes selected from the clusters with high mean performance to get desirable transgressive segregants. According to Patil *et al*. (11), the highly divergent genotypes would produce a broad spectrum of variability enabling further selection and improvement. The hybrids developed from these genotypes within the limit of compatibility of these clusters may produce high magnitude of heterosis or desirable transgressive segregants, which would be rewarding for successful breeding programme.

Table 3. Inter/ intra distance matrix among 5 clusters based on D^2 analysis.

Cluster	1	2	3	4	5
1	1226.1	2004.3	7395.9	3324.4	1641.1
2		719.1	5542.4	3902.1	2383.3
3			2067.0	6882.3	11005.8
4				0.0	4335.1
5					772.5

The study of cluster mean value of 5 clusters indicated considerable differences for the traits studied (Table 4). After leaving out the solitary cluster IV, range of variation for number of leaves, scape thickness, flower size, number of bulbs & bulblets were low among the multi-member cluster. The characters, *viz*., duration of flowering, bulb size and bulb weight exhibited moderate variations. The high range of variation was observed for days to sprout, days to flower, wrapper leaf area, percent sprouting & flowering, plant height and scape length among

Table 2. Cluster classification of 21 tulip genotypes based on D^2 analysis.

Cluster No.	Genotype(s)
I	Ali Bi, American Dream, Golden Melody, Golden Oxford, Oxford Wonder
II	Apeldoorn Elite, Cassini, Lean Vander Mark, Lle de France, Monte Carlo, Pretty Women
III	Character, Christian Dream, Hamilton, Horizon
IV	Ganders Rhapsody
V	Apeldoorn, Blushing Apeldoorn, Golden Apeldoorn, Strong Gold, Tulip Hb

Table 4. Cluster means of 21 genotypes for 16 traits based on D^2 analysis.

Cluster	Days to sprout	Percent sprouting	No. of leaves/ plant	Wrapper leaf area (cm^2)	Days to flower	Plant height (cm)	Spike length (cm)	Scape thickness (mm)	Flower size (cm)	Percent flowering	Flower duration (days)	No. of bulbs/ plant	Bulb wt. (g)	Bulb size (cm)	No. of bulblets/ plant	Bulblet wt. (g)
1	52.0	85.3	4.1	114.9	126.9	29.15	23.3	5.4	6.1	88.0	17.5	2.2	15.1	10.8	2.6	3.1
2	39.8	96.0	3.6	85.3	119.8	27.97	22.3	5.2	6.6	83.9	19.2	2.3	14.8	10.8	2.5	2.6
3	61.5	55.0	3.5	59.4	122	19.7	15.7	5.2	5.3	45.4	16.2	1.4	9.2	8.7	1.5	1.3
4	40.3	44.4	5.1	98.4	114	44.3	38.4	6.8	5.4	97.6	21.3	3.2	16.4	13.6	1.5	2.0
5	37.1	100.0	3.8	123.3	114.2	34.0	25.5	6.2	6.3	96.8	22.0	2.8	21.5	12.4	1.8	3.3

the different clusters. The variation observed in cluster means also point out the degree of variability. The cluster V includes genotypes with earliness and exhibited longest duration of flowering, highest wrapper leaf area and bulb weight. Genotypes of this cluster also possessed desirable floral traits (scape length, floral size and scape thickness) and bulb traits (number of bulbs per plant and bulb weight). Hence, genotypes from this cluster could serve as valuable parents to develop superior cultivars. The cluster IV that contained only one genotype (Ganders Rhapsody) was early with taller plant height and scape length. This cluster had thicker scape and high bulb multiplication potential. The cluster III genotypes were late and produced small sized flowers on thin and short scapes. The genotypes of this cluster were dwarf and produced least number of bulbs and bulblets with minimum bulb size and bulb weight. The genotypes of cluster II had larger flowers and were early in sprouting and flowering. The genotypes of cluster II possessed medium plant height, scape length and had moderate bulb multiplication potential. The genotypes of cluster I was late with medium plant height, scape length and produced relatively larger flowers. The cluster I genotypes showed moderate bulb multiplication potential and produced comparatively larger bulbs than cluster III. The intercrossing genotypes of cluster IV and V with other genotypes of cluster I, II and III may create wider variability, which is expected to produce high yielding transgressive segregants in tulip improvement programme.

In the present investigation, the first six principal components with eigen values more than 0.5 contributed to 91.88 per cent of cumulative variability among the 21 tulip genotypes evaluated for 16 morphological characters (Table 5). The first principal component accounted for 57.25 per cent of variability while, the second and third accounted for 12.37 and 7.54 per cent of total variability respectively. The percent of variability from fourth to sixth principal component accounted for 7.54, 6.67, 4.63 and 3.42 in decreasing order, respectively. The PCs from 7 to 16 which recorded the eigen values less than 0.5 were ignored as they were unlikely to have any practical significance. It was therefore inferred that essential features of dataset had been represented in the first 6 PCs. The significance of the variables in each PC was determined by comparing the loading with corresponding SC. Days to sprouting (0.90) and percent sprouting (0.43) explained the maximum variance in PC1. The PC2 which accounted for 12.37% of total variance showed higher variance for number of leaves (0.65), scape thickness (-0.43) and scape length (-0.42) signifying their importance

Table 5. Component loading of 16 traits, eigen values, proportion of the total variability represented by first 6 principal components (PC), cumulative percent variability and Selection Criterion (SC) in 21 tulip genotypes.

Trait	Principal component					
	1	2	3	4	5	6
Days to sprout	0.90	0.00	-0.39	0.00	0.00	0.00
Percent sprouting	0.43	0.03	0.70	0.00	0.00	0.01
No. of leaves/ plant	-0.01	0.65	-0.06	0.00	0.01	0.30
Wrapper leaf area (cm^2)	-0.08	-0.03	-0.59	0.00	0.00	-0.01
Days to flower	0.00	-0.13	0.00	0.97	0.11	0.16
Plant height (cm)	0.00	-0.34	0.00	-0.22	0.76	0.38
Spike length (cm)	0.00	-0.42	0.00	-0.10	-0.64	0.51
Scape thickness (mm)	0.00	-0.43	0.00	0.01	-0.01	-0.31
Flower size (cm)	0.00	-0.21	0.00	0.01	-0.01	-0.43
Percent flowering	0.00	0.16	0.00	0.09	-0.06	-0.19
Flower duration (days)	0.00	0.08	0.00	0.00	0.00	-0.32
No. of bulbs/ plant	0.00	-0.02	0.00	0.00	0.00	0.10
Bulb wt. (g)	0.00	0.02	0.00	0.00	0.00	0.21
Bulb size (cm)	0.00	0.00	0.00	0.00	0.00	0.00
No. of bulblets/ plant	0.00	0.00	0.00	0.00	0.00	0.00
Bulblet wt. (g)	0.00	0.00	0.00	0.00	0.00	0.00
Eigen values (variance)	9.16	1.98	1.21	1.07	0.74	0.55
Percent variability	57.25	12.36	7.54	6.67	4.63	3.42
Cumulative percent Variability	57.25	69.62	77.16	83.83	88.46	91.88
SC	0.16	0.36	0.45	0.45	0.58	0.68

in quality improvement in tulip. The PC3 reflected significant loadings for traits like percent sprouting (0.70) and wrapper leaf area (-0.59). The PC4 showed significant variance for days to flower (097). The PC5 reflected the significant loading for plant height (0.76) and scape length (-0.64).

Principal component analysis (PCA) reduces the large dataset to a small numbers of unrelated groups of variables of their components. Variables strongly associated with same group may share some underlying biological relationship. These associations are often useful for generating hypothesis or for understanding behavior of complex traits (Dey *et al*., 3). The components like days to sprout and percent sprouting, number of leaves, scape length and scape thickness explained considerably higher amount of variations in PC1 and PC2 that accounts for 69.62% of cumulative variability. Scape length is one of the economically important traits that determine the quality of cut tulips. Significant positive correlation of scape length with plant height, number of leaves per plant, wrapper leaf area, flower size, number of bulbs per plant, bulb size and bulb weight was earlier reported by Bhatia *et al.* (2). Hence, there is a large scope for improvement of these traits through selection based on scape length.

Based on hierarchical cluster analysis, 21 genotypes were divided into two major clusters at a distance co-efficient of 25 (Fig. 1). The first cluster had four genotypes, namely, Character, Hamilton, Horizon and Christmas Dream. Among them first three genotypes were very close to each other. The second major cluster had rest of the 17 genotypes. The second cluster had two sub-cluster with five genotypes in first sub-cluster had five genotypes (Cassini, Oxford Wonder, Leen Vender Mark, Monte Carlo and Lle De France) and rest of the 12 genotypes were represented in second sun-cluster. In the second sub-cluster the cultivars, Apeldoorn, Golden Apeldoorn and American Dream were very closely related to each other. Similarly, Strong Gold and Tulip Hb were also closely related with each other. Clustering pattern based on the dendrogram make it possible to visualize the distance among the cultivars very clearly. Selection of cultivars becomes easier with dendrogram. Similar results were reported by Kameswari *et al.* (6) in chrysanthemum.

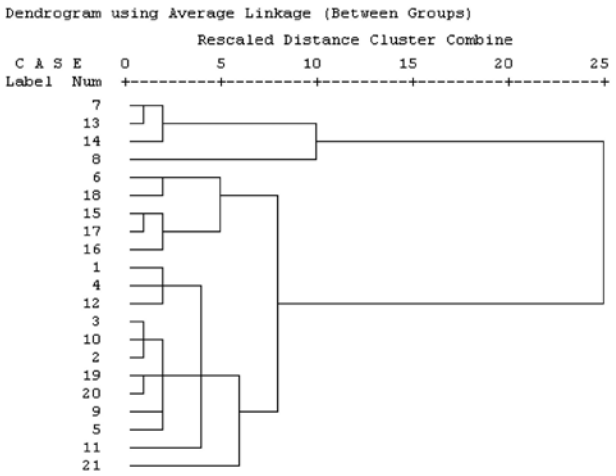


Fig. 1. Clustering pattern of tulip genotypes based on morphological traits.

In total, 8 distinct minor clusters were formed with largest distance between the genotypes, Character and Tulip Hb. The clustering pattern was similar to the D^2 analysis. However, they were separated through dendrogram with 8 minor clusters.

ACKNOWLEDGEMENT

Financial assistance from AICRP on Floriculture Crops (ICAR) is highly acknowledged.

REFERENCES

1. Archana, B., Patil, A.A., Hunje, R. and Patil, V.S. 2007. Studies on genetic variability analysis in gladiolus hybrids. *J. Orn. Hort*. **11**: 121-16.

2. Bhatia, R., Dhiman, M.R., Parkash, C. and Dey, S.S. 2013. Genetic variability and character association in tulip (*Tulipa gesneriana*) for various quantitative traits. *Indian J. Agric. Sci.* **83**: 773-80.

3. Dey, S.S., Behera, T.K., Sirohi, P.S. and Rao, A.R. 2005. Genetic diversity of bitter gourd (*Momordica charantia* L.) genotypes as revealed through principal component analysis. *Proc. Nat. Sem. Cucurbits,* India, pp. 251-57.

4. Jhon, A.Q., Khan, F.U. and Rather, Z.A. 2006. Genetic variability studies in tulip. *Appl. Biol. Res.* **8**: 37-39.

5. Kamble, B.S., Reddy, B.S., Patil, R.T. and Kulkarni, B.S. 2004. Performance of gladiolus (*Gladiolus hybridus* Hort.) cultivars for flowering and flower quality. *J. Orn. Hort.* **7**: 51-56.

6. Kameswari, P.L., Pratap, M., Anuradha, G. and Begum, H. 2014. Genetic divergence studies in chrysanthemum (*Dendranthema grandiflora* Tzvelev). *Indian J. Sci. Res. Tech*. **2**: 4-10.

7. Kavitha, R. and Anburani, A. 2009. Genetic diversity in African marigold (*Tagetes erecta* L.) genotypes. *J. Orn. Hort.* **12**: 198-201.

8. Kumar, R., Kumar, S., Kumar, P. and Mer, R. 2011. Genetic variability and divergence analysis in snapdragon (*Antirrhinum majus* L.) under tarai conditions of Uttarakhand. *Progr. Hort.* **43**: 332-36.

9. Mahalanobis, P.C. 1936. On the generalized distance in statistics. *Proc. Nat. Inst. Sci.*, *India*, **2**: 49-55.

10. Ovalles, F.A. and Collins. 1988. Variability in North West Florida soils by principal component analysis. *Soil. Sci. Soc. American J*. **52**: 1430-33.

11. Patil, M.S., Aswath, C. and Kumar, D.P. 2009. Multivariate analysis and the choice of parents for hybridization in anthurium (*Anthurium andreanum* L). *J. Orn. Hort.* **12**: 127-31.

12. Podwyszynska, M. and Sochacki, D. 2010. Micropropagation of tulip: Production of virus-free stock plants. **In**: *Protocols for In Vitro Propagation of Ornamental Plants*. Jain, M. and Ochatt, J. (Ed.), The Humana Press Inc. Totowa, NJ, USA, pp. 243-56.

13. Rao, C.R. 1952. *Advanced Statistical Methods in Biometrical Research*, John Wiley and Sons Inc., New York, pp. 236-72.

14. Sestra, R., Mihalte, L., Sestra, A., Bondrea, I. and Baciu, A. 2007. The variability and heritability of several traits at different cultivars of tulips *Bull. Usamv-CN* **64**: 1-2.

Received : January, 2016; Revised : September, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00109.8

Indian J. Hort. 74(4), December 2017: 568-573

In vivo bulblet multiplication in LA lilium hybrids through scaling technique

Asmita*, S.S. Sindhu and M.K. Singh

Department of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Lilium is a high value flower but difficult to multiply through conventional propagation methods. Therefore, an attempt has been made with an objective to multiply maximum bulblets through scaling under different storage durations and temperature conditions. The experiment was conducted in a completely randomized design (factorial) consisting of different storage durations (0, 3, 6, 9 and 12 weeks), type of scales (outer and inner) and temperature regime (4° and 2°C) on three lilium cultivars, namely/ Brindisi, Ercolana and Pavia. The results showed that bulblet production decreased from outer to inner scales and showed a positive correlation with the scale width. Nine week storage resulted in early bud sprouting and root initiation due to breaking of dormancy. However, size of bulblets were recorded more in the scales planted just after harvesting. Total weight of bulblets per scale was directly related to the number of bulblets per scale produced. Maximum number of bulblets (3.28) per scale was produced in the cv. Pavia followed by Ercolana (3.10) and Brindisi (2.18). Six weeks storage duration at 4°C was recorded best for cv. Brindisi. However, 9 week storage (4°C) was best for cvs Ercolana and Pavia with regard to maximum bulblet production. While, maximum rooting and root length were recorded in the cv. Brindisi followed by Ercolana and Pavia.

Key words: *In vivo* bulblet, lilium, multiplication, scaling, storage.

INTRODUCTION

Lilium is one of the leading cut flowers in the world market and amongst the most beautiful ornamental bulbous cut flowers. This high value crop is very popular pot plant as well due to different colours like white, pink, yellow *etc*. The genus *Lilium* belongs to the family Liliaceae and is native to Northern hemisphere in Asia, Europe and North America. In India, lilium is found growing naturally in Nilgiri Hills and Himalayan regions (Bose and Yadav, 2). Its bulbs are non-tunicated, consisting of fleshy scales joined at the basal plate. About 76% of the total world lily bulb production takes place in the Netherlands (Qu *et al.*, 13). In India suitable climatic conditions for lilium cultivation have been identified in different regions. Although, vernalized lilium bulbs could be made to flower anywhere in the country by providing optimum growing conditions, yet commercial cultivation of lilium is restricted to Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Tamil Nadu (Ooty and adjoining region) and North Eastern states without much environmental manipulation.

Lilium bulbs exhibit a distinct period of dormancy, which can be broken by regulating the storage duration and temperature to enable root and shoot development (De-Hertogh and Le Nard, 4). It triggers the developmental processes leading to dormancy breaking (De-Hertogh *et al*., 3; Bewley, 1). Earlier Dhiman (5) and Park (12) have studied the effect of temperature and scale position and storage in Asiatic lily. Sharma *et al.* (15) have standardized propagation medium and growth regulators for scaling in Oriental lily. Cultivation of liliums under north Indian plain conditions is a new intervention. However, availability of quality planting material to a common grower at an affordable price has always remained the bottleneck. Multiplication through bulbs, bulblets and bulbils is slow, whereas; micropropagation need controlled environment conditions and trained technician. Hence, multiplication through scaling holds an effective way for commercial multiplication of bulblets of lilium hybrids at comparatively cheaper and faster rates, hence the present study were carried out.

MATERIALS AND METHODS

The present experiment was conducted at the Experimental Farm of the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, India during 2016-17. The experiment was laid out in a completely randomized design(factorial) (Gomez and Gomez, 7) consisting of different storage durations, *viz*., 0, 3, 6, 9 and 12 weeks and storage temperature regimes, *viz.* 4 and 2°C on three LA lily cultivars, namely, Brindisi, Ercolana and Pavia. The bulbs were lifted from the experimental field during last week of April, 2016 and after removal of the soil particles adhered on the surface were dipped into the fungicidal solution of carbendazim (0.2%) for 1 h before storage. Cold

*Corresponding author's E-mail: asmitakumari97@gmail.com

storage with automatically controlled parameters like temperature and humidity were used for the storage of bulbs at desirable temperatures (4 and 2°C) and durations (3, 6, 9 and 12 weeks) except for the control lots maintained at room temperature. The relative humidity of the storage chambers was adjusted at 70-80% throughout the storage period. The bulbs were stored according to the storage durations and taken out at subsequent intervals for planting of the outer and inner scales under *in vivo* conditions in moist coco-peat as growing medium.

For *in viv*o propagation through scales, bulbs were taken from cold storage and individual bulb scales were excised from the mother bulb with hands in such a way so that a small portion of basal plate remained attached to ensure bulblet formation and rooting. Diseased, rotten, broken and ones without basal plate scales were discarded. The detached scales were then disinfected by dipping them in carbendazim (0.2%) for one hour and maintained in the shade. In the present experiment, coco-peat was used as the propagation medium for scaling. Commercial coco peat bricks were soaked overnight in tap water to make it sufficiently moist for scaling or bulblet induction on the scales. The medium was treated with a fungicidal solution (0.2% carbendazim) before use and then filled into plastic seed trays. The prepared scales were then planted in such a way so that a thin layer of 1 cm coco-peat remains above the scales. Both outer and inner scales were used for bulblet multiplication. Fifteen scales were used in each treatment with five scales per replication. Routine cultural operations like weeding, loosening of potting medium and watering *etc*. were done as per the requirement. Observations on important parameters were recorded and analyzed.

RESULTS AND DISCUSSION

Multiplication of bulblets through scales under *in vivo* conditions was found to be significantly influenced by storage duration, storage temperature and types of scale. Sprouting percentage of outer and inner scales was recorded 100% irrespective of storage duration and temperature in all the three cultivars. However, days to bulblet initiation varied significantly under different storage periods (Table 1). Scales of cv. Brindisi sprouted earlier than Ercolana and Pavia. Irrespective of cultivars, the earliest bud sprouting was recorded in 12 week storage period, which was statistically at par with 9 weeks storage period followed by 6 week storage period. Of the two scale type studied, outer scales sprouted earlier than inner scales and storage temperature of 4°C had marked affect than 2°C, which was statistically significant in all the three cultivars. Storing bulbs at cold temperature is known to trigger the increase of the growth promoters and or the decrease of inhibitors, which result in rapid bulblet induction. The time required for bulb sprouting decreased with enhancement in storage duration was earlier reported by Dhiman (5), Malik *et al*. (10) and Lee *et al*. (9).

Bulblet multiplication is a major consideration in the commercial floriculture. Maximum numbers of bulblets were produced in cv. Brindisi when stored for 6 week duration (Table 1). However, cvs Ercolana and Pavia produced the maximum bulblets when stored for 9 weeks. Irrespective of cultivars, outer scales produced more bulblets as compared to inner scales and 4°C storage temperature was found to be effective as compared to 2°C. Among all the three cultivars, maximum numbers of bulblets were produced in Pavia followed by Ercolana and Bridisi (Fig. 1). Interaction effect of the storage duration and type of scale revealed that 6 week storage duration in cv. Brindisi outer scales resulted in the highest number of bulblets per scale. However, 9 week storage period was sufficient for the highest bulblet induction from outer scales of cvs Ercolana and Pavia. Average number of bulblets per scale was more in the outer scales than inner scales, which showed that production of bulblet is directly related to the scale width. Among all the cultivar studied, cv. Pavia resulted in the highest number of bulblets per scale followed by Ercolana and Brindisi. The genotypic differences along with environmental conditions could be accounted for this variation (Table 1a). Interaction data of the storage duration and storage temperature showed that 4°C storage temperature had marked effect than 2°C on bulblet multiplication. In case of Brindisi, 4°C storage temperature combined with 6 week storage period had marked effect on bulbet multiplication. But in case of cvs Ercolana and Pavia, 9 week storage duration with 4°C storage temperature resulted in highest No. of bulblets per scale. Cultivar Pavia produced highest number of bulblets per scale followed by cvs Ercolana and Brindisi (Table 1b). The physical environment exerts a marked influence on dormancy, which is usually broken by a period of

Table 1a. Interaction of storage period × scale type on number of bulblets production per scale in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	Outer scale	Inner scale	Outer scale	Inner scale	Outer scale	Inner scale
0	1.80	1.73	2.73	2.00	2.20	2.20
3	2.13	2.00	2.23	2.33	2.30	2.37
6	2.33	2.03	2.27	2.20	2.97	2.17
9	2.07	2.00	3.23	2.97	3.43	3.13
12	2.30	2.07	3.07	2.70	3.27	3.07
CD at 5%	NS		0.33		0.23	

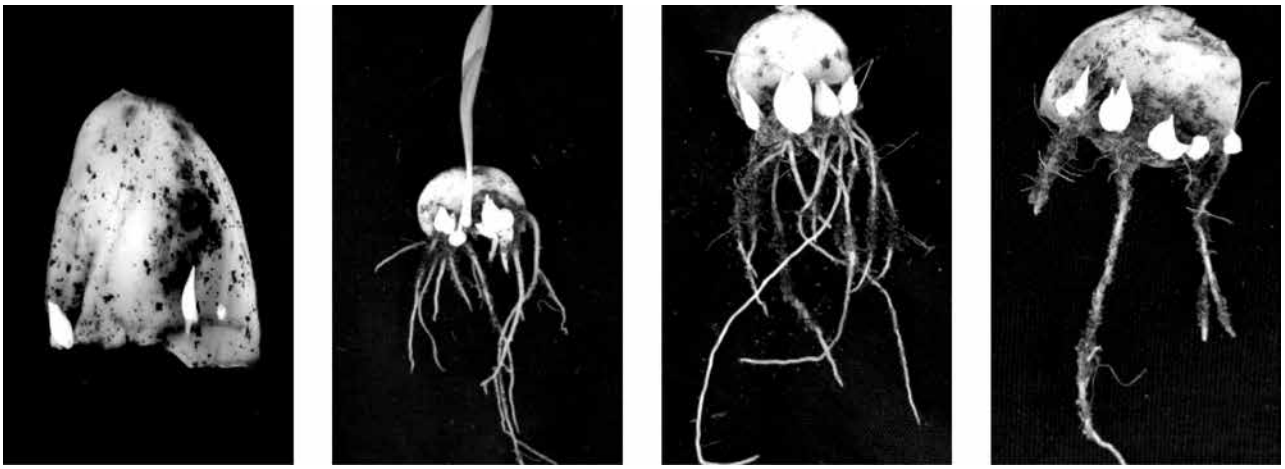


Fig. 1. Stages of vegetative propagation in LA lilium hybrids through scaling technique. (a) Bulblet induction from scales, (b) Bulblets produced from scales in cv. Brindisi, (c) Bulblets produced from scales in cv. Ercolana, (d) Bulblets produced from scales in cv. Pavia.

cold treatment. Not only storage at low temperature but also long storage durations at low temperatures lead to breaking of dormancy, thus resulting in early sprouting and maximum bulblet production. Abscisic acid concentration in the scales of Lilium bulbs decreased as storage duration extended, and it declined to a constant low level after bulbs had been stored for 6-9 weeks at 4°C. This result indicates that the decrease in the endogenous ABA concentration during bulb storage is related to dormancy release in lilium bulbs (Rong-Yan, 14). These findings have also been supported earlier findings of Moshrefi *et al*. (11) and Singh (16) in Asiatic lilies. Scale segments obtained from the outer scales tended to induction of higher number of bulblets, which can be correlated with the total carbohydrates content in the scales (Park, 12).

Significantly higher bulblet size was observed in the 6 week storage duration followed by control (scale planted just after harvest) and 3 week storage duration in the cv. Brindisi. However, in case of cvs Ercolana and Pavia, the maximum bulblet size was attained in the scales planted just after harvesting. This may be due to the fact that scales planted immediately after harvest remained in the coco-peat for a longer duration as compared to other treatments. Thus, one can recommend to multiply bulblets from the scales just after harvesting of bulbs if have enough scales because it will take shorter period to achieve commercial size of bulbs. As the number of bulblet per scale increased, individual size of bulblets is decreased. Hence, it will take comparatively longer period to get commercial size of bulbs. When compared to the types of scale, outer scales attained bigger bulblet size as compared to the inner scales in all the three cultivars studied. Similarly, 4°C storage temperature stored bulb's scales attained bigger bulblet size among all the three cultivar. Interaction of storage duration and types of scales influenced bulblet size in cv. Brindisi. However, it was statistically non-significant in cvs Ercolana and Pavia (Table

Table 1b. Interaction of storage period × storage temperature on number of bulblets per scale in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	4°C	2°C	4°C	2°C	4°C	2°C
0	1.77	1.77	2.37	2.37	2.20	2.20
3	2.20	1.93	2.47	2.10	2.30	2.37
6	2.27	2.10	2.30	2.17	2.70	2.43
9	2.07	2.00	3.13	3.07	3.33	3.23
12	2.20	2.17	3.07	2.70	3.20	3.13
CD at 5%	NS		NS		NS	

Table 1c. Interaction of storage period × scale type on the size of bulblets (mm) in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	Outer scale	Inner scale	Outer scale	Inner scale	Outer scale	Inner scale
0	11.43	11.54	10.85	10.67	10.77	10.43
3	11.38	10.78	10.31	10.47	9.94	9.81
6	11.97	11.49	10.27	10.31	10.19	9.99
9	10.22	10.39	9.65	9.67	9.85	9.85
12	10.06	9.88	10.41	10.31	8.73	8.78
CD at 5%	0.40		NS		NS	

1c). Interaction data of storage duration and storage temperature showed that 4°C storage temperature had significant effect on the size of bulblet in cvs Brindisi and Pavia (Table 1d). This may be due the fact that low temperature below 4°C may suspend metabolic activity in the bulbs, so slow rate of size enlargement. In case of scales planted just after harvesting, no cold treatment was given, hence once the scales sprouted, it attained the maximum growth. Longer duration in the cocopeat media is also the contributing factor affecting size enlargement. Among all the three cultivars, maximum bulblet size was observed in the cv. Brindisi followed by cvs Ercolana and Pavia. A significant difference in the bulblet size was also reported by Kapoor *et al*. (8) but on different growing media.

Table 1a revealed that there was a significant effect of the storage duration on weight of bulblets per scale produced in all the three cultivars. Maximum weight of bulblets was recorded in cv. Pavia followed by cvs Ercolana and Brindisi. Total weight of bulblets per scale was directly related to the number of bulblets per scale produced. Cultivar Brindisi produced maximum weight of bulblets at storage duration of 6 weeks, whereas, cvs Ercolana and Pavia produced the maximum weight at 9 week storage duration. Dhiman and Sindhu (6) and Sharma *et al.* (15) also worked on lilium and found significant result. Weight of the outer scales was more than the inner scales and 4°C storage was found to be significant among all the cultivars.

Table 1d. Interaction of storage period × storage temperature on the size of bulblets (mm) in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	4°C	2°C	4°C	2°C	4°C	2°C
0	11.49	11.49	10.76	10.76	10.60	10.60
3	11.48	10.68	10.56	10.22	10.09	9.65
6	11.70	11.76	10.22	10.36	10.07	10.11
9	10.56	10.05	9.85	9.48	10.02	9.68
12	9.99	9.94	10.41	10.31	9.19	8.31
CD at 5%	0.40		NS		NS	

When root system was compared in the three cultivars, statistically significant variation was observed in the days to root initiation under different storage periods (Table 2). Earliest root initiation was observed in the 12 week storage duration and was statistically at par with the 9 week storage period in all the cultivars. When compared with the types of scales, outer scales of cvs Brindisi and Pavia were earlier to root than inner scales. 4°C storage temperature had pronounced effect on early root initiation in cvs Ercolana and Pavia whereas, 2°C stored scales rooted early in cv. Brindisi. Number of primary roots (Roots arising directly from the bulblets) was recorded maximum under 9 week storage duration and outer scales produced more number of primary roots than inner scales in all the 3 cultivars

Table 1e. Effect of different storage duration, scale type and temperature on bulblet attributes in LA lilium hybrids.

Treatment	Days to bulblet initiation			No. of bulblets			Size of bulblets (mm)			Weight of bulblets per scale (g)		
	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia
Storage duration (week)												
0	36.57	36.00	45.30	1.77	2.37	2.20	11.49	10.76	10.60	1.93	2.57	2.40
3	21.00	25.53	26.72	2.07	2.28	2.33	11.08	10.39	9.87	2.25	2.48	2.53
6	19.03	20.33	22.18	2.18	2.23	2.57	11.73	10.29	10.09	2.38	2.47	2.60
9	18.05	19.42	21.18	2.03	3.10	3.28	10.30	9.66	9.85	2.25	3.30	3.47
12	17.42	18.93	20.57	2.18	2.88	3.17	9.97	10.36	8.75	2.38	3.08	3.37
CD at 5%	0.73	1.30	1.09	0.18	0.24	0.17	0.28	0.43	0.28	0.19	0.23	0.27
Scale type												
Outer	21.61	24.31	26.33	2.13	2.71	2.83	11.01	10.30	9.90	2.31	2.91	3.02
Inner	23.22	23.78	28.05	1.97	2.44	2.59	10.81	10.29	9.77	2.17	2.65	2.73
CD at 5%	0.46	NS	0.69	0.11	0.15	0.10	0.18	NS	NS	0.12	0.15	0.17
Storage temp. (°C)												
4	22.85	22.97	26.19	2.10	2.67	2.75	11.04	10.36	10.00	2.28	2.87	2.95
2	21.98	25.11	28.19	1.99	2.48	2.67	10.78	10.22	9.67	2.20	2.69	2.79
CD at 5%	0.46	0.82	0.69	NS	0.15	NS	0.18	NS	0.18	NS	0.15	NS

Table 2. Effect of different storage duration, scale and temperature on rooting parameters in LA lilium hybrids.

Treatment	Days of to root initiation			No. of primary roots			Length of primary roots (cm)			Dia. of primary roots (mm)			No. of secondary roots		
	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia
Storage duration (week)															
0	38.57	38.00	47.30	6.03	6.13	4.60	11.70	11.29	9.08	1.17	1.02	0.98	4.59	4.99	2.94
3	23.02	27.57	28.70	6.22	6.02	3.46	13.55	9.80	5.89	1.27	1.04	1.04	7.01	3.84	3.04
6	21.23	22.38	24.22	6.92	5.68	5.25	18.69	8.84	11.93	1.31	1.00	1.13	12.98	6.12	5.05
9	20.10	21.40	23.22	7.60	7.59	6.69	12.12	13.19	13.10	1.11	1.18	1.17	10.41	9.87	10.15
12	19.53	20.77	22.58	5.37	6.95	5.01	13.76	16.29	11.02	1.18	1.72	1.26	4.17	6.42	6.51
CD at 5%	0.71	1.28	1.07	0.89	1.00	0.88	2.72	1.78	1.73	0.12	0.16	0.13	1.91	1.59	0.99
Scale type															
Outer	23.67	26.24	28.41	6.40	6.91	5.00	12.82	12.48	10.16	1.23	1.23	1.14	8.03	6.88	5.86
Inner	25.31	25.81	30.00	6.46	6.04	5.00	15.11	11.28	10.25	1.19	1.15	1.09	7.64	5.62	5.22
CD at 5%	0.45	NS	0.68	NS	0.63	NS	1.72	1.13	NS	NS	NS	NS	NS	1.00	0.62
Temperature (°C)															
4	24.87	24.96	28.22	6.82	6.39	5.09	14.55	12.14	8.89	1.28	1.21	1.14	6.86	6.07	5.00
2	24.11	27.09	30.19	6.04	6.56	4.91	13.37	11.62	11.52	1.14	1.17	1.09	1.21	6.43	6.07
CD at 5%	0.45	0.81	0.68	0.57	NS	NS	NS	NS	1.09	0.08	NS	NS	1.21	NS	0.62

(Table 2). Among the cultivars, Brindisi produced the maximum number of roots followed by Ercolana and Pavia. Along with the number of roots, root length is also an important parameter because it can make uptake nutrient and water from the deeper region of growing medium. Maximum length of primary roots as observed under 6 week storage duration followed by 12 and 3 weeks in cv. Brindisi. The non-significant differences in the length of roots were observed in under different storage temperature in cvs Brindisi and Ercolana, however, differences were significant in cv. Pavia. More the branching of primary roots or number of secondary roots, higher is the rate of field survival, which was found to be maximum in cv. Brindisi among all the cultivars. Highest numbers of secondary roots were observed under 6 week storage duration followed by 9 week in cv. Brindisi, whereas, highest number of secondary roots was observed in cvs Ercolana and Pavia under 9 week storage. Higher level of endogenous auxin in the scales may be responsible for easy rooting and more branching.

Results of these investigations revealed that outer scales were more effective than inner scales for bulblet multiplication through scaling. Six week storage duration at 4°C was found to be best for bulblet multiplication in cv. Brindisi however, 9 week storage at 4°C was best for cvs Ercolana and Pavia.

ACKNOWLEDGEMENTS

The authors are grateful to the University Grants Commission, Ministry of Human Resource Development, Govt. of India for the financial assistance.

REFERENCES

1. Bewley, J.D. 1997. Seed germination and dormancy. *Plant Cell*, **9**: 1055-66.

2. Bose T.K. and Yadav, L.P. 1998. Lilium. *Commercial Flowers*, Naya Udyog, Kolkata, West Bengal, 789 p.

3. De-Hertogh, A.A., Carlson, W.H. and Kays, S. 1969. Controlled temperature forcing of planting lily bulbs. *J. American Soc. Hort. Sci.* **94**: 433-36.

4. De-Hertogh, A.M. and Le Nard. 1993. *The Physiology of Flower Bulbs*, Elseviers, Amsterdam, 812 p.

5. Dhiman, M.R. 2005. Effect of bulb storage duration and temperature on performance of lily. *J. Orn. Hort.* **8**: 108-11.

6. Dhiman, M.R. and Sindhu, S.S. 2007. Effect of propagation media and growth regulators on

bulblet formation through scale propagation in lilium. *J. Orn. Hort.* **10**: 181-83.

7. Gomez, L.A. and Gomez, A.A.1984. *Statistical Procedures for Agricultural Research* (3rd Edn.), John Wiley and Sons, Singapore, 680 p.
8. Kapoor, M., Grewal, H.S. and Arora, J.S. 2000. Effect of media on propagation of lilium. *J. Orn. Hort.* **3**: 58-59.
9. Lee, J.S., Kim, Y.A. and Wang, H.J. 1996. Effect of bulb vernalization on the growth and flowering of Asiatic hybrid lily. *Acta Hort.* **414**: 229-34.
10. Malik, K.M., Shiekh, M.Q., Nazki. and Mir, S.A. 2017. Influence of storage temperature, storage duration and disbudding on bulb production in Asiatic lilium cv. Royal Trinity. *Biosci. Biotech. Res. Asia*, **14**: 577-85.
11. Moshrefi, M.M., Moeini, A. and Tavasolian, E. 2004. Effects of plant growth regulators and scale on propagation of *Lilium ledebourii* (Boiss). *Iranian J. Agric. Sci.* **35**: 1033-41.
12. Park, N.B. 1996. Effect of temperature, scale position and growth regulators on the bulblet formation and growth during scale propagation of lilium. *Acta Hort.* **414**: 257-62.
13. Qu, L.W., Yin, D.S., Su, S.J., Pan, B.T., Zhao, X.H., Yang, J.M., Pei, X.H. and Fu, B. 2014. Production of lily bulbs and cut flowers in Liaoning Province of Northeast China. *Acta Hort.* **1027**: 105-12.
14. Rong-Yan, Xu. 2007. Effect of low temperature on changes in endogenous hormone level and plant development in lilium and tulipa. Ph.D. thesis, Niigata University, pp. 101-09.
15. Sharma, P., Sharma, Y.D. and Gupta, Y.C. 2007. Effect of growth regulators and growing media on propagation of Oriental lily hybrids through scaling. *J. Orn. Hort.* **10**: 148-52.
16. Singh, A. 2002. Effect of scale position, plant growth regulators and growing media on bulblet production of *Lilium* hybrids. Ph.D. thesis, Dr Y.S. Parmar University of Horticulture and Forestry, Solan, Himachal Pradesh.

Received : July, 2017; Revised : September, 2017;
Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00110.4

Indian J. Hort. 74(4), December 2017: 574-578

Occurrence of *Plantago asiatica mosaic virus* infecting oriental lily in India

S. Rajamanickam*, S. Rageshwari, P. Renukadevi and S. Nakkeeran

Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore 641 003

ABSTRACT

Lilium is a major cut flower grown under protected cultivation in Tamil Nadu and Bengaluru provinces by importing lilium bulbs from European countries. The oriental lilium grown under protected cultivation in Nilgiris provinces expressed the symptom of viral infection. The symptoms in Oriental lily include chlorotic and necrotic streaks on leaves and stunting of the infected plants. Leaf samples expressing the characteristic symptoms of virus infection were indexed using DAC-ELISA and reverse transcription polymerase chain reaction (RT-PCR). The coat protein (CP) gene of PlAMV was amplified with an amplicon size of 722 bp and sequence analysis confirmed the viruses as *Plantago asiatica mosaic virus* (PlAMV) with 99 to 100% nucleotide and 99.5 to 100% amino acid homology with other PlAMV isolates. Comparison of multiple sequence alignment analyses confirmed the close relationship between PlAMV and *Tulip virus* × (TVX), which had 70% nucleotide sequence identity. Phylogenetic analysis of the nucleotide confirms that our PlAMV isolates formed a single subgroup with other PlAMV isolates. The result provides important clues about spread of the virus and to the best of our knowledge it is the first detailed study of PlAMV infecting lily in India.

Key words: Lily, PlAMV, Occurrence, CP gene analysis.

INTRODUCTION

Lilies (*Lilium* sp.) belongs to the family *Liliaceae*, is a commercial high value cut flower crop cultivated in Nilgris province of South India under protected cultivation. The genus Lilium includes 294 genera with 4500 species with three commercially important divisions of lily including Easter lily (*Lilium longiflorum*), Asiatic and Oriental hybrids. In India, Asiatic and Oriental lilies are commonly grown in hilly areas and under temperate condition (Sharma *et al*., 15). Owing to the commercial value, lilium is grown throughout the year in Nilgris province of India (Hemamoorthy and Prakasam, 6). The *Cucumber mosaic virus* (CMV), *Lily symptomless virus* (LSV), *Strawberry latent ringspot virus* (SLRSV) and *Lily mottle virus* (LMoV) are the major viruses infecting lily in India (Sharma *et al*., 16). Apart from these viruses, *Prunus necrotic ringspot virus* (PNRSV) (Han and Liu, 2007), *Tobacco mosaic virus* (TMV), *Lily virus* × (LVX), *Tobacco rattle virus* (TRV), *Lily mild mosaic virus* (LMV), *Tomato ringspot virus* (ToRSV), *Narcissus mosaic virus* (NMV) and *Arabis mosaic virus* (ArMV) (Lee *et al*., 11; Asje, 1; Komatsu *et al*., 8) have also been reported in lily worldwide. The CMV, LSV, SLRSV predominate in India, whereas PlAMV has not been experienced. An unusual symptom including chlorotic and necrotic streaks was observed on the leaves in oriental lily in Nilgris province of Tamil Nadu during October 2015. However, it was not associated with infection by any of major viruses detected through serological reaction with tospovirus, potyviruses and ilarvirus specific antibody. Hence, the oriental lilium infection was suspected as *Plantago asiatica mosaic virus* (PlAMV). The *Plantago asiatica mosaic virus* (PlAMV) pertains to the genus *Potexvirus*, family *Flexiviridae*, a mechanically transmitted virus with unknown vectors (Komatsu *et al*., 8). The occurrence of PlAMV in oriental hybrid lilies has been reported in Southern Italy, Netherlands, South Korea, and USA (Parrella *et al*., 12; Hammond *et al*., 4). Though the occurrence of PlAMV has been reported from many countries, reports on molecular characterization of PlAMV in India are not known. However in the present investigation, we report the natural occurrence of PlAMV in lilum and its molecular properties and phylogenetic relationship.

MATERIALS AND METHODS

The lilium varieties grown under protected cultivation were observed for the presence of PlAMV symptoms in field at Nilgris district of Tamil Nadu, India. The characteristic symptoms were observed and described. The PlAMV infected plant samples collected from lilium was subjected to direct antigen coating-ELISA (DAC-ELISA) with tospovirus, potyviruses and ilarvirus specific antibody as per the procedure described by Hobbs *et al*. (7). The polystyrene plates were coated with 200 µl of plant extract ground in 0.05 M carbonate buffer pH 9.6 @ 1:10 dilution (1.59 g = Na_2CO_3; 2.93 g = $NaHCO_3$;

*Corresponding author's E-mail: rajamanickam.path@gmail.com

dissolved in 1 l deionized water) incubated at 37°C for 1.30 h. The plates were washed three times in PBS Tween (2.89 g of Na_2HPO_4; 0.4 g of KH_2PO_4; 0.4 g of KCl; 16.0 g of NaCl; dissolved in 2 l deionized water; pH 7.4 and add 0.5 ml/ l of Teen 20®) with an interval of 3 min. for each washing. The polyclonal antibody diluted in antibody buffer to a dilution of 1:1000 were added at the rate of 200 µl per well separately. Then the plates were incubated at 37°C for 3 h and washed with PBS-Tween with an interval of 3 min. for each washing. Universal conjugate was diluted in antibody buffer to a dilution of 1:2000 and then added to each well. The plate was incubated at 37°C for 3 h and washed in PBS-Tween. Substrate buffer containing 0.5 mg/ ml of PNPP (p-nitrophenyl phosphate pH 9.8) was then dispensed to each well @ 200 µl/ well and incubated at room temperature under dark for 20-30 min. Light orange to yellow colour development indicated a weak to strong positive reaction and the results were quantitatively recorded in an ELISA reader at 405 nm (Biotek EL × 800).

The total RNA was extracted from 100 mg lilium leaves using RNeasy plant extraction kit (Qiagen Inc., Chatsworth, CA, USA) according to the manufacturers' protocol and resuspended in 50 µl nuclease free water. For cDNA synthesis of PlAMV, 1 µg total isolated RNA (200 ng/ µl) was annealed with 0.3 µM downstream primers (PlAMV CPR - 5'AAACGGTAAAATACACACCGGG 3') at 70°C for 10 min. To the transcription mixture, various reaction components were added [(RNase inhibitor 1 µl (20 U); dNTPs 2 µl (10 mM); 4 µl 5´ reverse transcriptase buffer containing Tris-HCl 250 mM, pH 8.3 at 25°C, KCl 250 mM, $MgCl_2$ 20 mM, 1 µl DTT 50 mM)]. The reaction mixture was incubated at 37°C for 10 min., 40 U M-MuLV reverse transcriptase was added and the mixture was re-incubated at 37°C for 60 min. The reaction was stopped by heating the mixture at 70°C for 10 min.

PlAMV:cDNA product (5 µl) was added to 50 µl of PCR reaction mixture containing 0.20 mM each of dNTPs, 0.25 µM of each primer (PlAMV CPF-5' CAAGACATTC TCCACCATGGCACTC 3' and PlAMV CPR - 5'AAACGGTAAAATACACACCGGG 3', 5 µl 10X *Taq* polymerase buffer, 2.0 mM $MgCl_2$ and 2 U *Taq* DNA polymerase. The RT-PCR was performed in Eppendorf Mastercycler Gradient ES with the following thermal programme: initial denaturation at 94°C for 2 min., followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min. and final extension of 72°C for 10 min. The PCR product was analyzed on a 1.2% agarose gel, stained with ethidium bromide and viewed under transilluminator.

The amplicon of coat protein gene was purified using QIAGEN gel extraction kit (Qiagen Inc., Chatsworth, CA, USA) and cloned into pGEM-T Easy vector (Promega, Madison, WI, USA) following the manufacturer's instructions and transformed into *Escherichia coli* DH5α by following standard molecular biology procedures. Plasmid DNA was isolated from the potential recombinant clones using Wizard plus DNA purification kit (Promega, Madison, WI) according to the manufacturer's protocol and the positive clones were identified by restriction digestion analysis using *EcoRI* enzyme. The three independent clones were sequenced (Chromos Biotech Pvt. Ltd., Bengaluru) from both orientations for each fragment separately. The sequences were then edited using the BIOEDIT Software (Hall, 3). Sequence similarity search of the GenBank database was done using the Basic Local Alignment Search Tool (BLAST) program.

The sequence variability of PlAMV was analysed with the nucleotide and amino acid sequences of CP genes compared with sequences of the previously reported isolates available in GenBank. The amino acid sequences of the PlAMV coat protein gene was translated from the consensus nucleotide sequences using the EMBOSS Transeq program (Rice *et al*., 13). Both the nucleotide and amino acid sequences were then aligned with selected sequences of *Potexvirus* along with selected sequences of PlAMV using the CLUSTAL W program (Larkin *et al*., 10). Phylogenetic analysis of CP sequence was done on MEGA 5.1 (Tamura *et al*., 16) and trees were created using the Neighbour-joining method (Saitou and Nei, 14). The robustness of the trees was determined by bootstrap using 1,000 replicates. *Lily virus* × (LVX) was used as a reference out group member of the genus potexvirus for rooting the phylogenetic tree.

RESULTS AND DISCUSSION

Viruses are the major constraints in lily cultivation under protected condition that often decrease the yield and quality of flowers. Characterization of PlAMV infecting lily in Tamil Nadu provides knowledge on better understanding the occurrence and genetic composition. The oriental lily grown under protected cultivation expressed the characteristic symptoms of PlAMV on leaves, which showed severe chlorotic and necrotic symptoms. The severely infected plants were stunted. Symptom development of PlAMV starts with the end of the vegetative growth stage with brown coloured veins on the bottom side of a leaf along with chlorotic streaks and turns necrotic. In severe stage of infection brown-coloured and necrotic symptoms were noticed on the top side of the leaves (Fig. 1). Immunological assays have been developed and successfully used for a number of years for the

Fig. 1. Symptoms of PlAMV on lilium with chlorotic and necrotic streaks on the leaves.

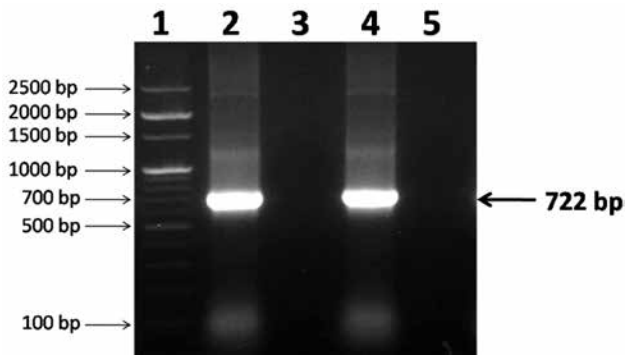


Fig. 2. RT-PCR amplification of PlAMV coat protein gene from naturally infected leaves of lilium. Lane 1:100 bp ladder; Lanes 2 & 4: Amplified DNA fragment from infected samples; Lane 3 & 5: Healthy samples (control).

detection of plant viruses. Bulbs of the infected plants were collected and the leaf samples were tested for the presence of tospovirus, potyviruses and ilarvirus by DAC-ELISA with specific antibody and conjugate (provided by ICRISAT, Hyderabad, Telangana, India). All the samples tested were negative to tospovirus, potyvirus and ilarvirus group antiserum, respectively. This could help in understanding that, virus infecting lily is different from previously reported lilium viruses. The PCR assay has been used as a tool for identification of unreported viruses from lily, since PCR has been shown to be effective in rapid and sensitive detection of many plant viruses (Kwon *et al*., 9). Parrella *et al*. (12) has reported severe necrotic streaking in midstem leaves caused by PlAMV on plants of lily hybrids (*Lilium* sp., Liliaceae) in several greenhouses of Campania region of Southern Italy. Hammond *et al*. (4) reported the infection of PlAMV in Asiatic and Oriental lilies in the United States.

RNA isolated from infected sample was subjected to RT-PCR assay using self designed primers corresponding to coat protein gene based on alignment of KM205357 with all PlAMV sequences available in GenBank. RT-PCR using PlAMV CPF/ PlAMV CPR yielded an amplicon size of 722 bp from infected leaves, while amplification was not observed with total RNA extracted from healthy plants (Fig. 2). The amplified fragments were separated on agarose gel and cloned into pGEM-T easy vector. The three independent clones were sequenced (Chromos Biotech Pvt. Ltd., Bengaluru) from both the orientations for each fragment separately. The nucleotide sequence analysis using the NCBI BLAST confirmed the association of *Plantago asiatica mosaic virus* in lilium. The CP gene sequence of PlAMV virus lilum isolate TN-1 from Ooty and TN-2 from Devashola of Nilgris district were submitted in NCBI GenBank database (Acc. No. KU845394 and KX130954). The CP gene of PlAMV isolate was compared with corresponding gene from known PlAMV isolates and other genus of potexvirus at the nucleotide levels. The sequence analysis revealed the high homologies between the PlAMV isolates, including Ko-JJ-2-2 isolate from South Korea (KU159091), CES5 isolate from Italy (LN827658), kr isolate from South Korea (KT717325) and Concador isolate from Hungary (LN794199), respectively. They showed the highest nucleotide sequence identity of 100 per cent with CES5 isolate from Italy (LN827658), kr isolate from South Korea (KT717325) and Concador isolate from Hungary (LN794199). Similarly, sequence had 99 per cent similarity with Sorbonne isolate from the Netherlands (KF471012), LIL6 isolate from Italy (LN651194) and SEG2 isolate from Italy (LN827660). Analysis of the 239 deduced amino acid sequence of coat protein gene revealed that our isolates had 99.5 to 100% homology with other strains of the same virus (Table 1). Phylogenetic analysis of nucleotide sequences of CP gene supported a single cluster PlAMV, indication the absence of geographical variation. The combined analysis of species representing the *Potexvirus* revealed a distinct group of PlAMV, which was closely related to *Tulip virus* × (TVX) (Fig. 3). Similarly, Hammond *et al*. (2015) amplified the sequence of PlAMV using RT-PCR from *C. quinoa* and *N. benthamiana*, which yielded 1.3 kb product and the consensus sequence (KM205357) had 98.7% nucleotide identity to a Dutch isolate of PlAMV (PlAMV, KF471012). Similarly, Parrella *et al*. (12) demonstrated that,

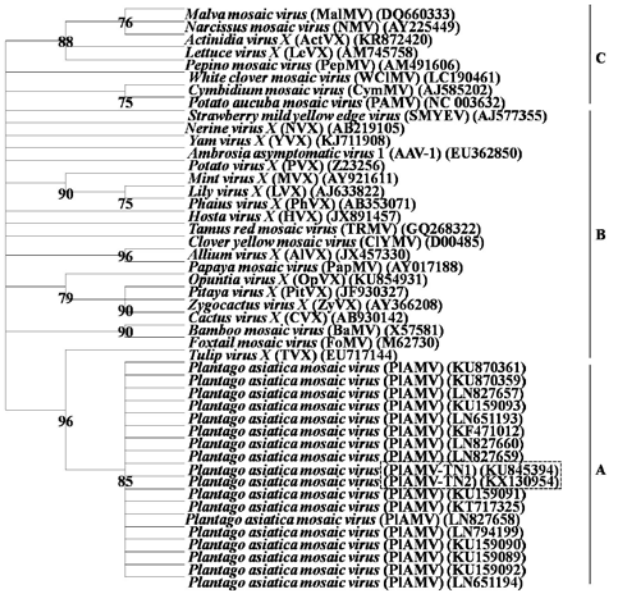


Fig. 3. Neighbor-joining phylogenetic tree based on the nucleotide sequences of the coat protein gene of PlAMV (KU845394 and KX130954) and *Lily virus* × (LVX) is defined as an out-group. Numbers above each branch are the neighbor-joining bootstrap scores given as percentage of 1,000 replicates. Bootstrap scores lower than 70% are not shown. Group B and C contain potexviruses, while group A contains members of representative species of PlAMV within potexviruses collected from NCBI database.

the amplified coat protein gene with an amplicon size of approximately 1.0 kb encompassing the complete ORF had the highest nucleotide sequence identity with the Dutch isolate Sorbonne of PlAMV (KF471012), ranging from 99.4 to 99.8%. This clearly indicate that Indian isolates of PlAMV infecting lily showed a high genetic stability that may be due to these isolates having evolved from the same parental source in lily, which is propagated vegetatively through bulbs and bulblets. The combined analysis of species representing the *Potexvirus* revealed a distinct group of PlAMV, which was closely related to TVX as reported previously by Fajolu *et al*. (2). He illustrated that, the TVX and PlAMV are sister group in the genera potexvirus based on nucleotide percentage identity of CP from TVX. Based on the sequence similarity of PlAMV isolates TN-1 and TN-2 detected from India, closely mimics the identity of CES5 isolate from Italy or Sorbonne isolate from the Netherlands, indicating that the virus might have been introduced through the import of bulbs from either Netherlands or Italy. This is the first report of PlAMV in *Lilium* spp. in India, since, PlAMV infection of lilies have not been reported earlier.

Table 1. Nucleotide (nt) and amino acid (aa) identities of the coat protein gene of *Plantago asiatica mosaic virus* (PlAMV) lilium strain (KU845394 and KX130954) with corresponding sequences of selected strains of PlAMV and *Lily virus* × (LVX) is defined as an out-group.

Accn. No.	Strain	Country	Percentage (%) identity	
			nt	aa
KU159091	PlAMV	South Korea	100.00	100.00
KT717325	PlAMV	South Korea	100.00	100.00
LN827658	PlAMV	*Lilium* sp.	100.00	100.00
LN794199	PlAMV	Hungary	100.00	100.00
KU159090	PlAMV	South Korea	99.80	100.00
KU159089	PlAMV	South Korea	99.80	100.00
KU159093	PlAMV	South Korea	99.80	100.00
KU159092	PlAMV	South Korea	99.80	100.00
LN651194	PlAMV	South Korea	99.80	99.50
LN827657	PlAMV	Italy	99.80	100.00
LN651193	PlAMV	Italy	99.60	100.00
LN827660	PlAMV	Italy	99.60	99.50
LN827659	PlAMV	Italy	99.60	99.50
KF471012	PlAMV	Netherlands	99.60	99.50
KU870361	PlAMV	Netherlands	99.50	100.00
KU870359	PlAMV	Netherlands	99.50	100.00
AJ633822	LVX	Italy	46.10	36.00

ACKNOWLEDGEMENT

The authors acknowledge the Tamil Nadu Agricultural University, Coimbatore for support of facilities.

REFERENCES

1. Asjes, C.J. 2000. Control of aphid-borne lily symptomless virus and lily mottle virus in *Lilium* in the Netherlands. *Virus Res*. **71**: 23-32.

2. Fajolu, O.L., Wen, R.H., Windham, A.S., Windham, M.T., Moulton, J.K. and Hajimorad, M.R. 2009. Genetic variability and phylogenetic analysis of *Hosta virus X*. *Arch. Virol*. **154**: 1909-16.

3. Hall, T.A. 1999. Bioedit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. *Nucleic Acids Symp. Series*, **41**: 95-98.

4. Hammond, J., Bampi, D. and Reinsel, M.D. 2015. First report of *Plantago asiatica mosaic*

virus in imported Asiatic and Oriental lilies (*Lilium* hybrids) in the United States. *Plant Dis.* **99**: 292.

5. Han, L.J. and Liu, W.H. 2007. Studies on *Prunus* necrotic ringspot virus (PNRSV) occurring on lily. *Agric. Sci. China*, **6**: 1201-08.

6. HemaMoorthy, T. and Prakasam, V. 2013. First report of *Penicillium expansum* causing bulb rot of *Lilium* in India. *American-Eurasian J. Agric. Env. Sci*. **13**: 293-95.

7. Hobbs, H.A., Reddy, D.V.R., Rajeshwari, R. and Reddy, A.S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. *Plant Dis.* **71**: 747-49.

8. Komatsu, K., Yamaji, Y., Ozeki, J., Hashimoto, M., Kagiwada, S., Takahashi, S. and Namba, S. 2008. Nucleotide sequence analysis of seven Japanese isolates of *Plantago asiatica mosaic virus* (PlAMV): A unique potexvirus with significantly high genomic and biological variability within the species. *Arch. Virol*. **153**: 193-98.

9. Kwon, J.Y., Ryu, K.H. and Choi, S.H. 2013. Reverse transcription polymerase chain reaction-based system for simultaneous detection of multiple lily-infecting viruses. *Plant Pathol. J*. **29**: 338-43.

10. Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J. and Higgins, D.G. 2007. Clustal W and Clustal × version 2.0. *Bioinformatics*, **23**: 2947-48.

11. Lee, K.H., Choi, H.S., Choi, G.S. and Kim, J.S. 1996. Virus diseases of lilies in Korea. *Acta Hort*. **414**: 195-201.

12. Parrella, G., Pasualini, A., Greco, B. and Nappo, A.G. 2015. *Plantago asiatica mosaic virus* found in protected crops of lily hybrids in southern Italy. *Plant Dis.* **99**: 1289.

13. Rice, P., Longden, I. and Bleasby, A., 2000. EMBOSS: The European molecular biology open software suite. *Trends Genet*. **16**: 276-77.

14. Saitou, N. and Nei, M. 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. **4**: 406-25.

15. Sharma, A., Mahinghara, B.K., Singh, A.K., Kulshrestha, S., Raikhy, G., Singh, L., Verma, N., Hallan, V., Ram, R. and Zaidi, A.A. 2015. Identification, detection and frequency of lily viruses in Northern India. *Scientia Hort.* **106**: 213-27.

16. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*. **28**: 2731-39.

Received : February, 2017; Revised : September, 2017; Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00111.6

Indian J. Hort. 74(4), December 2017: 579-585

Varietal evaluation and biochemical changes due to field incidence of *Fusraium* wilt in gladiolus genotypes

Meenu Kumari*, Kishan Swaroop, Kanwar Pal Singh and Prabhat Kumar

Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Thirty gladiolus genotypes were evaluated for agronomic traits and natural screening to *Fusarium* wilt during 2015 and 2016. Among the genotypes, Suchitra × Melody took the minimum days (11.24) to bud sprout post planting, while Tha Barton registered 72 days for spike emergence. Pusa Unnati recorded the maximum spike and rachis length, while Pusa Manmohak, Pusa Red Valentine and Pusa Unnati recorded maximum number of florets per spike. Maximum field-life of spikes was recorded in Pusa Unnati followed by Pusa Red Valentine. After about 45 days of planting, different genotypes showed varied disease incidence (3.47-45.13%) and plant mortality index (0-35.47%). Genotypes, Swarnima, Pusa Unnati and Suryakiran were found more resistant to fusarium wilt, while Urmi and white Friendship were found highly susceptible. Leaves and corms were sampled at 75 days of planting for estimation of different biochemical parameters, namely, total chlorophylls, total sugars, total phenols, and malondialdehyde (MDA) content. The resistant genotypes had higher contents of total chlorophylls; higher phenols but lower TSS and MDA contents in corms. Negative correlations were estimated for DSI and biochemical parameters like total chlorophyll (r = -0.974), total phenol (r = 0.925**). Positive correlations were reported among DSI and total sugars (r = 0.938**) and MDA content (r = 0.918**). Hence, these parameters can be employed as biochemical markers for screening of gladiolus genotypes for *Fusarium* tolerance.**

Key words: Agronomic traits, biochemical changes, gladiolus, *Fusarium* wilt.

INTRODUCTION

Gladiolus (*Gladiolus hybridus* Hort.) is one of the most popular ornamental bulbous flowering crop grown for cut flower and garden display and it belongs to the family Iridaceae and sub family Ixioideae. It is native to South Africa and has been cultivated globally. Gladiolus is becoming very popular among the bulbous flowering crops due to its beautiful dazzling coloured spikes, varied range of spike length, florets size and number as well as long vase-life. Despite of having good ornamental characters, this crop is very susceptible to different *Fusarium* species that leads to spike loss upto 40%. *Fusarium* is soil borne fungus belongs to Deuteromycetes class. It is very difficult to control, since it survives in soil and corms as both spores and mycelium in latent form for prolonged period. There is genetic variation in resistance levels among the gladiolus genotypes and also variation in virulence may present in *Fusarium oxysporum* pv. *gladioli*. There are many races found in *F. oxysporum* pv. *gladioli* that means not all resistance hosts are resistance/ susceptible to all isolates of *fusarium*. Hence, there is need of continuous screening of genotypes that might be used further in improvement programme.

MATERIALS AND METHODS

Thirty gladiolus genotypes, namely, Shabnam, Gulal, Mohini, Dhanwantri, Berlew-OS, Little Fawn, Pusa Kiran, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsal, Urvashi, Hunting Song, Melody-OS, Surya Kiran (Melody × Mayur) × H. Heady, GW × Oscar, S. Lady × Oscar, Suchitra × Melody, White Friendship, Green Lilac-OS, Pusa Vidhushi, Swarnima, Pusa Red Valentine, Pusa Srijana, Gunjan, Jyotsana, Suchitra and Pusa Unnati were planted in field in October 2015 and 2016. Experiments were conducted in a randomized block design. Ninety six corms of each genotype were planted in four replications. Different vegetative traits, *i.e.* days to sprouting, plant heights, leaf No., leaf length and width, corm weight and diameter; and reproductive traits, namely, spike length, rachis length, No. of florets per spike were recorded.

The natural incidence of *Fusarium* wilt and plant mortality was calculated using the method suggested by Riaz *et al.* (14). The disease severity was determined by the adopting the suggested disease rating scale, *i.e.* 0 = No disease symptoms,1 = Yellowing of leaves, 2 = Wilting, and 3 = Dead and were accordingly grouped as highly resistant (0-5%), tolerant (6-10%), moderately tolerant (11-20%), moderately susceptible (21-30%) and susceptible (31-50%).

Different biochemicals were estimated following standard procedures. Total leaf chlorophyll content

*Corresponding author's E-mail: meenubhu08111@gmail.com

was measured by DMSO method (Hiscox and Israelstam, 10), total soluble sugars by the anthrone reagent method (Hedge and Hofreiter, 8), total phenols (Malick and Singh, 11) and malondialdehyde level by thiobarbituric acid (TBA) method (Hagage, 7) at 45 days after planting. Analysis of variance (ANOVA) was carried out for different parameters following Completely Randomized Design. Pearson's correlation coefficient (r) among DSI and biochemical traits was computed using statistical analysis system software (SPSS version 2.1).

RESULTS AND DISCUSSION

There was a marked variation recorded in vegetative growth, flowering and *Fusarium* wilt incidence in the 30 gladiolus genotypes when evaluated under natural field conditions (Tables 1-4). As evident, the days to corm sprout rangedfrom 11.67-

Table 1. Variation in vegetative growth and corm parameters in gladiolus genotypes under field conditions (pooled data).

Genotype	Days to 50% corm sprouting	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Corm wt. (g)	Corm dia. (cm)
Shabnam	15.66	106.87	9.00	42.44	1.94	22.75	77.25	6.47
Gulal	17.67	85.50	8.50	35.69	3.24	15.63	67.5	6.73
Mohini	15.67	91.62	9.50	41.00	3.13	33.88	68.63	5.93
Dhanwantri	12.67	107.87	10.25	51.37	3.37	15.37	85.13	6.70
Berlew-OS	13.67	117.75	8.62	50.12	3.21	16.13	74.50	5.87
Little Fawn-OS	16.00	92.62	9.00	36.12	3.25	15.75	53.50	5.81
Pusa Kiran	13.00	86.37	8.13	33.75	2.38	14.93	60.63	5.91
Pusa Shubham	23.67	76.50	7.25	35.12	3.33	20.25	53.00	5.41
Pusa Manmohak	21.34	97.63	8.37	42.75	3.98	10.37	53.63	6.08
Tha Barton	16.34	92.25	8.00	34.50	2.93	15.75	51.13	6.28
Urmi	17.67	81.50	7.00	46.00	3.27	13.00	49.63	5.43
Pink Parsal-OS	21.67	108.00	10.12	51.62	2.73	17.75	66.37	6.58
Urvashi	14.80	87.50	7.87	46.25	4.03	16.37	52.37	5.66
Hunting Song	16.00	100.25	8.25	36.37	2.76	20.13	76.25	6.51
Melody-OS	17.00	95.50	7.37	36.50	3.30	17.87	45.87	4.85
Suryakiran	15.00	111.37	8.13	38.00	2.45	17.25	44.63	4.81
(Melody × Mayur) × H. Wine	17.34	94.37	7.13	32.37	2.88	15.75	49.00	5.25
Green Willow × Oscar	23.84	77.25	7.00	34.62	3.61	19.25	44.37	4.21
Smokey Lady × Oscar	15.67	88.62	8.37	35.75	2.50	14.37	47.13	4.90
Suchitra × Melody	11.67	98.50	8.87	49.87	4.81	16.25	101.75	6.51
White Friendship	18.33	114.00	7.75	37.87	3.78	22.63	81.63	6.53
Green Lilac-OS	16.00	95.12	7.75	31.25	2.78	16.50	86.25	6.01
Pusa Vidushi	12.50	94.87	7.62	34.37	3.91	9.25	56.50	4.95
Swarnima	13.17	91.62	8.87	47.50	3.76	14.13	44.37	5.03
Pusa Red Valentine	14.33	119.12	7.87	39.87	4.5	16.75	70.63	6.10
Pusa Srijana	11.89	87.50	7.62	29.75	3.30	17.37	61.88	4.94
Gunjan	19.17	91.75	7.87	33.25	3.41	18.25	93.63	5.08
Jyotsana	17.50	91.00	8.75	34.12	3.55	15.75	96.38	5.38
Suchitra	15.34	102.25	8.12	31.00	3.20	18.87	89.75	5.40
Pusa Unnati	18.34	129.75	9.00	39.38	4.05	24.88	52.75	5.25
Range	11.67-23.84	129.75-81.50	7.00-10.25	31.00-51.37	4.81-1.94	33.88-9.25	101.75-44.37	6.73-4.22
$CD_{0.05}$	1.73	1.98	0.77	3.21	0.34	3.12	13.65	0.75
CV (%)	6.40	1.45	6.67	5.86	7.36	12.76	14.94	9.36

Table 2. Variation in floral traits in gladiolus genotypes under field conditions (pooled data).

Genotype	Spike emergence (days)	Spike length (cm)	Rachis length (cm)	Florets No./ spike	Spike field-life (days)
Shabnam	86.37	70.00	41.37	16.87	15.13
Gulal	80.25	53.63	38.12	13.25	14.87
Mohini	87.12	73.25	46.25	13.87	15.37
Dhanwantri	80.25	65.13	45.00	14.50	12.87
Berlew-OS	79.00	78.87	54.87	18.00	16.50
Little Fawn-OS	91.75	61.75	45.62	17.75	16.37
Pusa Kiran	86.87	54.00	36.62	12.87	15.62
Pusa Shubham	86.25	55.00	38.25	12.50	18.50
Pusa Manmohak	100.62	69.63	49.37	18.62	13.13
Tha Barton	72.25	65.75	45.75	13.37	15.00
Urmi	86.50	52.00	27.00	9.75	12.75
Pink Parsal-OS	109.62	79.13	46.75	14.00	14.25
Urvashi	84.75	55.50	38.37	13.37	15.37
Hunting Song	84.50	80.63	58.87	14.37	16.25
Melody-OS	74.62	73.12	45.37	15.87	18.00
Suryakiran	81.25	75.00	52.62	16.87	16.63
(Melody × Mayur) × H. Wine	78.37	56.12	41.87	10.00	11.50
Green Willow × Oscar	88.37	52.87	41.25	10.50	11.50
Smokey Lady × Oscar	96.00	58.50	45.00	11.75	13.87
Suchitra × Melody	87.25	75.38	53.12	13.62	20.75
White Friendship	80.75	74.37	58.50	17.37	16.62
Green Lilac-OS	86.50	54.50	40.00	8.87	17.50
Pusa Vidushi	87.00	73.62	42.75	14.00	16.37
Swarnima	100.25	68.63	50.87	13.37	13.87
Pusa Red Valentine	84.5	80.50	61.75	18.50	20.37
Pusa Srijana	91.375	57.37	42.37	15.87	15.75
Gunjan	91.375	69.62	49.12	16.00	13.87
Jyotsana	79.125	75.75	53.50	17.26	16.37
Suchitra	85.75	76.50	46.12	14.12	14.62
Pusa Unnati	104.75	92.37	62.75	18.37	21.25
Range	109.62-72.25	92.37-52.00	62.75-27	18.62-8.87	21.25-11.50
$CD_{0.05}$	2.98	1.877	1.627	1.47	1.93
CV (%)	2.43	1.971	2.478	7.23	8.75

23.84 days. The earliest corm sprouting was recorded in Suchitra × Melody (11.27 days), followed by Pusa Srijana (11.89 days), while it was most delayed in Green willow W × Oscar (23.84 days). Data revealed in genotypes Dhanwantri, Pusa Kiran, Suchitra × Melody, Pusa Srijana and Pusa Vidhushi sprouted in 11-13 days could be rated as early sprouting types, while Green Willow × Oscar, Pusa Shubham, Pusa Manmohak and Pink Parsal-OS had late sprout (21-23 days). Most of the genotypes exhibited intermediate sprouting duration (14-20 days). Plant height at induction complete emergence of spike was noted maximum in Pusa Unnati (129.75 cm) significantly followed by Pusa Red Valentine (119.12 cm). Shortest plants were seen in Urmi (81.50 cm). Most of the genotypes had plant height over 1 m. The leaf length varied significantly, *i.e.* from 31.00 cm in Suchitra to 51.37 cm in Dhanwantri. The leaf number ranged from 7.00 to 10.25 per plant. Minimum number of leaves were found in Urmi and Green willow × Oscar

Table 3. Variation in biochemical status of gladiolus genotypes after field screening for *Fusarium* wilt incidence.

Genotype	Leaf chlorophyll (mg /g FW)	Corm		
		Total soluble sugars (mg/g FW)	Total phenols (mg/g FW)	MDA (µmol g^{-1}/ FW)
Shabnam	2.32	20.77	0.77	26.12
Gulal	2.47	18.78	0.87	24.40
Mohini	2.39	19.64	0.75	25.08
Dhanwantri	2.52	18.38	0.94	23.54
Berlew-OS	2.17	21.09	0.74	26.65
Little Fawn-OS	2.13	21.47	0.75	26.85
Pusa Kiran	2.31	20.57	0.78	26.39
Pusa Shubham	2.08	21.64	0.66	26.89
Pusa Manmohak	2.25	20.93	0.73	25.79
Tha Barton	2.15	21.25	0.69	26.56
Urmi	2.00	22.28	0.65	27.61
Pink Parsal-OS	2.15	21.50	0.69	26.01
Urvashi	2.22	21.26	0.71	25.96
Hunting Song	2.53	19.43	0.88	23.67
Melody-OS	2.02	22.16	0.66	26.85
Suryakiran	2.72	18.29	1.11	22.28
(Melody × Mayur) × Heady Wine	2.16	21.57	0.68	26.82
Green Willow × Oscar	2.16	21.54	0.71	26.04
Smokey Lady × Oscar	2.79	18.32	0.94	22.86
Suchitra × Melody	2.58	19.37	0.92	23.47
White Friendship	2.06	21.91	0.65	27.13
Green Lilac-OS	2.36	21.31	0.75	26.12
Pusa Vidushi	2.57	18.51	0.92	23.25
Swarnima	2.89	18.18	1.17	22.19
Pusa Red Valentine	2.34	20.57	0.77	26.28
Pusa Srijana	2.64	18.74	0.94	23.32
Gunjan	2.32	20.52	0.80	25.58
Jyotsana	2.36	21.30	0.77	26.33
Suchitra	2.15	21.67	0.68	26.16
Pusa Unnati	2.81	18.25	1.13	21.89
Range	2.89-2.00	22.28-18.18	1.17-0.65	27.61-21.89
$CD_{0.05}$	0.103	0.816	0.082	0.712
CV (%)	2.64	2.443	6.22	1.72

(7.00) compared to maximum noted in Dhanwantri and Mohini (10.24). Longest leaf was recorded in Pink Parsal (51.62 cm) compared to shortest in Pusa Srijana (29.75 cm). The widest leaves were observed in Suchitra × Melody (4.81 cm) significantly followed by Urvashi (4.03 cm), whereas it was most narrow in Shabnam (1.94 cm).There was significant variation found in corm parameters (Table 1). Average corm weight varied significantly among the genotype and was found maximum in Suchitra × Melody (101.75 g) non-significantly followed by Jyotsana (96.38 g) and Gunjan (93.63 g), whereas lowest was in genotype Green willow × Oscar and Swarnima (44.37 g). Maximum corm size was observed in Gulal (6.73 cm) non-significantly followed by Pink Parsal-OS (6.58 cm), while smallest was in Smokey Lady × Oscar (4.21 cm).

Significant variations were recorded for various characters studied among the 30 genotypes. These differential expressions of traits are the direct result of genotype and the physio-biochemical status of the corms. Similar finds have been studied by several workers in gladiolus (Kadam *et al.*, 9). Plant height at flowering stage and spike fully opened and number of leaves at flowering stage were important traits.

Similarly, root length varied considerably, *i.e.* from 9.25 cm in Pusa Vidushi to 33.88 cm (Mohini) (Table 1). Field incidence of *Fusarium* wilt was noted at 75 days of planting. Genotypes Urmi and Pusa Manmohak showing symptoms of fusarium wilt had root length of 13.00 and 10.37 cm, respectively. However, Pusa Vidhushi having smaller roots was categorized as moderately tolerant. Most of tolerant genotypes had longer roots, suggesting that for penetration of fungus mycelium it is succulence of root and not length, which is important for susceptibility (Chandra *et al.*, 4).

Different genotypes exhibited significant variation for flowering characters, *i.e.* days required to spike initiation and floret opening (Table 2). Tha Barton had early spike initiation (72.25 days), whereas Pink Parsal-OS was late (109.62 days). Maximum spike length was found in Pusa Unnati (92.37 cm) significantly followed by Hunting Song (80.63 cm) and Pusa Red Valentine (80.50 cm), whereas shortest spikes were noted in Urmi (52.00 cm). Rachis length was maximum in Pusa Unnati (62.75 cm), which was at par with Pusa Red Valentine (61.75 cm), while the shortest length was recorded in Urmi (27.00 cm) significantly followed by Pusa Kiran (36.62 cm). Floret number per spike was maximum in Pusa Manmohak (18.62) followed by Pusa Red Valentine (18.50), Pusa Unnati (18.37) and Berlew-OS (18.00). Minimum number of florets per spike was found in Green Lilac-OS (8.87) followed by Urmi (9.75). The variation in

Table 4. Correlation among different parameters with respect to *Fusarium* wilt incidence in gladiolus.

Parameter	Chlorophyll	TSS	Phenols	MDA	DSI	PMI
Chlorophyll	1.000					
TSS	-0.931**	1.000				
Phenols	0.958**	-0.916**	1.000			
MDA	0.873**	-0.688**	0.857**	1.000		
DSI	-0.974**	0.938**	-0.971**	-0.918**	1.000	
PMI	-0.598**	0.633**	-0.587**	-0.730**	0.618**	1.000

**Significant at the 0.01 level (2-tailed).

different floral traits expressed under field conditions by the genotypes may be due to their unique genetic makeup and partially due to the effect of prevailing environmental conditions. Maximum field spike-life was recorded in Pusa Unnati (21.25 days), which was at par with Suchitra × Melody (20.75 days) and Pusa Red Valentine (20.37 days). In gladiolus, ideal cut flower genotypes should produce big sized daughter corms and good number of corms and cormels, which are the genotypic traits owing unique recombinant of individual germplasm and their pedigree (Balaram and Janakiram, 2; Poon *et al*., 12). Owing to unique genotype, the gladiolus germplasm varied considerably for flowering traits, too.

Based on Disease Severity Index (DSI) noted for the 30 genotypes, Swarnima was rated highly resistant (0-55); Pusa Unnati tolerant (6-10%); Suryakiran, S. Lady × Oscar, Pusa Srijana, Dhanwantri and Pusa Vidushi were moderately tolerant (11-20%), Shabnam, Gulal, Mohini, Pusa Kiran, Hunting Song, Suchitra × Melody, Green Lilac, Pusa Red Valentine, Gunjan and Jyotsana were found moderately susceptible (21-30), BL Little, Little Fawn, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsol, Urvashi, Melody, Melody × Mayur × H. Wine, GW × Oscar, White Friendship, Suchitra (31-50%) were found susceptible to disease in field (Table 5). Among the gladiolus genotypes, Urmi and White Friendship recorded the maximum disease severity index (45.14 and 44.45) with maximum number of plant died. While, Gulal, Smokey Lady × Oscar, Swarnima, Pusa Red Valentine, Pusa Unnati exhibited only symptoms of *Fusarium* wilt, but with no mortality of plants (Fig. 1).

Disease reaction of different genotypes against *Fusarium* wilt has previously been examined under different growing conditions (Riaz *et al*.,14; Shanmugam, 15). Natural variations in disease susceptible and tolerant genotypes due to different rot causing pathogens have also been earlier noticed in gladiolus (Riaz *et al*., 14; Amrutha *et al*., 1). Earlier reports have suggested that fungal infection induces increase in antioxidative abilities, such as SOD, guaiacol peroxidase, catalase, APX, and flavonoid content, suggesting that biotic stress lead to their enhanced level. The importance of PPO, POD, chlorogenic acid, and total soluble phenols in defense mechanisms against pathogen cannot be ruled out (Ray *et al*., 13). Resistant genotypes showed higher content of phenols in corm tissue compared to susceptible ones.

There was a sharp reduction in total chlorophyll contents in disease affected leaves of susceptible genotypes as compared with resistant genotypes. Pusa Swarnima and Pusa Unnati recorded 44 and 40% higher chlorophyll content as compared to susceptible genotype Urmi. Total chlorophylls showed highly negative correlation (r = -0.974**) and moderate

Table 5. *In vivo* reaction of gladiolus genotypes to *Fusarium* wilt disease.

Category	Genotype(s)
Highly resistant (0-5)	Swarnima
Tolerant (6-10)	Pusa Unnati
Moderately tolerant (11-20)	Suryakiran, Smokey Lady × Oscar, Pusa Srijana, Dhanwantri, Pusa Vidushi
Moderately susceptible (21-30)	Shabnam, Gulal, Mohini, Pusa Kiran, Hunting Song, Suchitra × Melody, Green Lilac-OS, Pusa Red Valentine, Gunjan, Jyotsana
Susceptible (31-50)	Berlew-OS, Little Fawn-OS, Pusa Shubham, Pusa Manmohak, Tha Barton, Urmi, Pink Parsal-OS, Urvashi, Melody, (Melody × Mayur) × Heady Wine, Green Willow × Oscar, White Friendship, Suchitra

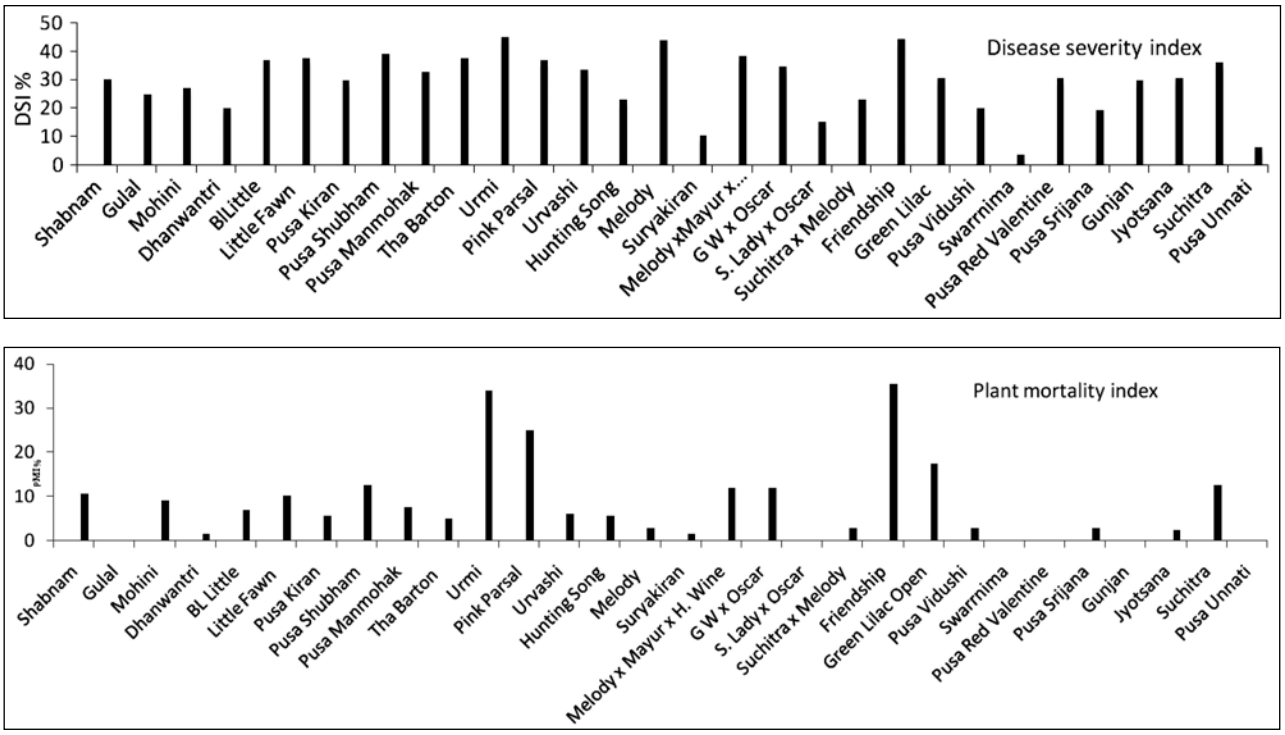


Fig. 1. Plant mortality and disease severity index (%) due to *Fusarium* wilt incidence in gladiolus genotypes (at 75 days of planting).

negative correlation (r = 0.598**) with DSI and PMI, respectively. The results are in agreement with Gurjar *et al*. (2015), where susceptible genotypes were identified with lower levels. Corms of susceptible genotypes had higher total soluble sugars compared to resistant. Corms of Urmi (22.28 mg/g FW) non-significantly followed by Friendship (21.91 mg/g FW) and Suchitra (21.67 mg/g FW) had highest content, and these genotypes showed susceptibility to disease. Lowest content found in Swarnima (18.18 mg/g FW) was non-significantly followed by Pusa Unnati (18.25 mg/g FW), that was resistant to disease. The correlation studies revealed that total sugars were positively correlated (r = 0.938**) with DSI. Higher total soluble sugars in the tissue facilitate the fungus invasion and growth by dissolving cell wall and plasma membrane. Similar results were reported by Gurjar *et al*. (6) and Sharma *et al.* (16). The highly resistant genotype was Swarnima (1.17 mg/g FW) followed by Pusa Unnati (1.13 mg/g FW) and Suryakiran (1.11 mg/g FW) as compared to Urmi and White Friendship (0.65 mg/g FW). As evident from the resistant genotypes had higher total phenols than susceptible genotypes. Total phenols content showed high negative correlation (r = -0.971**) with DSI. The highest level of MDA was observed in Urmi (27.61 µmol /g FW), whereas, Pusa Unnati showed the lowest (21.89 µmol /g FW), in diseased leaves, which was significantly different (Table 3). The MDA levels were higher in susceptible as compared to resistant genotypes. Total MDA content in corms showed high negative correlation (r = -0.918**) with DSI and (r = -0.730**) with PMI (Table 4). Our results showed increased phenolic compounds facilitating resistance in genotypes to counter fungal infection and growth. These findings are in agreement with those of Goswami *et al.* (5). The phenolic limits the pathogen invasion during plant defense responses. Peroxidase enzyme oxidizes and polymerizes phenolics into quinones, which possess ability to inactivate enzymes. These oxidized phenolic compounds possess increased antimicrobial activity and could play role in inhibiting pathogen development. The enzyme also polymerizes phenolic acids to form lignin, thereby further enhancing resistance to pathogens. Fungus infection enhances loss of cell membrane integrity, increased the production of reactive oxygen species and cause antioxidative enzymes inefficient leading to cellular disruption. Increased ROS acted toxic agents for lipid peroxidation and membrane damage (Bao *et al.*, 3).

Based on above results, it may be concluded that among the 30 gladiolus genotypes, the agronomic performance of cvs Pusa Unnati, Pusa Red Valentine,

Suryakiran, Swarnima and Dhanwantri were found most promising. Based on biochemical parameters Pusa Unnati, Swarnima and Dhanwantri were found tolerant to Fusarium infection owing to high phenolic compounds, high leaf chlorophyll and low MDA and sugar contents in corm tissue. These biochemical parameters can be used as markers for screening genotypes for *Fusarium* wilt.

ACKNOWLEDGEMENT

Senior author acknowledges the Union Grant Commission, Ministry of Human Resources Development for Providing National Fellowship.

REFERENCES

1. Amrutha, N., Madhavi, K. and Suchitra, V. 2014. Genetic variability studies in gladiolus (*Gladiolus grandifloras* L.). *Trends Biosci.* **7**: 1227-29.
2. Balaram, M.V. and Janakiram, T. 2009. Genetic variability in gladiolus genotypes for corm characters. *J. Orn. Hort.***12**: 123-26.
3. Bao, G., Bi, Y., Li, Y., Kou, Z., Hu, L., Ge, Y., Wang, Y. and Wang, D. 2014. Overproduction of reactive oxygen species involved in the pathogenicity of *Fusarium* in potato tubers. *Physiol. Mol. Plant Pathol.* **86**: 35-42
4. Chandra, K.J., Negi, S.S., Raghava, S.P.S. and Sharma, T.V.R.S. 1985. Evaluation of gladiolus cultivars and hybrids for resistance to *Fusarium oxysporum* f. sp. *gladioli. Indian J. Hort.* **42**: 304-05.
5. Goswami, R.S. and Punja, Z.K. 2008. Molecular and biochemical characterization of defense responses in ginseng (*Panax quinquefolius*) roots challenged with *Fusarium equiseti*. *Physiol. Mol. Plant Path.* **72**: 10-20.
6. Gurjar, P.S., Singh, S.K., Singh, A.K. and Verma, M.K. 2015. Field reaction and biochemical response of grape genotypes to anthracnose incidence under sub-tropical conditions. *Int. J. Sci. Nat.* **6**: 248-55
7. Hagege, D., Nourelot, A., Boucard, J. and Gaspar, T.H. 1990. *Phytochemical Analysis*, **1**: 86.
8. Hedge, J.E. and Hofreiter, B.T. 1962. *Carbohydrate Chemistry* 17, Whistler, R.L. and Be Miller, J.N. (Eds.), Academic Press, New York.
9. Kadam, G.B., Kumar, G., Saha, T.N., Tiwari, A.K. and Kumar, R. 2014. Varietal evaluation and genetic variability studies on gladiolus. *Indian J. Hort.* **71**:379-84.
10. Hiscox, J.D. and Israelstam, G.F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration using dimethyl sulphoxide. *Canadian J. Bot.* **57**: 1332.
11. Malick, C.P. and Singh, M.B. 1980. *Plant Enzymology and Histo Enzymology*, Kalyani Publication, New Delhi, 286 p.
12. Poon, T.B., Rao, T.M., Kumar, D.P. and Dhananjaya, M.V. 2010. Evaluation of different genotypes of gladiolus for corm and cormel production. *Nepal Agric. Res. J.* **10**: 49-53.
13. Ray, S., Mondal, S., Supriyo, C. and Kundu, S. 2015. Differential responses of resistant and susceptible tomato varieties to inoculation with *Alternaria solani*. *Physiol. Mol. Plant Path.* **90**: 78-88.
14. Riaz, Tariq, Khan, Salik, Nawaz and Javaid, Arshad 2010. Management of corm-rot disease of gladiolus by plant extracts. *Natural Prod. Res.* **24**: 1131-38.
15. Shanmugam, V., Ajit, Singh, N., Raja, R. and Devendra, D. 2009. Screening carnation and gladiolus cultivars for vascular wilt resistance. *Indian Phytopath.* **62**: 117-18.
16. Sharma, Sweety, Dastagiri, M.B. and Narsi Reddy, M. 2017. Morphological variation and evaluation of gladiolus (*Gladiolus hybridus* Hort.) cultivars. *J. Hort.* **4**: 212.doi: 10.4172/2376-0354.1000212

Received : April, 2017; Revised : August, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00112.8

Indian J. Hort. 74(4), December 2017: 586-590

Effect of foliar spray of calcium chloride and boric acid on shelf-life of guava

Anil Kumar, Navin Singh, K.K. Misra* and Vishal Nirgude

Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology Pantnagar 263145, Uttarakhand

ABSTRACT

The present investigation was conducted during 2015-16 on guava cv. Pant Prabhat having 9 treatments of different combinations of calcium and boron sprays made in 3 replications. The results obtained showed that minimum physiological loss in weight (PLW) was found under T_2 (calcium chloride @ 0.4%) treatment. Whereas, TSS (11.99ºB), ascorbic acid (252.4 mg/100 g) and total sugars (7.51%) were found to be maximum in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) treatment and overall acceptability of fruits in storage up to six days was also found maximum under the same treatment. The findings of the present investigation revealed that the pre-harvest foliar application of calcium chloride and boric acid twice was an effective way for improvement of fruit quality and shelf-life of guava. Calcium chloride (0.4%) and boric acid (0.2%) were found to be effective in increasing quality, whereas, calcium chloride (0.4 %) alone was effective for maintaining fruits under ambient storage conditions for six days.

Key words: Boric acid, calcium chloride, guava, storage condition, shelf-life.

INTRODUCTION

Guava (*Psidium guajava* L.) is a popular fruit tree of the subtropical climate and is considered as one of the exquisite, nutritionally valuable and remunerative crops. Guava fruits are used for both fresh consumption and processing (Singh, 9). Foliar nutrient feeding has been universally used and established as an essential part of crop production, mainly on horticulture crops. It has been well established that calcium (Ca) is involved in the regulation of maturation and ripening processes of fruits. Fruit with low Ca content are prone to many biotic and abiotic disorders, and such fruit have usually short shelf-life and hence, foliar applications of Ca may extend the aging process significantly. Another nutrient supposed to have an important role in fruit quality is boron (B). It is an essential nutrient element and it is essential for cell division, reproduction, formation of pollen germination and pollen tube growth, also aids in the translocation of calcium, sugars and is required for protein synthesis. Therefore, the present study was conducted with the objectives to find out the most effective treatment for improving shelf-life and fruit quality of guava cv. Pant Prabhat.

MATERIALS AND METHODS

The present investigation was carried out at Horticultural Research Centre, Patharchatta, Department of Horticulture, GBPUA&T, Pantnagar during 2015-16. The soil of the experimental plot has been classified as series VI (sandy loam under the order Mollisol) of Patharchatta (Deshpande *et al.*, 2). The experiment was conducted on six-year-old guava trees of cv. Pant Prabhat where all the selected trees were uniform in growth and vigour. The trees were given uniform cultural operations during the course of investigation. The experiment was laid out in completely randomized design. The number of treatments were 9, T_1 {Calcium chloride(0.2%)}, T_2 {Calcium chloride (0.4%)}, T_3 {Boric acid (0.1%)}, T_4 {Boric acid (0.2%)}, T_5 {Calcium chloride (0.2%) + Boric acid (0.1%)}, T_6 {Calcium chloride (0.2%) + Boric acid (0.2%)}, T_7 {Calcium chloride (0.4%) + Boric acid (0.1%)}, T_8 {Calcium chloride (0.4%) + Boric acid (0.2%)} and T_9 {control (water spray)} and each treatment was replicated three times. All the treatments were given on 15th August, on fruit set and repeated again on 30th August after 15 days of first application. The chemicals, *viz.*, calcium chloride, boric acid and their combination, respectively were sprayed at different concentrations. The total soluble solids were measured by hand refractometer. Titratable acidity of fruits was calculated by titration method. The ascorbic acid was estimated by 2, 6-dichlorphenol-indophenol visual titration method and expressed in terms of mg per 100 g pulp. The sugars were estimated as described by Ranganna (6). Physiological loss in weight was expressed into percentage. Organoleptic evaluation was done by a panel of four judges taking into consideration of fruit colour, appearance, flavour and taste.

*Corresponding author's E-mail: misrakk_hort@raddiffmail.com

RESULTS AND DISCUSSION

Calcium and boron treatments and storage periods had significant effect on physiological loss in weight (PLW) of guava fruits under ambient condition (Table 1). Minimum PLW (18.02%) was recorded in calcium chloride (0.4%) (T_2) treatment followed by 18.51% PLW in calcium chloride 0.2% (T_1) treatment, whereas, maximum PLW (24.58%) was recorded in control (T_9). From the above finding it's clearly indicated that storage day's affected PLW significantly which increased gradually irrespective of the treatment as the storage period progressed. Application of calcium chloride 0.4% (T_2) retarded the weight loss of guava fruits during storage period compared to the control. Loss of weight in fresh fruit is mainly due to the loss of water caused by transpiration and respiration processes. Fruits treated with higher concentrations of calcium chloride recorded less loss of weight, which may be due to the role of calcium in maintaining fruit firmness, limiting respiratory rate and transpiration during pre-climacteric and climacteric phases, which was attributed to the altered membrane permeability as reported by Tingwa and Young (10). The calcium could also have reduced the endogenous substrate catabolism during respiration by limiting the diffusion of substrate from the vacuole to the cytoplasm and favoured the uptake of sorbitol, thus, disallowing its involvement in reactions related to internal breakdown. Results of present findings are in support with those of Jayachandran *et al.* (4) and Raychaudhary *et al.* (7) on guava.

The data pertaining to TSS varied significantly due to different treatments (Table 1). The maximum TSS (11.99°B) was observed in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) treatment, followed by 11.27°B in T_6 (calcium chloride @ 0.2% + Boric acid @ 0.2%) and 10.90°Brix in T_7 (calcium chloride @ 0.4% + boric acid @ 0.2%) treatments compared to 8.57°B TSS in T_9 (control). The results on increased TSS under these treatment was supported by findings of Bhat *et al.* (1) on pear. The maximum acidity (3.71%) was recorded under T_9 (control) followed by 3.44% in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%). Whereas, minimum acidity (2.57%) was observed in T_4 (boric acid @ 0.2%). Lower acidity in fruits may be ascribed to the higher accumulation of sugars, better translocation of sugars into fruit tissues and conversion of organic acid into sugars. Similarly, maximum ascorbic acid content (252.4 mg/100 g) was recorded in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) followed by (237 mg/100 g) in T_7 (calcium chloride @ 0.4% + boric acid @ 0.1%). Whereas, minimum ascorbic acid (157.6 mg/100 g) was in T_9 (control) (Table 2). The increased ascorbic acid content in guava fruit might be due to higher synthesis of organic acids, on account of maximum availability of plant metabolism. The data presented on reducing sugars clearly indicates that the maximum reducing sugar (11.33%) was recorded in T_2 (calcium chloride @ 0.4%) treatment followed by 10.95% in T_1 (calcium chloride @ 0.2%), (10.54%). Whereas, minimum reducing sugar (10.01%) was obtained in the control (T_9). However, non-reducing sugar was recorded maximum (12.21%) under treatment T_9 (control). The total sugar content of guava fruit was found maximum (15.89%) in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) followed by 15.80% total sugars in T_1 (calcium chloride @ 0.2%) (Table 2). The possible reason for increase in sugar content of fruits with the application of these nutrients might be due to hydrolysis of polysaccharides to simpler from, *i.e.*, mono-and dis-accharides and better transportation of assimilates from leaves to their place of utilization, which helps in increase the sugar content of fruits and consequently reduces the acidity. These results corroborate the earlier records of Kaur and Dhillon (5) and Dutta and Banik (3) on guava.

The findings on appearance of guava fruit at different interval (Table 3) clearly indicates that on harvest day fruits were rated with maximum appearance (7.81) under T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) followed by 7.71 in T_6 (calcium chloride @ 0.2% + boric acid @ 0.2, whereas, minimum rating for appearance (6.28) was in fruits from T_9 (control). Similarly, maximum flavour (6.87) was rated under treatment T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%) followed by 6.75 in T_1 (calcium chloride @ 0.2%) and 6.64 in T_7 (calcium chloride @ 0.4% + boric acid @ 0.1%) compared to control (5.57). In similar manner the fruits on harvest day were rated for maximum texture (6.21) in T_8 (calcium chloride @ 0.4% + boric acid @ 0.2%). Guava fruits treated with calcium and boron spray develops good appearance, desirable flavour, which might be due to loss of organic acids during senescence and change in carbohydrates, proteins, amino acids, lipids and phenolic compounds. The fruit softening (textural integrity) becomes faster with foliar spray of calcium chloride, this softening is due to deterioration in the cell structure, the cell wall composition and the intracellular materials. The above findings were in conformity with the results of Seymour *et al.*(8) and Bhat *et al.* (1).

Table 1. Effect of foliar spray of calcium chloride and boric acid on physiological loss of weight and chemical parameters in guava.

Treatment	PLW (%)					Chemical parameters									
						TSS (ºB)					Acidity (%)				
	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean
T_1 Calcium chloride @ 0.2%	0.00 (0.00)	9.33 (17.77)	17.33 (24.58)	27.60 (31.67)	13.57 (18.51)	8.43	8.93	9.20	9.65	9.05	0.36	0.33	0.29	0.26	0.31
T_2 Calcium chloride @ 0.4%	0.00 (0.00)	8.86 (17.34)	16.25 (23.76)	26.59 (31.02)	12.93 (18.02)	8.70	9.21	9.47	9.86	9.31	0.30	0.29	0.26	0.23	0.27
T_3 Boric acid @ 0.1%	0.00 (0.00)	9.66 (18.09)	17.94 (25.05)	27.99 (31.92)	13.90 (18.76)	9.50	9.70	10.30	10.70	10.05	0.29	0.27	0.24	0.22	0.25
T_4 Boric acid @ 0.2%	0.00 (0.00)	10.20 (18.62)	18.94 (25.78)	29.14 (32.66)	14.57 (19.26)	10.10	10.65	10.74	10.87	10.59	0.23	0.21	0.19	0.18	0.20
T_5 Calcium chloride @ 0.2% + Boric acid @ 0.1%	0.00 (0.00)	10.62 (19.01)	19.72 (26.35)	30.34 (33.41)	15.17 (19.69)	9.50	9.80	10.10	10.33	9.93	0.29	0.27	0.25	0.23	0.26
T_6 Calcium chloride @ 0.2% + Boric acid @ 0.2%	0.00 (0.00)	9.64 (18.07)	20.89 (27.18)	32.14 (34.52)	15.67 (19.94)	11.70	11.23	11.31	11.45	11.27	0.38	0.34	0.29	0.26	0.31
T_7 Calcium chloride @ 0.4% + Boric acid @ 0.1%	0.00 (0.00)	9.38 (17.82)	17.42 (24.65)	28.14 (32.02)	13.74 (18.62)	10.63	10.76	1095	11.26	10.90	0.39	0.36	0.34	0.28	0.34
T_8 Calcium chloride @ 0.4% + Boric acid @ 0.2%	0.00 (0.00)	9.94 (18.37)	18.46 (25.42)	28.40 (32.19)	14.20 (18.99)	11.74	11.83	1.97	12.40	11.99	0.41	0.39	0.35	0.31	0.36
T_9 Control (water spray)	0.00 (0.00)	11.21 (19.55)	31.15 (33.91)	49.84 (44.89)	23.05 (24.58)	8.00	8.40	8.73	9.13	8.57	0.47	0.44	0.40	0.37	0.42
Mean	0.00 (0.00)	9.87 (18.29)	19.78 (26.30)	31.13 (33.81)	-	9.74	10.06	10.31	10.63	-	0.35	0.32	0.29	0.26	-
CD at 5%															
Treatments (T)			0.131					0.140					0.037		
Storage Interval (S)			0.087					0.210					0.25		
Interaction (T × S)			0.262					0.420					0.75		

*The data under parenthesis are angular transformed values.

Table 2. Effect of foliar spray of calcium chloride and boric acid on chemical parameters of guava fruits.

Treatment	Ascorbic acid (mg/100 g)					Reducing sugars (%)					Non-reducing sugars (%)					Total sugars (%)				
	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean
T_1 Calcium chloride @ 0.2%	180.0	178.5	176.8	175.0	177.6	3.57	3.60	3.62	3.65	3.61	3.80	3.79	3.81	3.82	3.81	7.37	7.39	7.43	7.47	7.42
T_2 Calcium chloride @ 0.4%	210.0	208.5	206.8	205.0	207.6	3.83	3.85	3.87	3.90	3.86	3.38	3.39	3.38	3.37	3.38	7.21	7.24	7.25	7.27	7.24
T_3 Boric acid @ 0.1%	225.0	223.5	221.8	220.0	222.6	3.11	3.14	3.15	3.17	3.14	3.74	3.77	3.79	3.79	3.77	6.85	6.91	6.94	6.96	6.92
T_4 Boric acid @ 0.2%	163.3	161.8	160.2	158.3	160.9	3.28	3.29	3.32	3.35	3.31	3.70	3.71	3.81	3.82	3.76	6.98	7.00	7.13	7.17	7.07
T_5 Calcium chloride @ 0.2% + Boric acid @ 0.1%	235.0	233.5	231.8	230.0	232.6	3.11	3.14	3.17	3.20	3.16	3.38	3.38	3.38	3.37	3.38	6.49	6.52	6.55	6.57	6.53
T_6 Calcium chloride @ 0.2% + Boric acid @ 0.2%	180.0	178.5	176.8	175.0	177.6	3.06	3.11	3.15	3.18	3.13	3.34	3.49	3.66	3.74	3.56	6.40	6.60	6.81	6.92	6.68
T_7 Calcium chloride @ 0.4% + Boric acid @ 0.1%	239.8	238.3	236.7	234.8	237.4	3.26	3.27	3.29	3.31	3.28	3.42	3.44	3.45	3.46	3.44	6.68	6.71	6.74	6.77	6.73
T_8 Calcium chloride @ 0.4% + Boric acid @ 0.2%	254.8	253.3	251.7	249.8	252.4	3.30	3.33	3.37	3.40	3.35	4.33	4.33	4.32	3.65	4.16	7.63	7.66	7.69	7.05	7.51
T_9 Control (water spray)	160.0	158.5	156.8	155.0	157.6	2.96	3.00	3.05	3.10	3.03	4.67	4.66	4.64	3.95	4.48	6.28	6.31	6.33	6.34	6.32
Mean	205.3	203.8	202.2	200.3	-	3.28	3.30	3.33	3.36	-	3.75	3.77	3.80	3.66	-	6.88	6.93	6.99	6.95	-
CD at 5%																				
Treatment (T)			2.14					0.10					0.12					0.16		
Storage interval (S)			3.21					0.67					0.08					0.14		
Interaction (T × S)			6.42					0.29					0.28					0.32		

*The data under parenthesis are angular transformed values.

Table 3. Effect of foliar spray of calcium chloride and boric acid on organoleptic parameters of guava fruits.

Treatment	Appearance					Flavour					Texture				
	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean	0 day	2nd day	4th day	6th day	Mean
T_1 Calcium chloride @ 0.2%	8.25	8.45	7.35	5.61	7.42	8.18	7.03	6.53	5.26	6.75	7.65	6.08	4.62	3.07	5.36
T_2 Calcium chloride @ 0.4%	8.10	8.31	7.2	5.46	7.27	8.14	7.00	6.5	5.24	6.72	8.34	6.78	5.32	3.77	6.05
T_3 Boric acid @ 0.1%	7.71	7.90	6.80	5.05	6.87	8.11	6.96	6.46	5.20	6.68	6.90	6.33	4.84	3.32	5.35
T_4 Boric acid @ 0.2%	7.51	7.70	6.60	4.86	6.67	7.40	6.25	5.75	4.49	5.97	7.85	6.28	4.82	3.27	5.56
T_5 Calcium chloride @ 0.2% + Boric acid @ 0.1%	7.35	7.55	6.45	4.71	6.52	7.85	6.70	6.20	4.94	6.42	8.10	6.53	5.02	3.52	5.79
T_6 Calcium chloride @ 0.2% + Boric acid @ 0.2%	8.55	8.75	7.64	5.91	7.71	7.75	6.60	6.10	4.84	6.32	7.55	5.98	4.52	2.97	5.26
T_7 Calcium chloride @ 0.4% + Boric acid @ 0.1%	8.31	8.50	7.40	5.66	7.47	8.23	7.08	6.25	5.00	6.64	7.80	6.22	4.77	3.22	5.50
T_8 Calcium chloride @ 0.4% + Boric acid @ 0.2%	8.65	8.84	7.75	6.01	7.81	8.34	7.20	6.60	5.32	6.87	8.50	6.93	5.47	3.92	6.21
T_9 Control (water spray)	7.11	7.31	6.21	4.47	6.28	7.00	5.85	5.34	4.09	5.57	7.14	5.57	4.11	2.56	4.85
Mean	7.95	8.15	7.04	5.30	-	7.89	6.74	6.19	4.93	-	7.76	6.30	4.83	3.29	-
CD at 5%															
Treatment (T)			0.10					0.09					0.07		
Storage interval (S)			0.16					0.14					0.10		
Interaction (T × S)			0.32					0.29					0.21		

REFERENCES

1. Bhat, M.Y., Hafiza, A., Banday, F.A., Dar, M.A., Wani, A.I. and Hassan, G.I. 2012. Effect of harvest dates, pre-harvest calcium sprays and storage period on physico-chemical characteristics of pear cv. Bartlett. *J. Agric. Res. Develop.* **2**: 101-6.
2. Deshpande, S.B., Fehrenbacher, J.B. and Beavers, A.H. 1971. Mollisols of *Tarai* region of Uttar Pradesh, northern India. International Morphology and Mineralogy. *Geoderma*, **6**: 179-93.
3. Dutta, P. and Banik, A.K. 2007. Effect of foliar feeding of nutrients and plant growth regulators on physico-chemical quality of guava cv. Sardar grown in red and lateritic tract of West Bengal. *Acta Hort.* **735**: 407-11.
4. Jayachandran, K.S., Srihari, D. and Reddy, Y.N. 2005. Pre-harvest sprays of different sources of calcium to improve the shelf-life of guava. *Indian J. Hort.* **62**: 68-70.
5. Kaur, G. and Dhillon, W.S. 2006. Effect of foliar application of chemicals on physico and chemical characters of guava variety Allahabad Safeda during winter. *J. Res. Punjab Agric. Univ.* **43**: 114-16.
6. Ranganna, S. 1986. *Handbook of Analysis and Quality Control for Fruits and Vegetable Products* (2nd Edn.), Tata McGraw Hill Pub. Co. Ltd., New Delhi.
7. Raychaudhary, R., Kabier, J., Dutta, P. and Dhua, R.S. 1992. Effect of calcium on fruit quality of guava. *Indian J. Hort.* **49**: 27-30.
8. Seymour, M., Carrington, C.S., Greve, L.C. and Labavitch, J.M. 1993. Cell wall metabolism in ripening fruit (VI. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes). *Plant Physiol.* **103**: 429-34.
9. Singh, G. 2007. Strategies for improved production of guava. **In**: *Souvenir*, *1st International Guava Symposium*, December 5-8, 2005, CISH, Lucknow, India. pp. 26-39.
10. Tingwa, P.O. and Young, R.E. 1974. The effect of calcium on ripening of avocado (*Persica arminicana* Mill.) fruits. *J. Amer. Soc. Hort. Sci.* **99**: 540-42.

Received : February, 2017; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00113.X

Indian J. Hort. 74(4), December 2017: 591-595

Physico-chemical and enzymatic changes in low temperature stored plum fruits in response to putrescine application

Amandeep Kaur*, S.K. Jawandha and Harminder Singh
Department of Fruit Science, Punjab Agricultural University, Ludhiana 141004

ABSTRACT

Plum is a perishable fruit and its ripening coincides with the hot and dry summer months under north Indian conditions. It has very short storage life at ambient temperature and high postharvest losses. An experiment was planned to extend the post-harvest life of plum fruits with putrescine application under cold storage conditions. Physiologically mature and uniform fruits of plum cv. Satluj Purple were dipped in different concentrations of putrescine (0.0, 1.0, 2.0 and 3.0 mmol l^{-1}) for 5 min. Treated fruits were air-dried in shade and packed in corrugated fiber board boxes with paper lining before storage at 0-1°C and 90-95% RH for 35 days. Results revealed that fruits treated with putrescine @ 3.0 mmol l^{-1} retained the acceptable quality by reducing physiological loss in weight, spoilage, pectin methyl esterase activity and maintaining the fruit firmness, sensory quality, total soluble solids, titrable acidity, total sugars, reducing sugars and non-reducing sugars up to 28 days of storage.

Key words: Cold storage, plum, postharvest, putrescine.

INTRODUCTION

Plum belongs to genus *Prunus* and family *Rosaceae*. It includes European (*Prunus domestica* L.) and Japanese species (*Prunus salicina* Lindl.). Japanese plum is native to China that bears the edible juicy fruits. Under sub-tropical conditions of north India it ripens in the first fortnight of May. Hot and dry weather during this period leads to short post-harvest life of plum fruits. Plum is a climacteric fruit and its storage life is limited even at low temperature because of high susceptibility to physiological disorders. Several chemicals have been reported to delay ripening and extend the shelf-life of fruits. Polyamines have also been found to be anti-senescence agents and the concentration of polyamines decreases during tissue senescence with accelerated ethylene production (Valero *et al*., 12). Novita and Purvoko (10) studied that in papaya fruits polyamine infiltration inhibited the change of colour and reduced the physiological loss in fruit weight and fruit softening process. Inhibition of ethylene production, low respiration rate and higher flesh firmness was also reported in 'Hayward' kiwi fruit with the application of 1 mM putrescine treatment (Wen *et al*., 13). Hence, the present investigation was carried out to study the effect of post-harvest treatments of putrescine on physico-chemical and enzymatic changes of cv. Satluj Purple plum fruits under low temperature storage.

MATERIALS AND METHODS

Physiologically mature and uniform fruits of plum cv. Satluj Purple were harvested from Fruit Research Farm, Punjab Agricultural University, Ludhiana. Selected fruits were treated with aqueous solutions of different concentrations of putrescine @ 1.0 (T_1), 2.0 (T_2), 3.0 (T_3) and 0.0 mmol l^{-1} (T_4). Each treatment was replicated thrice and comprised of 1.0 kg fruit/ replication. Treated fruits were air-dried in shade and packed in corrugated fibre board (CFB) boxes with paper lining. Packed fruits were kept at 0-1°C and 90-95% RH for 35 days. Stored fruits were analysed at weekly interval for various physico-chemical and enzymatic changes. The percent loss in weight after each interval of cold storage was calculated by subtracting final weight from the initial weight of the fruits and then converted into percentage value. The fruit colour was recorded with the help of Color Flex EZ spectrophotometer (Hunter Lab) and expressed as a^* value (Hunter, 3). Firmness of randomly selected fruits was measured with the help of a penetrometer (Model FT-327, USA) using stainless steel probe. About one square centimeter of the peel from both sides of each fruit was removed with the help of peeler and firmness was expressed in terms of lbf. Sensory quality evaluation of fruits was conducted by a panel of five judges following the Hedonic scale (1-9) as described by Amerine *et al.* (2). Spoilage percentage of fruits was also calculated. Total soluble solids (TSS) were determined with the help of hand refractrometer at room temperature and expressed in per cent. These readings were corrected with the help of temperature correction chart at 20°C temperature. The tritratable acidity, total sugars, reducing sugars and non-reducing sugars were estimated by the

*Corresponding author's E-mail: adhillon76@gmail.com

standard methods described by AOAC (1). Pectin methyl esterase enzyme activity was determined by using method of Mahadevan and Sridhar (6). The data obtained were subjected to statistical analysis by following Factorial Completely Randomized Design (CRD) as described by Singh *et al*. (11).

RESULTS AND DISCUSSION

Physiological loss in weight (PLW) of plum fruits increased with extension in storage period (Table 1). The maximum PLW (6.84%) was recorded in untreated fruits, whereas minimum (4.87%) PLW was observed in putrescine @ 3.0 mmol l^{-1} treated fruits after 35 days of storage. All the treatments showed significantly less PLW as compared to control. Physiological loss in weight is due to various metabolic activities, *i.e.* respiration and transpiration processes. Polyamine treatments reduced the weight loss of fruits during storage, which might be due to lower rate of respiration in treated fruit as compared to control. Similarly, putrescine treated lemon fruit showed lower weight loss than untreated fruit during storage (Valero *et al*., 12). Polyamines infiltration reduced the loss of fruit weight during ripening process in papaya fruits (Novita and Purvoko, 10).

Plum fruit colour is associated with the accumulation of carotenoids and anthocyanins. Both groups of pigments are more abundant in the peel but anthocyanins are mainly responsible for the surface colour of the fruit. The colour development was improved with the advancement of storage period. After 35 days, maximum *a** value (25.72) was recorded in untreated fruits, whereas minimum a^* value (23.92) was observed in putrescine @ 3.0 mmol l^{-1} treated fruits (Table 1). Similarly, Malik *et al*. (9) also observed that both pre- and post-harvest putrescine applications retarded fruit colour development of mango fruits.

Spoilage during storage leads to quantitative and qualitative losses of fruits. Putrescine application significantly reduced the spoilage in fruits during storage. After 14 days of cold storage only untreated fruits showed the rotting. However, the putrescine treated fruits showed a little spoilage only after 35 days of storage. At the end of storage, maximum (9.02%) spoilage was noticed in control fruits and minimum (0.35%) spoilage was observed in fruits treated with putrescine @ 3.0 mmol l^{-1} (Table 1). Zheng and Zheng (14) also reported a reduced decay percentage with postharvest treatment of polyamines, *viz*., putrescine, spermidine, spermine and salicylic acid in 'Ponkan' mandarin as compared to control.

Fruit softening is a suitable predictor of potential shelf-life for plums. The decrease in fruit firmness

Table 1. Effect of of putrescine treatments on physiological loss in weight (PLW), fruit colour and spoilage of cold stored plum fruits.

Treatment	PLW (%)						Fruit colour (*a** value)						Spoilage (%)					
Days	7	14	21	28	35	Mean	7	14	21	28	35	Mean	7	14	21	28	35	Mean
T_1	1.65	1.84	2.57	4.47	5.00	3.10	8.92	16.88	18.92	20.79	24.42	17.98	0.00	0.00	0.00	0.00	0.60	0.12
T_2	1.52	1.82	2.49	4.20	4.92	2.99	8.72	16.72	18.79	20.59	24.01	17.76	0.00	0.00	0.00	0.00	0.48	0.09
T_3	1.38	1.80	2.36	4.15	4.87	2.91	8.68	16.68	18.58	20.42	23.92	17.65	0.00	0.00	0.00	0.00	0.35	0.07
T_4	2.97	3.98	4.23	5.74	6.84	4.75	9.42	18.02	19.88	21.61	25.72	18.93	0.00	1.92	5.23	6.86	9.02	4.60
Mean	1.88	2.36	2.91	4.64	5.07		8.93	17.07	19.04	20.85	24.51		0.00	0.48	1.30	1.71	2.61	
$CD_{0.05}$																		
Treatment (T)				0.02						0.07						0.17		
Storage interval (S)				0.03						0.08						0.19		
T × S				0.04						0.16						0.39		

during storage was inhibited significantly by postharvest treatments of putrescine. Putrescine application suppressed and delayed the softness of plum fruits during storage. Reduction in fruit firmness was progressively decreased with increase in putrescine concentration (Fig. 1A). The reduction in fruit softening with putrescine application may be due to decrease in PLW and cell wall degenerating enzyme (PME) activity. At the end of storage, the putrescine @ 3.0 mmol l^{-1} treated fruits retained the maximum (2.42 lbf) fruit firmness and the minimum (1.03 lbf) fruit firmness was recorded in untreated fruits. Softening of fruits is caused either by the breakdown of insoluble protopectins into soluble pectins or by the cellular disintegration leading to increased membrane permeability.

Post-harvest treatment of putrescine @ 3 mmol l^{-1} significantly lowered the pectin methyl esterase (PME) activity as compared to control fruits. After 21 days of storage, the maximum (2.26 ml 0.02 N NaOH used) PME activity was recorded in control fruits and the minimum (1.68 ml 0.02 N NaOH used) was noticed in putrescine @ 3 mmol l^{-1} treated fruits (Fig. 1B). But after 28th and 35th days of storage the trend was reversed, and at the end of storage maximum (1.52 ml 0.02N NaOH used) PME activity was registered in fruits treated with putrescine @ 3 mmol l^{-1}, while the minimum (1.22 ml 0.02 N NaOH used) PME activity was noticed in the reference fruits. This might be due to the presence of high substrate level for PME activity at later stages of storage in putrescine @ 3 mmol l^{-1} treated fruits, which was already decomposed to the higher extent at the early stages of storage in other treatments. The decrease in PME activity at later stage of storage was also reported by Jawandha *et al.* (4) in *ber* fruits.

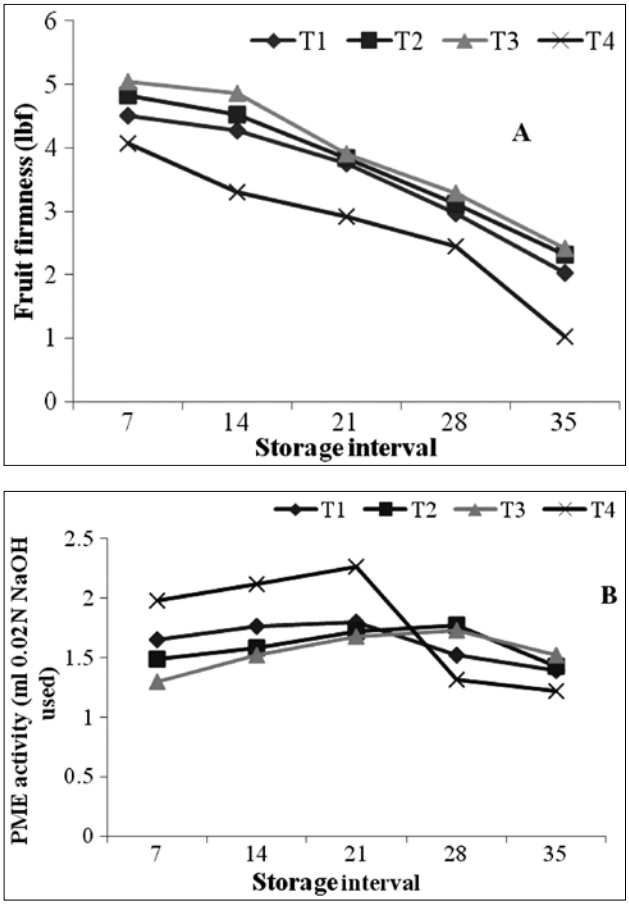


Fig. 1. Effect of putrescine treatments on firmness (A) and PME activity and (B) of cold stored plum fruits.

The data pertaining to sensory quality of plum fruits during storage is given in (Table 2). Sensory quality of stored fruits was improved up to three weeks of storage in all the treatments, afterwards a decline was noticed in T_1 and T_4. After 14 days of storage, highest (7.73) sensory quality was recorded in untreated fruits, whereas, after 28 days of storage highest (8.43) sensory quality was recorded in putrescine @ 3.0 mmol l^{-1} treated fruits. Putrescine treated fruits retained acceptable appearance, flavour and taste for longer period of storage that might be due to the fact that putrescine treatments retarded the moisture, respiration and transpiration losses. Similarly, Malik and Singh (7) also reported a higher sensory quality rating in putrescine treated mango fruits as compared to control.

Total soluble solids content is an important indicator to judge the quality of fruits. TSS content of plum fruits increased upto 21 days of storage in all the treatments, afterwards a decrease in TSS content was observed during storage (Table 2). The mean minimum (12.61%) TSS content was recorded in fruits treated with putrescine @ 3.0 mmol l^{-1} and the mean maximum (13.09%) TSS content was noticed in control fruits. The increase in TSS with advancement of storage period may be due to numerous metabolic processes taking place in the fruits during ripening and senescence processes. The increase in TSS could also be attributed to water loss and hydrolysis of complex polysaccharides to simple sugars. The results on TSS in the present study are in agreement with the findings of Malik *et al*. (8) who reported a slow increase in total soluble solids in 'Kensington Pride' mango fruits treated with putrescine as compared to control.

The titratable acidity of plum fruits showed a declining with an advancement of storage period. During storage the mean maximum (0.85%) titratable acidity was maintained by the fruits treated with putrescine @ 3.0 mmol l^{-1} and the mean minimum (0.52%) acidity was observed in control fruits (Table 2).

Table 2. Effect of putrescine treatments on sensory quality rating, total soluble solids and titratable acidity of cold stored plum fruits.

Treatment	Sensory quality rating (1-9 scale)						Total soluble solids (%)						Titratable acidity (%)					
Days	7	14	21	28	35	Mean	7	14	21	28	35	Mean	7	14	21	28	35	Mean
T_1	7.02	7.29	7.93	7.38	5.87	7.09	12.31	12.70	13.12	13.00	12.61	12.74	0.92	0.81	0.73	0.63	0.55	0.72
T_2	6.82	7.06	7.55	7.88	6.59	7.18	12.07	12.50	12.74	13.19	12.82	12.66	1.05	0.92	0.79	0.69	0.62	0.81
T_3	6.70	6.93	7.29	8.43	6.70	7.21	12.01	12.43	12.60	13.13	12.90	12.61	1.09	0.99	0.81	0.73	0.65	0.85
T_4	7.49	7.73	7.80	5.63	4.91	6.71	13.28	13.59	13.85	12.59	12.18	13.09	0.64	0.59	0.53	0.46	0.41	0.52
Mean	7.00	7.05	7.64	7.33	6.01		12.41	12.80	13.07	12.97	12.62		0.92	0.82	0.71	0.62	0.56	
$CD_{0.05}$																		
Treatment (T)			0.17						0.02						0.02			
Storage interval (S)			0.19						0.03						0.03			
T × S			0.39						0.06						0.04			

Table 3. Effect of putrescine treatments on total sugars, reducing sugars and non-reducing sugars of cold stored plum fruits.

Treatment	Total sugars (%)						Reducing sugars (%)						Non-reducing sugars (%)					
Days	7	14	21	28	35	Mean	7	14	21	28	35	Mean	7	14	21	28	35	Mean
T_1	9.21	9.49	9.68	9.50	8.76	9.32	6.46	6.64	6.78	6.63	6.13	6.52	2.55	2.65	2.69	2.66	2.44	2.59
T_2	8.92	9.24	9.43	9.67	9.00	9.25	6.36	6.48	6.60	6.76	6.30	6.50	2.47	2.56	2.63	2.70	2.51	2.57
T_3	8.90	9.12	9.37	9.63	9.12	9.22	6.26	6.38	6.56	6.73	6.38	6.46	2.45	2.54	2.60	2.69	2.55	2.56
T_4	9.87	10.22	10.42	9.18	8.47	9.63	6.98	7.12	7.28	6.41	5.93	6.74	2.68	2.88	2.92	2.57	2.36	2.68
Mean	9.22	9.51	9.72	9.49	8.83		6.98	7.12	7.28	6.41	5.93		2.53	2.65	2.71	2.65	2.46	
$CD_{0.05}$																		
Treatment (T)			0.10						0.04						0.03			
Storage interval (S)			0.11						0.04						0.04			
T × S			0.23						0.09						0.08			

Decrease in fruit acidity with the progress of storage is due to the utilization of acids in respiration and other metabolic processes (Khader *et al*., 5). The main sugars found in fresh plums are glucose, fructose and sucrose; although sorbitol (a sugar alcohol) is also present. In untreated fruits, total sugars (10.42%), reducing sugars (7.28%) and non-reducing sugars (2.92%) increased upto 21 days of cold storage, but in putrescine @ 2 and 3 mmol l^{-1} treated fruits this increase was recorded upto 28 days of storage (Table 3). Similarly, total and non-reducing sugars were found less in 'Kensington Pride' mango fruits after pre- and post-harvest application of putrescine as compared to control (Malik *et al*., 8). From present study, it can be concluded that 'Satluj Purple' plum fruits, harvested at colour break stage, followed by postharvest treatment of putrescine @ 3.0 mmol l^{-1} for five minutes retained acceptable quality upto 28 days under cold storage conditions (0-1˚C and 90- 95% RH).

REFERENCES

1. A.O.A.C. 2000. *Official Methods of Analysis* (15th Edn.), Association of Official Analytical Chemists, Washington, DC, USA.
2. Amerine, M.A., Pangborn, R.M. and Roessler, E.B. 1965. Principles of sensory evaluation of food. **In**: *Food and Technology Monographs*. Academic Press, New York, pp. 338-39.
3. Hunter, S. 1975. *The Measurement of Appearance*. John Wiley and Sons, New York, pp. 304-05.
4. Jawandha, S.K., Gupta, N. and Randhawa, J.S. 2012. Effect of post-harvest treatments on enzyme activity and quality of cold stored ber fruit. *Notes Sci. Biol.* **4**: 86-89.
5. Khader, S.E.S.A., Singh, B.P. and Khan, S.A. 1988. Effect of gibberellic acid as a postharvest treatment of mango fruits on ripening, amylase and peroxidase activity and quality during storage. *Scientia Hort.* **36**: 261-66.
6. Mahadevan, A. and Sridhar, R. 1982. *Methods in Physiological Plant Pathology*, Sivagami Pub. Madras.
7. Malik, A.U. and Singh, Zora. 2005. Pre-storage application of polyamines improves shelf-life and fruit quality of mango. *J. Hort. Sci. Biotech.* **80**: 363-69.
8. Malik, A.U., Singh, Z. and Dhaliwal, S. 2003. Exogenous application of putrescine affects mango fruit quality and shelf-life. *Acta Hort.* **628**: 121-27.
9. Malik, A.U., Tan, S.C. and Singh, Z. 2006. Exogenous application of polyamines improves shelf life and fruit quality of mango. *Acta Hort*. **699**: 291-96.
10. Novita, T. and Purvoko, B.S. 2004. Role of polyamines in ripening of Solo papaya fruits (*Carica papaya* L.). *J. Stigma*, **11**: 78-81.
11. Singh, S., Bansal, M.L., Singh, T.P. and Kumar, R. 1998. *Statistical Methods for Research Workers*, Kalyani Publ. New Delhi.
12. Valero, D., Perez-Vicente, A., Martinez-Romero, D., Castillo, S., Guillen, F. and Serrano. 2002. Plum storability improved after calcium and heat postharvest treatments: Role of polyamines. *J. Food Sci.* **67**: 2572-75.
13. Wen, H.H., Prista, T. and Sfakiotakis, E. 2003. Effect of dipping and pressure infiltration of putrescine on the propylene induced autcatalytic ethylene production and ripening of 'Hayward' Kiwi fruit. *Acta Hort.* **610**: 261-66.
14. Zheng, Y. and Zhang, Q. 2004. Effects of polyamines and salicylic acid postharvest storage of 'Ponkan' mandarin. *Acta Hort.* **632**: 317-20.

Received : December, 2016; Revised : October, 2017; Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00114.1

Indian J. Hort. 74(4), December 2017: 596-600

Effect of modified atmosphere packaging and storage duration on keeping quality of gladiolus spikes

Shalini Jhanji* **and Kiranjeet K. Dhatt**

Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana 141001

ABSTRACT

Gladiolus has elegant spikes with bright florets and good keeping quality. The storage of spikes during production period will be beneficial for the farmers during low production period. Thus, the present study was planned to investigate the effect of long term modified atmosphere storage on gladiolus spikes. The spikes of gladiolus cv. Alexander the Great, harvested at tight bud stage, were treated with sucrose (20%) + aluminum sulphate (300 ppm) for 20 h followed by sealing in LDPE and PP sleeves (25 µm) with and without perforations and stored vertically at 3-4°C for 0, 6, 12 and 18 days. After storage, vase-life was evaluated. The basal florets opened in all sleeves after 18 days of storage. Increase in storage duration hastened the opening of basal florets, whereas packaging did not affect significantly. Vase-life, opening of florets (%), floret diameter and physiological weight declined with increase in storage duration.

Key words: Gladiolus, modified atmosphere packaging, storage life, vase-life.

INTRODUCTION

Commercial floriculture is an emerging profitable agro industry in the world (Ezilmathi *et al.*, 2). The cut flowers like gladiolus, tuberose, chrysanthemum, rose *etc*. have a great demand in both local and international markets. However, the constraints faced by the growers are decrease in cut flowers' quality from harvesting to marketing and short vase-life. The storage and packaging systems play a pivotal role not only in preservation of keeping quality of flowers but also in regulation of supply of flowers in the markets for better remunerative prices. At lower temperatures, flowers have low respiration rate and other metabolic activities that provide time for proper handling, packaging and marketing (Farazi *et al.*, 3). Dry refrigerated storage or modified atmosphere (MA) storage in which flowers are packed in water retentive plastic films and stored at low temperature, holds most promising post harvest management device for transportation and long duration storage of flowers (Zelter *et al.*, 14). The key to successful passive MAP of fresh flowers is to use film of suitable permeability to gases like CO_2, O_2, water vapour *etc.* so as to ensure and establish the optimal equilibrium Modified Atmosphere (EMA) at low temperature during storage (Day, 1).

Gladiolus is known as the 'Queen of bulbous flowers' due to its spikes with florets of massive form, brilliant colours, attractive shapes, varying size and excellent shelf-life. An appropriate storage technique for gladiolus spikes is required during the periods of over production to sustain the supply of spikes during decline in production. It is immensely important to determine the optimum storage duration of cut flowers that keeps the quality and potential vase-life at its best. The research work done on storage of gladiolus is meagre. However, some reports on the technique of MAP at low temperature contributing to good flower quality during storage (Grover *et al.*, 4) and shipment (Zelter *et al.*, 14) have been documented.

Thus, keeping in view constraints in cut flower marketing and importance of gladiolus among different cut flowers, the present experiment was conducted.

MATERIALS AND METHODS

The plants of gladiolus cultivar, *viz.*, Alexander the Great were raised in the field area of the Department of Floriculture and Landscaping, PAU, Ludhiana from the uniform-sized corms (3.5-4.0 cm dia) following all recommended agronomical practices to raise the healthy crop. The spikes were harvested at tight bud stage (when 1-2 basal florets showed colour) to study the effect of long term modified atmosphere storage on post harvest quality of gladiolus spikes. The harvested spikes were pre-cooled at 4°C for 6 h and subjected to pre-storage treatments with sucrose (20%) + aluminum sulphate (300 ppm) for 20 h. After pre-storage treatment, the spikes were sealed in Low Density Polyethylene (LDPE) and Polypropylene (PP) sleeves of 25 µ thickness with and without perforations and stored vertically in cold room at 3-4°C temperature for 0, 6, 12 and 18 days. The freshly-harvested spikes served as control. Thus,

*Corresponding author's E-mail: shalinijhanji@pau.edu

there were 5 packaging treatments that included PP (with perforations), PP (without perforations), LDPE ((with perforations), LDPE (without perforations) and unsealed/ unwrapped spikes; and four storage treatments that included 0, 6, 12 and 18 days. After storage, the stems were placed in plain water for evaluation of keeping quality. The observations recorded were number and days to the opening of basal floret, vase-life, opening of florets (%), floret size, loss in weight (%) and membrane stability index (Grover *et al.*, 6).

Data were subjected to statistical analysis to calculate critical difference by using analysis of variance (ANOVA) for completely randomised block design (Khaderand and Saltveit, 7).

RESULTS AND DISCUSSION

The results pertaining to number of florets opened during storage revealed that after 6 days of storage, no floret showed opening, whereas 1-2 florets were half and fully opened, respectively, after 12 and 18 days of storage in all packaging treatments (Table 1). The freshly harvested spikes (without storage) took 3.22 days to opening of basal florets whereas increase in storage duration from 6 to 12 to 18 days declined the days to opening of basal florets respectively from 1.78 to 1.07 to 0.65 days. The days taken to opening of basal florets were at par in different packaging treatments (Table 1). The florets exhibited decreased metabolic activity during MA storage, which did not enable the florets to expand (Sharma *et al.*, 8). The delayed floret opening during MA storage is advantageous because spikes with unopened florets can be easily transported as such buds are less prone to damage during transport.

The vase-life of freshly harvested spikes was found to be highest (8 days), whereas vase-life declined in all other treatments (Table 2). The decline in vase-life was 14% after 6 days of storage whereas the corresponding values were 29 and 35% respectively after 12 and 18 days of storage in comparison to control. The mean vase-life of spikes stored without packaging was 5.31 days which was at par with packaging in LDPE {(with perforations) mean vase life of 5.57 days}. The significant difference was observed between mean vase-life of control and LDPE (without perforations) and PP packaging. The mean vase-life of spikes packed in PP with perforations and without perforations was at *par*. The precise mechanism for storage-induced decline in vase-life is not yet fully understood but increased sensitivity to ethylene and loss of membrane permeability after storage could be some of the causes, which have been reported to shorten post-storage vase-life of flowers (Singh *et al.*, 9).

The storage duration significantly declined the per cent opening of florets in all packaging treatments (Fig. 1). The mean per cent of florets that opened declined from 76.5 to 66.32 to 60.48 to 46.82, respectively, when storage duration increased from 0 to 6 to 12 to 18 days. This might be due to the fact that the spikes of gladiolus cut at tight bud stage contain high starch content but low levels of soluble sugars and hence, the upper florets fail to expand which lead to low percentage of floret opening (Singh *et al.*, 10). Physiological processes involved in reduction in floral bud opening might be due to increase in respiration rate during storage, decline in content of soluble sugars, increased sensitivity to ethylene and production of toxic metabolites during storage that contribute to poor post storage opening of the buds

Table 1. Effect of storage and packaging on number of florets open during storage and days to opening of basal floret of gladiolus spikes.

Packaging material (B)	No. of florets open in storage			Days to opening of basal floret			
	Storage duration (days, A)			Storage duration (days)			
	6	12	18	6	12	18	Mean
LDPE (with perforations)	No floret show opening	Basal floret half open	1-2 florets fully open	1.78	1.22	0.56	1.19
LDPE (without perforations)	-do-	-do-	-do-	2.00	0.89	0.44	1.11
PP (with perforations)	-do-	-do-	-do-	1.67	1.11	0.67	1.15
PP (without perforations)	-do-	-do-	-do-	1.44	1.00	0.67	1.04
Control	-do-	-do-	-do-	2.00	1.11	0.89	1.33
Mean	-	-	-	1.78	1.07	0.65	
	-	-	-	Control (0 day storage) = 3.22			
$CD_{0.05}$				CD (5%) A = 0.31; B = NS; A × B = NS			

Table 2. Effect of storage and packaging on vase-life (days) and floret size (cm) of gladiolus spikes.

Packaging material (B)	Vase-life (days)				Diameter of second floret (cm)			
	Storage duration (days, A)				Storage duration (days)			
	6	12	18	Mean	6	12	18	Mean
LDPE (with perforations)	6.31	5.53	4.86	5.57	8.47	7.67	6.91	7.68
LDPE (without perforations)	6.97	5.42	5.30	5.90	8.67	7.81	7.94	8.14
PP (with perforations)	7.19	6.19	5.64	6.34	8.37	7.61	6.87	7.62
PP (without perforations)	7.53	5.97	5.54	6.35	7.94	7.71	7.71	7.79
Control	6.08	5.19	4.64	5.31	8.04	7.61	6.54	7.40
Mean	6.82	5.66	5.20		8.30	7.68	7.19	
	Control (0 day storage) = 8.00				Control (0 day storage) = 8.98			
$CD_{0.05}$	A = 0.34; B = 0.44; A × B = NS				A = 0.25; B = NS; A × B = NS			

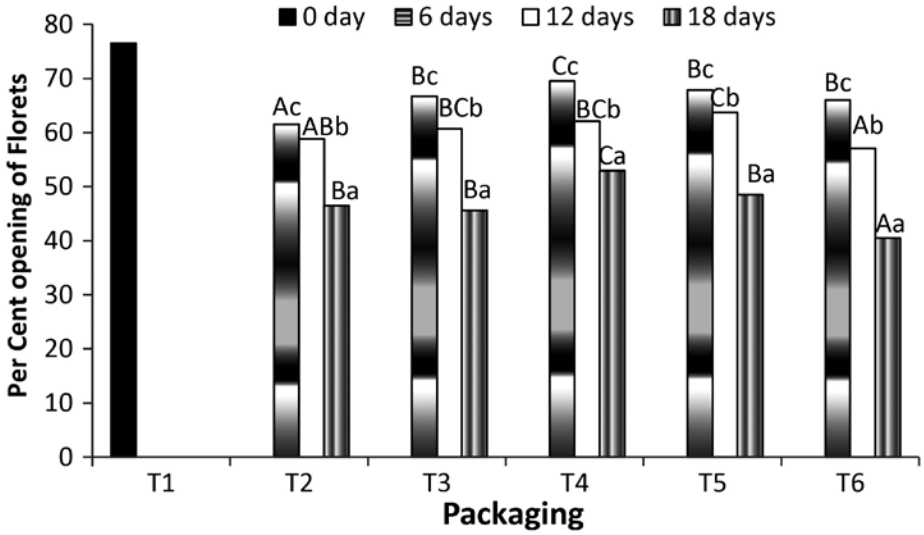


Fig. 1. Effect of storage and packaging on per cent opening of florets of gladiolus spikes. T1 = without packaging and storage; T2 = LDPE (with perforations); T3 = LDPE (without perforations); T4 = PP (with perforations); T5 = PP (without perforations); T6 = stored without packaging. **Different lower case letters indicate statistically significant differences between storage durations for the same packaging treatment, whereas different upper case letters indicate significant differences between packaging treatments for the same storage duration.

(Singh *et al.*, 11). The packaging material significantly influenced the per cent opening of florets. The both PP packaging have significantly higher per cent of opened florets in comparison to LDPE packaging and unpacked spikes. The higher number of floret opening in packed spikes could be attributed to turgidity of the spikes on account of higher water uptake and optimum cell metabolism with sustainable levels of carbohydrates in florets (Singh *et al.*, 12).

Floret size showed significant decrease with increase in storage duration (Table 2). The highest diameter of floret was recorded for freshly harvested spike (8.98 cm), which decreased by 8 per cent after 6 day storage, 14 and 20 per cent, respectively as storage duration increased to 12 and 18 days. The floret size of unpacked spikes was least in all storage treatments but the effect of packaging in all polymeric sleeves was found to be non significant.

The per cent weight loss by the spikes showed increase with increase in the storage duration (Fig. 2). The weight loss was least after 6 day of storage and reached the maximum value after 18 day of storage. Among the packaging treatments, the weight lost after storage by unpacked spikes was highest (7.95%). The packaging treatments significantly improved the retention of weight of spikes during storage as indicated by decreased per cent of weight loss in all packaging treatments. The longer the periods

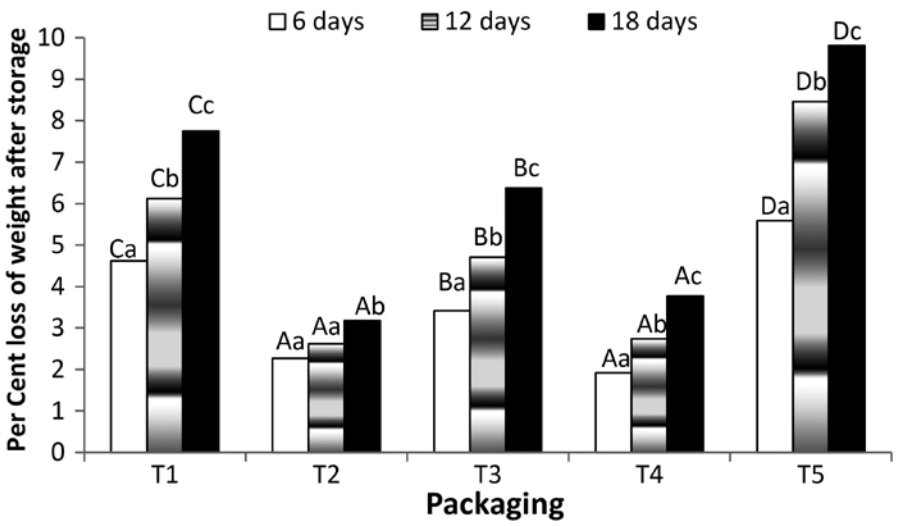


Fig. 2. Effect of storage and packaging on per cent loss of weight after storage of gladiolus spikes.

*T1 = LDPE (with perforations); T2 = LDPE (without perforations); T3 = PP (with perforations); T4 = PP(without perforations); T5 = stored without packaging

**Different lower case letters indicate statistically significant differences between storage durations for the same packaging treatment whereas different upper case letters indicate significant differences between packaging treatments for the same storage duration.

of storage of spikes more is the depletion of stored water and food. Hence, cut stems stored for longer periods with reduced amount of energy resulted in loss of weight and florets of smaller diameter and shorter vase-life as compared to those stored for short durations. Both polymeric sleeves (LDPE & PP without perforations) significantly retained higher weight after storage than other treatments. The reduced loss of weight of spikes packed in sleeves (Fig. 2) might be due to the reason that packaging prevented the water loss and maintained high relative humidity, which helped in reducing weight loss from cut stem 3).

Tepals excised from florets of freshly harvested spikes exhibited higher MSI (80.54). MSI decreased with increase in the storage duration and was 69.43, 60.72 and 45.85 after 6, 12 and 18 days of storage, respectively (Table 3). The loss of membrane integrity during storage could further explain the decrease in the ability of florets to open, decreased vase-life and loss of floret opening after storage. MSI of tepals of unpacked spikes after all storage durations exhibited significantly higher decline in comparison to packaging in polymeric sleeves, but different packaging treatments were at par in terms of maintaining membrane integrity. The maintained MSI of tepals stored under MA using PP and LDPE was a result of minimal damage during cold storage as attributed from per cent weight loss (Fig. 2). This further supports our findings of improved vase life of spikes packed in different sleeves. The membrane

Table 3. Effect of storage and packaging on Membrane Stability Index (MSI) on tepals of gladiolus spikes.

Packaging material (B)	Storage duration (days)			
	6	12	18	Mean
LDPE (with perforations)	72.24	68.43	45.52	62.06
LDPE (without perforations)	75.78	66.21	46.81	62.93
PP (with perforations)	68.37	58.22	48.05	58.21
PP (without perforations)	70.98	59.34	49.23	59.85
Control	59.78	51.42	39.63	50.28
Mean	69.43	60.72	45.85	
	Control (0 day storage) = 80.54			
$CD_{0.05}$	A = 4.86; B = NS; A × B = NS			

deterioration in unpacked spikes during storage as revealed by lower value of MSI (Table 2) could be explained by lipid peroxidation in cut flowers (Zeltzer *et al.*, 14).

The spikes packed in PP sleeves exhibited longer vase life followed by LDPE sleeves as compared to unwrapped ones (Table 2). This might be due to the fact that this wrapping material modifies internal atmosphere with high CO_2, low O_2 and high relative humidity within the package as a consequence of product's respiration and low permeability of films to gas, which further minimizes the respirational loss of carbohydrates as well as transpirational loss of water

and helps in retaining its fresh weight. This condition in turn reduces depletion of stored food and helped to supply adequate energy to the florets for successful opening and to be larger in diameter (Zencirkiranm and Menguc, 15). This is in concomitant with our findings where florets packed in sleeves have higher diameter than unpacked spikes (Table 2).

The differential behaviour of different sleeves as depicted by different parameters, *viz.* number of florets opening, days to basal floret opening, vase life, per cent opening of florets, loss in weight, MSI etc. could be explained on the basis of differential permeability of sleeves to different gases and storage temperature that influence the processes of respiration and transpiration and ultimately quality in gladiolus (Singh *et al.*, 10; Tripathi and Tuteja, 13; Zencirkiranm and Menguc, 15).

Thus, the gladiolus spikes stored for 18 days after MAP exhibited good post-harvest keeping quality and indicated that through packaging some loss of spikes during peak flowering seasons could be reduced.

REFERENCES

1. Day, B.P.F. 2001. Modified atmosphere packaging of fresh fruit and vegetables- An overview. *Acta Hort*. **553**: 585-90.
2. Ezhilmathi, K., Singh, V.P., Arora, A. and Sairam, R.K. 2007. Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of gladiolus cut flowers. *Plant Growth Regul.* **51**: 99-108.
3. Faraji, S., Naderi, R., Ibadli, O.V., Basaki, T., Gasimov, S.N. and Hosseinova, S. 2011. Effects of post harvesting on biochemical changes in gladiolus cut flower cultivar (*White Prosperity*) *Middle-East J. Sci. Res*. **9**: 572-77.
4. Grover, J.K., Gupta, A.K., Singh, K., Kumar, A. and Singh, P. 2006. Studies on passive modified atmosphere storage of gladiolus spikes. *Adv. Hort. Sci.* **20**: 175-80.
5. Grover, J.K. and Singh, K. 2010. Postharvest characteristics of gladiolus spikes as affected by quality and stages of harvest. *J. Orn. Hort.* **13**: 239-40.
6. Grover, J.K., Singh, K., Gupta, A.K. and Kumar, A. 2005. Effect of pre-storage pulsing treatment on storage of gladiolus spikes under modified atmosphere. *Indian J. Plant Physiol.* **10**: 248-53.
7. Kader, A.A. and Saltveit, M.E. 2003. Atmosphere modification. **In**: *Postharvest Physiology and Pathology of Vegetables*, J.A. Bartz and J.K. Brecht (Ed.), Marcel Dekker Inc. pp. 226-46.
8. Sharma, B.P., Beshir, H.M., Dilta, B.S. and Chaudhary, S.V.S. 2008. Effect of various wrapping material and storage durations on post harvest life of Asiatic hybrid lily cv. Apeldoorn. **In**: *4th Nat. Symp. Scenario of Agriculture in Changing Climatic Conditions*, pp. 69-74.
9. Singh, A., Dhaduk, B.K. and Desai, J.R. 2007. Postharvest technology and value addition in cut flowers. *Floriculture Today*, Sept., pp. 40-52.
10. Singh, K., Singh, P. and Kapoor, M. 2007. Effect of vase and pulsing solutiuons on keeping quality of standard carnation (*Dianthus caryophyllus* Linn.) cut flowers. *J. Orn. Hort.* **10**: 20-24.
11. Singh, K., Arora, J.S. and Bhattacharjee, S.K. 2001. *Postharvest handling of Cut Flowers*. Tech Bull No. 10. All India Research Project on Floriculture, IARI, New Delhi, 39 p.
12. Singh, S., Bansal, M.L., Singh, T.P. and Kumar R. 2001. *Statistical Methods for Research Workers*, Kalyani Publishers, New Delhi.
13. Tripathi, S.K. and Tuteja, N. 2007. Integrated signaling in flower senescence: An overview. *Plant Signal Behav*. **6**: 437-45.
14. Zeltzer, S., Mier, S. and Mayak, S. 2001. Modified atmosphere packaging for long term shipment of cut flowers. *Acta Hort.* **553**: 631-34.
15. Zencirkiranm, M. and Menguc, A. 2003. Cold storage of *Alstroemeria pelegrina* Ostara. *New Zealand J. Crop Hort. Sci.* **31**: 255-59.

Received : December, 2016; Revised : September, 2017; Accepted : October, 2017

DOI : 10.5958/0974-0112.2017.00115.3

Indian J. Hort. 74(4), December 2017: 601-603

Short communication

Evaluation of genetic diversity of pecan nut [*Carya illinoensis* (Wang) K. Koch.] in Jammu region

Sohnika Rani*, Arti Sharma, V.K. Wali, Kiran Kour, Manish Sharma and Manmohan Sharma

Faculty of Agriculture, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha 181101, Jammu & Kashmir

ABSTRACT

Pecan is one of the most important deciduous horticultural nut crops and valued for its high calorific value. It is superior to walnut in quality (flavour, 65-70% fats, 8-10% proteins, high in phosphorous, potassium and vitamins A, C, E and B complex) and thrives best in the area experiencing somewhat warmer climate. The present study was carried out during 2015-17 in pecan nut growing areas of Jammu division to assess the extent of genetic divergence and select the superior genotypes of seedling origin pecan nut. Nut weight, kernel weight, nut length, nut width (lateral), nut width (ventral) and shell thickness varied from 3.56-8.63 g, 2.06-6.95 g, 20.03-52.40 mm, 15.50-28.40 mm, 15.54-33.40 mm and 0.30-1.89 mm, respectively. Five out of 60 genotypes were selected on the basis of higher and desired nut and kernel characteristics, which can be further used for trait-specific breeding programmes.

Key words: Genotypes, pecan nut, seedling origin, genetic diversity.

Pecan [*Carya illinoensis* (Wang) K. Koch.] an important edible nut crop, belongs to family Juglandacae. Pecan is one of the most important deciduous horticultural nut crop in the world and is valued nut for high calorific value (~680 calories/100 g kernel). Pecan is superior to walnut in quality (flavour, 65-70% fats, 8-10% proteins, high in phosphorous, potassium and vitamins A, C, E and B complex) and thrives best in the areas, which are considered somewhat lower and hotter for walnut cultivation (Herrera, 2; Sparks, 4; Singh *et al*., 3). In India, its cultivation is limited to mid-hill areas of Himachal Pradesh, Jammu & Kashmir, Uttarakhand and climatically similar areas of North-Eastern states and Nilgiri hills of Tamil Nadu. Its importance lies in nuts having relatively thin shell, which cracks easily and having high nutritional value of the kernel in terms of more than 11 to 12 per cent protein, 70 per cent fat, good amount of phosphoric acid and high calorific value. Pecan nut has been introduced in J&K from Himachal Pradesh by State Horticulture Department. The area under pecan nut in Jammu region is around 464.68 ha with production of about 104.03 metric tonnes in 2015-16. Maximum area under pecan nut is in Rajouri district, *i.e.* 163.0 ha with production of 62.00 MT followed by Poonch having 125.0 ha area under pecan nut with production of 7.50 MT (Anon, 1).

These pecan nut trees raised from seeds being highly heterozygous provide an enormous wealth for carrying out the selection of desirable strains to improve the varietal wealth of pecan. However, still this nut crop could not assume commercial status for the want of suitable cultivars among orchardists due to many constraints such as lack of ideal varieties. Almost all pecan plantations in Jammu division owe their origin to un-descriptive seedlings and are extremely heterogeneous in quality attributes. The existing population comprising the trees of seedling origins exhibit tremendous variability in growth, yield and quality attributes there by providing a platform for exploitation of vast gene pool (Singh *et al.*, 3). Nevertheless, there is huge potential of this nut crop to commercialize being hardy to climatic vagaries and having export value. Meagre efforts have been made for selection of superior seedling pecan genotypes with desirable traits especially economically important nut and kernel traits. To commercialize this nut crop following strategies should be made such as development/ selection of ideal varieties of pecan nut: There is a need to identify suitable trees from native seedling populations or to introduce cultivars from other countries, which are suitable for the different climatic conditions prevailing in the state of Jammu and Kashmir.

Since the seedling pecan plants around Rajouri and Poonch districts of J&K exhibit wide genetic variability expressed through different tree and nut characters, it seems worthwhile to study variations in seedling trees. It appears to be more relevant, in view of the fact that all pecan cultivars introduced

*Corresponding author's E-mail: sohnikaskuastj@gmail.com

Table 1. Range of variability present in nut and kernel characters of pecan nut trees of seedling origin.

Trait	Nut wt. (g)	Kernel wt. (g)	Nut length (mm)	Nut width (lateral) (mm)	Nut width (ventral) (mm)	Shell thickness (mm)	Kernel recovery (%)
Range	3.56-8.63	2.06-6.95	20.03-52.40	15.50-28.40	15.54-33.40	0.30-1.89	40.39-86.55

Table 2. Nut and kernel characters of selected superior genotypes of pecan nut.

Selection/ Trait	SKJPP8	SKJPP13	SKJPM21	SKJPP23	SKJPP25	$CD_{0.05}$
Nut weight (g)	8.63	7.37	8.03	8.05	8.08	0.657
Kernel weight (g)	6.42	6.03	6.95	5.05	6.41	0.078
Nut length (mm)	52.40	39.00	42.40	38.71	48.63	2.113
Nut width (lateral) (mm)	26.25	23.84	28.40	24.77	25.57	1.101
Nut width (ventral) (mm)	32.84	29.98	32.53	30.53	33.40	0.541
Shell thickness (mm)	1.30	1.71	1.55	1.65	1.40	0.051
Kernel recovery (%)	74.39	81.82	86.55	62.73	79.33	1.012

in the country are deficient in one or other trait and there are still better pecan cultivars needs to be selected in pecan growing belts. Thus, the present investigation was carried out in the Division of Fruit Science, FoA, Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu, Chatha, J&K to study the extent of variability and selection of superior pecan trees from a population of seedling origin in Jammu Division during year 2015-16, and 2016-17. Various locations of Rajouri (33.77°N 74.1°E) and Poonch (33°23′N 74°18′E) districts of Jammu Division, which are at elevation of 915 and 981 m, respectively were surveyed for selecting pecan nut trees of seedling origin. Out of total population 60 seedling origin pecan nut genotypes have been selected based on superior nut and kernel characteristics. The observations on nut and kernel characters were recorded using the pecan nut descriptors given by UPOV so as to estimate the extent of genetic relationship by morphological characterization. The selected genotypes have been named as SKJPP (SKUAST Jammu Pecan nut Poonch), SKJPR (SKUAST Jammu Pecan nut Rajouri) and SKJPM (SKUAST Jammu Pecan nut Miran Sahib) and have been given numbers for the ease of identification. Physical dimensions of nut and kernels were determined using Mitutoyo digital Vernier callipers as per Thompson and Grauke (5). The kernel recovery was worked out as average of 20 randomly selected nuts from each selection using formula: % Kernel recovery = Kernel wt./ Nut wt. × 100.

In all the 60 trees studied, high range of variation was recorded for all horticulturally important traits (Table 1), *viz.*, nut weight (3.56-8.63 g), kernel weight (2.06-6.95 g), nut length (20.03-52.40 mm), nut width (lateral) (15.50-28.40 mm), nut width (ventral) (15.54-33.40 mm), shell thickness (0.30-1.89 mm) and kernel recovery per cent (40.39-86.55).

Out of the 60 pecan nut genotypes, five superior genotypes were selected on the basis of nut and kernel traits. As presented (Table 2), selection SKJPR8 exhibited the maximum nut weight (8.63 g), which was followed by SKJPP25 (8.08 g). The kernel weight was observed maximum in genotype SKJPM21 (6.95 g) followed by SKJPP25 (6.41 g). Genotype SKJPP8 recorded the maximum nut length (52.40 mm) followed by SKJPP25 (48.63 mm), lateral nut width was recorded maximum in genotype SKJPM21 (28.40 mm), which was followed by SKJPP8 (26.25 mm), whereas ventral nut width was observed maximum in genotype SKJPP25 (33.40 mm) followed by SKJPP8 (32.84 mm). The shell thickness was observed minimum in genotype SKJPP8 (1.30 mm) and maximum in SKJPP13 (1.71 mm). Kernel recovery per cent was observed maximum (86.55%) in genotype SKJPM21.

These genotypes having broad genetic base can serve as pertinent genetic sources for initiating trait-specific breeding programmes and have greater potential for commercialization of pecan nut in Jammu Division.

REFERENCES

1. Anonymous, 2016. *Area and Production of different Fruits in Jammu*, Directorate of Horticulture, Jammu, Jammu and Kashmir.

2. Herrera, E.M. 1995. Pecan growing in the western United States. *Hort. Tech.* **5**: 200-01.

3. Singh, D., Gupta, R.K., Singh, M. and Sharma, V.K. 2009. Characterization of pecan (*Carya illinoensis*) germplasm of seedling origin through regression and principal component analysis. *Indian J. Agric. Sci*. **79**: 324-26.

4. Sparks, D. 2000. Pecan in warm climate. **In**: *Temperate Fruit Crops in Warm Climates*, Amnon Erez (Ed.), Kluwer Academic Pub., the Netherlands, pp. 381-403.

5. Thompson, T.E. and Grauke, L.J. 2003. Pecan nut and kernel traits are related to shelling efficiency. *Hort. Sci.* **38**: 586-90.

Received : July, 2017; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00116.5

Indian J. Hort. 74(4), December 2017: 604-607

Short communication

Temporal modeling for forecasting of the incidence of litchi stink bug using ARIMAX analysis

T. Boopath*, S.B. Singh, T. Manju, S.K. Dutta, A.R. Singh, Samik Chowdhury, Y. Ramakrishna, Vishambhar Dayal and Lungmuana**

ICAR Research Complex for NEH Region, Mizoram Centre, Kolasib 796 081, Mizoram

ABSTRACT

The litchi, *Litchi chinensis* Sonn. is an important sub-tropical evergreen fruit crop. Among various insect pests of litchi, stink bug, *Tessaratoma papillosa* (Drury) is a major one causing extensive damage in Mizoram. The forecasting model to predict stink bug incidence in litchi was developed by ARIMAX model of weekly cases and weather factors. In exploring different prediction models by fitting covariates to the time series data, model × (mean maximum and minimum temperature, morning and evening relative humidity and rainfall) was found best model for predicting the stink bug incidence; all covariates were found significant predictors except evening RH, which did not have any significant covariates as predictor of stink bug incidence.

Key words: Prediction models, weather factors, stink bug.

The litchi is an important sub-tropical evergreen fruit crop contributing significantly to the growers' economy in India. It is highly specific to climatic requirements and probably due to this reason its cultivation is restricted to few countries in the world. Among fruit crops, litchi ranks seventh in area (90,000 ha.) and ninth in production (5,59,000 MT) but is sixth in terms of value in India. Since, it has adapted to variable climatic conditions, the production and productivity are limited by insect pests and yield loss can approach 70% (Boopathi *et al*., 2). Among various insect pests of litchi, stink bug, *Tessaratoma papillosa* (Drury) (Hemiptera: Tessaratomidae) is one of the most widespread and destructive pest species that up to 25-30% of fruit damaged on litchi in India (Butani, 5). Nymphs and adults suck the sap of the growing buds, leaf petioles, tender branches, flowering and fruiting shoots, causing inflorescence fall or shedding, stem necrosis, fruit discoloration and premature drop. Attacked fruits typically have a tan lesion on the seed testa. Liu and Lai (8) stated that up to 30% of fruit damaged by litchi stink bug in commercial orchards are damaged despite chemical applications. *Tessaratoma papillosa* infestation normally reduces the fruit yield by 20-30%, and may reduce it by 80-90% if the infestation is heavy. Liu (7) gave detailed information on the reduction in litchi and longan yield in Dongguan county, Guangdong Province in South China. Recently, an outbreak of litchi stink bug was observed in the Chotanagpur plateau of Jharkhand, India, during February-April (Choudhary *et al*., 6).

*Corresponding author´s E-mail: boopathiars@gmail.com

**Krishi Vigyan Kendra, Ukhrul District, Manipur 795145

Influence of weather parameters on stink bug incidence is lacking, which is essential for developing management strategies. Current studies showed that though the infestation was recorded throughout the year, it was found low in rainy season, moderate during post rainy season and high in summer. Therefore, these studies clearly show that besides the availability of new shoots and flowers, weather parameters also play an important role in the stink bug incidence in litchi orchards. The population buildup of any insect is very intimately related with the weather parameters (Boopathi *et al*., 1). Forecasting enables to prevent outbreaks and epidemics of stink bug incidence. Hence, this study also aimed at proposing a prediction model to use management practices well in advance.

The investigation was conducted for two years on eight-year-old litchi orchard (cv. Shahi) at ICAR Research Complex for NEH Region, Mizoram Centre, Kolasib, Mizoram. Ten trees were randomly selected and were kept free from insecticidal sprays during the period of investigation. Sampling was done at weekly interval accounting all stages of stink bug except eggs. In each tree, four terminal shoots were selected at random from the entire plant canopy. Thus, 40 shoots growing in all directions were sampled per week. The weather record from March 2009 to March 2010 was obtained from the Meteorological Unit, ICAR Mizoram Centre, Kolasib, Mizoram. Daily reported weather parameters include mean minimum and maximum temperature, morning and evening relative humidity (RH) and rainfall; these variables were collected and recorded at the weather station.

ARIMAX model is an extension of autoregressive integrated moving average (ARIMA) modeling in attempt to predict the stink bug incidence using the weather factors and the stink bug population in corresponding weeks (Bowerman and O'Connell, 3; Box *et al*., 4). The predictors in the model included the stink bug population in the corresponding week, mean maximum and minimum temperature, morning and evening RH and rainfall lagged at one week. The modeling of ARIMAX model was performed using SAS Software Version 9.3 (SAS, 9).

The population of stink bug varied from 2.29 to 8.34 (Fig. 1). The highest population of stink bug was during May (8.34), March (7.99) and June (7.73). A rapid decline in the stink bug population observed during October 2009 and December 2009 with small fluctuation in the stink bug incidence. An increase in minimum and maximum temperature, decrease in morning and evening RH were occurred during summer months, April (7.99), May (8.34), June 2009 (7.73) and July (5.87), favours the population build-up of stink bug.

The moving average intervals as 2, 4, 6 and 8. The moving average for 2 was the average of the previous one data point and the current data point. Similarly, the moving averages for 4, 6 and 8 were the average of the previous 3, 5 and 7 data points, respectively and the current data points. As a result, peaks and valleys were smoothed out. An increasing and decreasing trend was observed during the period of observation of the stink bug incidence (Fig. 2). The larger the interval (interval, 8), the more the peaks and valleys were smoothed out. The smaller the interval (interval, 2), the closer the moving averages were to the actual data points.

The covariates fitted in the models included the stink bug incidence in the corresponding weeks,

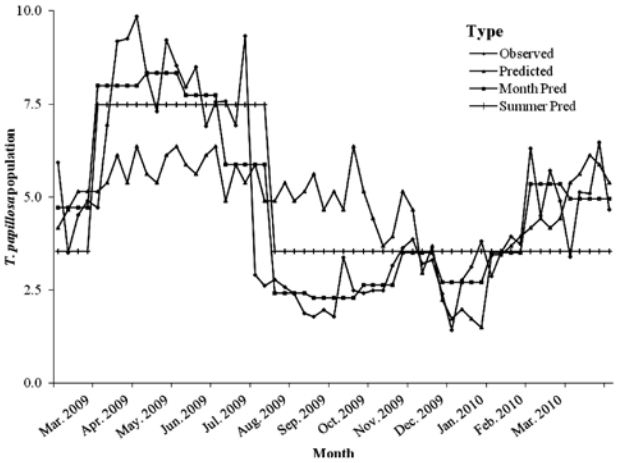


Fig. 1. Graphical representation of the observed, predicted, monthpred, summerpred of stink bug population in litchi.

mean maximum and minimum temperature, morning and evening RH and rainfall, all lagged at one week period. The significant predictors was mean maximum temperature (p = 0.0191), minimum temperature (p = 0.0012), morning RH (p = 0.0002) and rainfall (p = 0.0001) when the stink bug incidence of the corresponding week (Table 1). However, model × was the best model with the lowest SBC of 199.31. The different predictors that were fitted in the model × were stink bug incidence, maximum temperature, minimum temperature, evening RH, morning RH and rainfall. The highest litchi stink bug population observed during summer months, April (7.99), May (8.34), June (7.73) and July 2009 (5.87). The average of litchi stink bug population was the highest during summer (7.48) than winter (3.55). Earlier, Liu and Lai (8) and Boopathi *et al*. (2) reported that stink bug

Table 1. P-values and BIC of significant covariates of litchi stinkbug incidence in different ARIMAX models.

Model	Covariate	SE	t value	P value	AIC	BIC
I	*T. papillosa* population, Max.Temp	0.1301	-2.42	0.0191*	262.69	266.74
II	*T. papillosa* population, Min Temp	0.1239	-3.43	0.0012**	264.15	268.20
III	*T. papillosa* population, Max RH	0.1357	-1.11	0.2707ns	467.79	471.84
IV	*T. papillosa* population, Min RH	0.1240	-4.08	0.0002**	471.85	475.90
V	*T. papillosa* population, Rainfall	0.1083	-5.67	0.0001**	511.19	515.24
VI	*T. papillosa* population, Max Temp, Min Temp	0.2818	-0.34	0.7339ns	264.43	270.51
VII	*T. papillosa* population, Max Temp, Max RH	0.1559	3.57	0.0008**	457.09	463.16
VIII	*T. papillosa* population, Max Temp, Min RH	0.1972	0.12	0.9062ns	466.81	472.89
IX	*T. papillosa* population, Max Temp, Rainfall	0.1599	-1.01	0.3179ns	502.22	508.30
X	*T. papillosa* population, Max Temp, Min Temp. Max-RH, Min-RH, Rainfall	0.1322	-2.24	0.0291*	195.26	199.31

**,* Significant at 0.01 and 0.05 levels; AIC = Akaike's information criterion; SBC = Schwarz's ayesian criterion

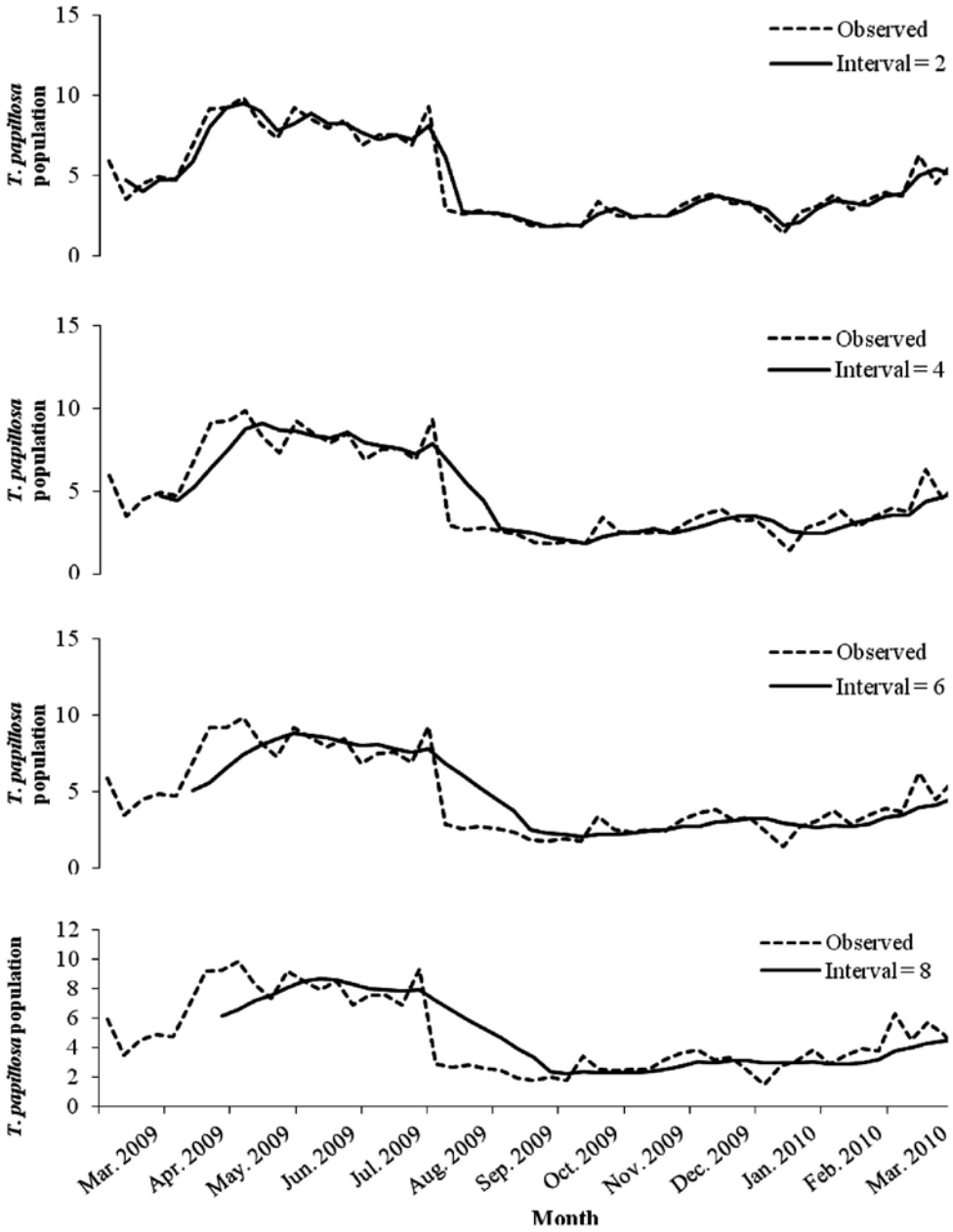


Fig. 2. Weekly litchi stinkbug population time series plot and 2, 4, 6 and 8 weeks moving average forecasts.

incidence found the highest during summer months than other seasons, which is in accordance with the present findings.

In exploring different prediction models by fitting covariates to the time series data, model × was found to be best model for predicting the stink bug incidence; all covariates were found significant except maximum RH. These covariates included the stink bug population in corresponding week, mean maximum and minimum temperature, maximum and minimum RH and rainfall. Meteorological factors such as temperature, humidity and rainfall are an important extrinsic factors that are directly associated with the development of stink bug. However, evening RH did not have any significant covariates as predictors of stink bug incidence. This can be explained by the fact that the stink bug incidence was rather low with less stink bug incidence in certain weeks, therefore, the stink bug incidence of the corresponding week was not a significant predictor, or at worst could lead to inaccurate prediction. Temperature was found as an important predictor for stink bug incidence.

Temperature affects the stink bug bionomics through the time required for developmental period decreases as temperature increases from 24° to 33°C. A decrease in temperature would have the opposite effect. All weather factors had a negative effect in the stink bug incidence. In all, using weather factors as predictors for stink bug incidence were different from one location to another; this pattern has been observed by several other studies.

This method is highly useful for estimating the incidence of stink bug and saves precious time by avoiding field observations. The knowledge of the spatial distribution of the stink bug would also deeply abet in the targeting the management measures. The prediction model based on the time series and weather factors were developed and showed different predictors.

REFERENCES

1. Boopathi, T., Mohankumar, S., Karuppuchamy, P., Kalyanasundaram, M., Ravi, M., Preetha, B. and Aravintharaj, R. 2014. Genetic evidence for diversity of spiralling whitefly, *Aleurodicus dispersus* (Hemiptera: Aleyrodidae) populations in India. *Fla. Entomol.* **97**: 1115-22.

2. Boopathi, T., Singh, S.B., Manju, T., Ramakrishna, Y., Akoijam R.S., Samik Chowdhury, Hemanta Singh, N. and Ngachan. S.V. 2015. Development of temporal modeling for forecasting and prediction of the incidence of the litchi stink bug, *Tessaratoma papillosa*, using time-series (ARIMA) analysis. *J. Insect Sci*. **15**: 1-5.

3. Bowerman, B. and O'Connell, R. 1987. *Forecasting and Time Series: An Applied Approach* (3rd Edn.), Belmont, CA.

4. Box, G.E.P., Jenkins, G.W. and Reinsel, G.C. 1994. *Time Series Analysis* (3rd Edn.), South Windor, New South Wales, Australia.

5. Butani, D.K. 1977. Pests of litchi in India and their control. *Fruits*, **32**: 269-73.

6. Choudhary, P., Prabhakar, C., Moanaro, Das, B. and Kumar, S. 2013. Litchi stink bug (*Tessaratoma javanica*) outbreak in Jharkhand, India, on litchi. *Phytoparasitica*, **41**: 73-77.

7. Liu, C.C. 1965. A preliminary study of biology of litchi stink bug, *Tessaratoma papillosa* Drury and its control. *Acta Phytophyl. Sin*. **4**: 329-40.

8. Liu, X.D. and Lai, C.Q. 1998. Experiment on control of litchi stink bug by using *Anastatus japonicus* Ashmead. *South China Fruits*, **27**: 31.

9. SAS Institute Inc. 2011. SAS® 9.3 System Options: Reference, Second Edition. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina.

Received : June, 2017; Revised : September, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00117.7

Indian J. Hort. 74(4), December 2017: 608-611

Short communication

Effect of method and interval of irrigation on plant growth, yield and quality of grape cv. Pusa Navrang

Ranjit Pal* and S.N. Ghosh**

Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, West Bengal

ABSTRACT

An investigation was carried out at the Malancha Farm under Srinekatan, Shantinekatan Development Authority, Government of West Bengal, Bolepur, Birbhum, West Bengal on 4-year-old vines of Pusa Navrang planted at 2 m × 3 m on Y-trellis to know the effects of method and interval of irrigation on growth dynamics, yield and fruit quality. There were 6-irrigation treatments, *viz*., basin irrigation @ 16 t/vine daily, 1-day interval, 2-day interval; drip irrigation @ 16 l/vine daily, 1-day interval and 2-day interval. Drip irrigation at one day interval resulted in highest yield increment (33.3%) with higher bunch and berry weight and better fruit quality. Regarding irrigation strategy, vines should be irrigated daily after pruning to 2-leaf stage, 1-day interval from flowering to near fruit maturity and 2-day interval during fruit maturity for better growth dynamics, fruit yield and fruit quality.

Key words: Grape, growth dynamics, irrigation, quality, laterite soil and yield.

Grape (*Vitis vinifera* L.) is one of the important export oriented fruit crops in India, commercially grown in different regions in the country having varied climatic conditions. In all commercial regions of the country, table grapes, which accounted for 80%, are produced for fresh consumption and export, 18% for raisin and rest 2% for juice and wine only (Singh, 10). Demand of grape juice and wine is increasing worldwide due to its health benefits. Among the different cultivars available in India, Pusa Navrang (Madeleine Angevine × Ruby Red) is one performing well on laterite soils of West Bengal (Ghosh *et al.*, 5,6). It is established that irrigation in grape is an essential requirement for vine growth after pruning, fruit production and maintaining its vigour for longer vine-life. For irrigating vineyards, drip irrigation is commonly practiced in commercial grape growing states. There are published literatures on effect of method of irrigation, quantification of irrigation water through drip *etc*. are applicable to respective grape growing region in the country. However, in West Bengal, no work on any aspect of irrigation has been done earlier. Generally, ring or basin and drip are followed. Although, drip is the best method of irrigation but it requires initial high monetary investment and electricity availability.

The investigation was taken up during 2011-13 on 4-year-old grapevine of cv. Pusa Navrang planted at spacing of 2 m (plant to plant) × 3 m (row to row). The farm is situated at 23°67' N latitude and 87°72' E longitude at an elevation of 58 m above msl. The top soil of the orchard was collected before starting of the experiment and analysed. Soil of vineyard was laterite, acidic, porous with low water holding capacity. The pH of the soil was 5.7, available N, P and K were 313.5, 32.5 and 111.0 kg/ha. The vines have been trained on Y trellis system. There were six irrigation treatments, *viz*., Basin irrigation (@ 16 l/ vine daily, one day and two day intervals; drip irrigation (@ 16 l/ vine daily, one day and two day intervals. The treatments were applied following randomized block design having six replications with three plants in each replication. Basin irrigation was done manually. Discharge rate of water per dripper was 2.0 l/h. The irrigation treatment was given after pruning to fruit maturity. The irrigation was stoped at 2 leaf stage to panicle emergence. The irrigation treatment was finally stopped 7 days before harvest in each year. Observations were made on fruitfulness of spur, mortality of fruiting spur, renewality of spur, length of renewal and fruiting shoots, leaf number of renewal and fruiting shoots, fruit yield, bunch weight, 10-berry weight and fruit quality with regard to juice per cent, TSS, acidity and ascorbic acid. Fruitfulness of fruiting spur was measured by total number of fruiting shoots kept per vine at the time of pruning and total number of fruiting shoots produced bunches. Mortality of fruiting pruned spur was observed by total number of fruiting shoots kept per vine at the time of pruning and total number of fruiting shoots died after two month of pruning. Renewality of pruned shoots was noted by total number of renewal shoots kept

*Corresponding author's E-mail: profsnghosh@yahoo.co.in

**Rani Lakshmi Bai Central Agricultural University, Jhansi 284003, Uttar Pradesh

per vine at the time of pruning and total number of renewal shoots sprouted after two month of pruning. Length of renewal and fruiting shoot, leaf number of renewal and fruiting shoot were observed 45 days after pruning. Five bunches were weighed from each plant and average bunch weight was calculated and expressed in grams. The TSS was measured by hand refractometer while acidity, total sugars and ascorbic acid content of fruit were determined following standard methods (AOAC, 1).

The results of the present investigation clearly indicated that method and interval of irrigation had significant effect on pruned shoots (spurs), kept for fruiting and renewality. Fruitfulness and renewality of spurs is directly related to fruit production in the current and subsequent year. Fruitfulness and renewality of spurs significantly varied due to method and interval of irrigation (Table 1). Drip irrigated vines had higher fruitfulness (90-98%) and renewality of spurs as compared to basin irrigation (72.8-90.8%) at corresponding irrigation intervals. Renewality of spurs, which determines the next year's yield, was highest in daily irrigated vines (96.3-96.5%) irrespective of method of irrigation. Besides, mortality of fruiting spurs was also lowest in daily irrigated vines irrespective of method of irrigation. These findings clearly indicated that vines to be irrigated daily after pruning and to be continued before emergence of floral primordial (2-leaf stage). Fruitfulness of spurs was highest (98.0%) in the vines with drip irrigation at one-day interval. It indicated that the vines to be irrigated at one-day interval after panicle emergence to get more yield.

Length of renewal and fruiting shoots were more in both the cases in drip irrigated vines as compared to basin irrigated vines at corresponding irrigation intervals (Table 1). Shoot length of renewal and fruiting shoot was highest in daily irrigated vines irrespective of the method of irrigation. Like shoot extension, leaf production in renewal and fruiting shoot was also more in drip irrigated vines as compared to basin irrigation irrespective of interval of irrigation (Table 1). Leaf number was maximum in daily irrigated vines irrespective of the method of irrigation. This growth dynamics in respect of shoot extension and leaf production indicated to adopt irrigation daily after pruning. The practice will be helpful for better accumulation of carbohydrates & other assimilates in the shoots for better vine growth and fruiting.

Method of irrigation as well as irrigation interval had a significant effect on vine yield. In general, drip irrigation resulted in higher yield as compared to basin irrigation irrespective of irrigation intervals throughout the period of investigation. In basin method, irrigation at 1-day interval gave the highest average yield (5.7 kg/ vine) as compared to daily irrigation (3.2 kg/ vine) (Table 1). Results suggested that this practice should be followed in the areas where drip irrigation may not be possible. In drip method, irrigation at one-day interval also resulted in highest yield (7.6 kg/ vine) as compared to all irrigation treatments (Table 1). Highest yield in the vines with drip irrigation at 1-day interval was due to maximum number of fruitful spurs (98.0%). Fimbres-Fontes *et al.* (4) opined that increased yield in drip irrigated vines may due to higher fruitfulness, increase berry set and reduce berry drop. There are many reports indicating superiority of drip method of irrigation over basin or ring under various locations and cultivars (Sarkar and Hanamashetti, 8; Sharma

Table 1. Effect of method and irrigation interval on growth dynamic and fruit yield in grape cv. Pusa Navrang.

Treatment	Growth dynamic (Av. of 2-years)							Fruit yield (kg)/vine		
	Spur fruitfulness (%)	Spur renewality (%)	Fruiting spur mortality (%)	Length of renewal shoot (cm)	Leaf No. of renewal shoot	Length of fruiting shoot (cm)	Leaf No. of fruiting shoot	1st year	2nd year	Pooled
Basin irrigation										
Daily	72.8	96.5	3.3	34.2	11.8	46.7	15.2	2.6	3.8	3.2
1-day interval	90.8	92.5	5.3	31.2	10.7	44.5	15.0	4.9	6.5	5.7
2-day interval	89.3	91.4	6.0	31.2	10.5	39.0	14.0	3.9	5.9	4.9
Drip irrigation										
Daily	97.0	96.3	2.3	41.5	12.7	53.5	16.0	6.4	7.6	7.0
1-day interval	98.0	96.0	3.2	35.7	11.3	44.7	14.0	7.0	8.2	7.6
2-day interval	90.0	93.0	9.6	32.7	10.8	43.7	13.2	5.8	6.2	6.0
$CD_{0.05}$	3.9	2.5	1.5	2.1	0.9	3.6	1.1	1.7	1.9	1.8

Table 2. Effect of method and irrigation interval on bunch & berry weight and fruit quality of grape cv. Pusa Navrang.

Treatment	Bunch wt. (g)	10-berry wt. (g)	Juice (%)	TSS (°Brix)	Acidity (%)	TSS/ acid ratio	Total sugars (%)	Ascorbic acid (mg/ 100 ml juice)
Basin irrigation								
Daily	176	11.2	63.8	15.6	1.00	15.6	11.3	2.8
1-day interval	189	11.6	64.7	15.8	1.00	15.8	11.9	2.5
2-day interval	220	16.1	74.5	16.6	0.67	24.8	12.8	4.8
Drip irrigation								
Daily	212	11.4	64.9	16.4	0.98	16.7	12.7	3.2
1-day interval	221	18.7	70.0	17.4	0.66	26.4	13.2	3.5
2-day interval	219	18.6	72.1	17.8	0.66	26.9	13.9	3.4
$CD_{0.05}$	27.81	1.63	2.90	0.36	0.16	1.44	0.83	NS

et al., 9). The present investigation was aimed to establish how much yield improvement can be made over conventional basin method in red laterite soil. It was calculated that 33.3% yield increment was observed by drip irrigation at 1-day interval over basin irrigation at same intervals using equal volume of water/vine.

Bunch and berry weight are considered to be important parameters for yield determination as well as market price. Method and irrigation intervals had significant effect on both these yield contributing attributes. Drip irrigated vines produced higher bunch and berry weight as compared to basin irrigation irrespective of intervals of irrigation. Among the treatments, drip irrigation at 1-day interval resulted in the highest bunch weight (221 g) and 10-berry weight (18.7 g). The lowest bunch and 10-berry weight were recorded from the vines with basin irrigation daily (176.0 and 11.2 g, respectively). Higher bunch and berry weight in drip irrigated vines may be to more efficient use of applied water for better physiological activity in vines, which in turns resulted in synthesis of more carbohydrates and other reserved foods and translocation of water to berry.

Significantly higher TSS/ acid ratio was noted from the vines received drip irrigation as compared to basin irrigation at corresponding intervals of irrigation (Table 2). Maximum TSS/ acid ratio of 26.9 was determined from the vines received irrigation through drip at 2-day interval and minimum (15.6) was with daily basin irrigation. Irrigation at 2-day interval resulted in maximum TSS, total sugars and TSS/ acid ratio irrespective of method of irrigation. It is suggested that drip irrigation at near maturity stage should be followed at 2-day interval instead of 1-day. Araujo *et al.* (2) also opined that different irrigation amounts were required for the vines, which varied from pruning to fruit set, fruit set to veraison and veraison to fruit maturity. Quality improvement in grapes due to drip irrigation was also observed by Burg (3) and Matouk *et al.* (7). Ascorbic acid content in grapes was generally low irrespective of the treatments and did not vary due to different irrigation treatments.

REFERENCES

1. A.O.A.C. 1990. *Official Methods of Analysis*, *Association of Official Agriculture Chemists* (*15th Edn.*), Washington, D.C.
2. Araujo, F.J., Faria, A., Sanchez, C., Nickel, W., Rivero, Y. and Urdaneta, T. 1999. A drip irrigation strategy for maximizing grapevine water use efficiency in tropical vineyards of Venezuela. *Acta Hort.* **493**: 117-40.
3. Burg, P. 2008. The influence of drip irrigation on the quality of vine grapes. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, **56**: 31-35.
4. Fimbres-Fontes, A., Martinez Diaz, G. and Valenzuela Ruiz, M. de J. 2000. Regular and high application of water with drip irrigation and its effect on the floral buds of table grapes. *Terra,* **18**: 219-24.
5. Ghosh, S.N., Bera, B., Roy, S. and Kundu, A. 2012. Adaptation and commercialization of viticulture in West Bengal - A new area in India. *Acta Hort.* **931**: 389-99.
6. Ghosh, S.N., Tarai, R. and Pal, P.P. 2008. Performance of eight grape cultivars in laterite soil of West Bengal. *Acta Hort.* **785**: 73-77.

7. Matouk, A.M., El-Gindy, A.M., El-Adl, M.A. and Bondok, M.Y. 2000. Grape yield response to subsurface drip irrigation in old valley. *Egyptian J. Agric. Res.* **78**: 1411-33.

8. Sarkar, A.N. and Hanamashetti, J.S. 2002. Financial viability of drip irrigation system for sugarcane and grape cultivation in Maharashtra. *Asia Pacific J. Rural Dev.* **12**: 1-31.

9. Sharma, J., Upadhyay, A.K. and Adsule, P.G. 2005. Effect of drip water application at sub-surface on grapevine performance- a case study. *J. Appl. Hort.* **7**: 137-38.

10. Singh, H.P. 2011. Research and development in grapes: Achievements and perspectives. *Indian Hort.* **56**: 3-12.

Received : January, 2017; Revised : October, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00118.9

Indian J. Hort. 74(4), December 2017: 612-614

Short communication

Validation of molecular marker AVRDC-PP12 linked to male sterility gene *ms10* of chilli

Parjeet S. Aulakh, M.S. Dhaliwal* and S.K. Jindal

Department of Vegetable Science, Punjab Agricultural University, Ludhiana 141004

ABSTRACT

The newly identified SSR marker AVRDC-PP12 linked to the male sterility gene *ms10* distinguished the heterozygous (*Ms10ms10*) fertile from the homozygous (*Ms10Ms10*) fertile plants in chilli segregating populations. To assess efficiency of AVRDC- PP12 in marker-assisted backcrossing for transfer of *ms10* gene; the marker was screened in four backcross progenies. The marker co-segregated with *ms10* gene in three progenies derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev' with recombination frequency of 3.22, 4.16 and 3.27%, respectively. The marker failed to differentiate the parents in the cross 'MS-12 × DCL- 524' and hence was not used for further genotyping of the population.

Key words: *Capsicum*, nuclear male sterility, *ms10*, SSR markers.

Hybrids in chilli (*Capsicum annuum* L.) are very popular due to their superior performance and the F_1 seed is produced manually as well as by utilizing male sterility. By using male sterility, hybrid seed cost is reduced by about 50% (Yang *et al*., 11). Nuclear male sterility was first documented in *Capsicum* spp. by Martin and Crawford (7). Till-date, approx. 20 independently inherited NMS genes have been identified. Male sterility in all, except one, is controlled by a single recessive gene (Dhaliwal and Jindal, 3). Unlike the CMS, which is influenced by low temperature, the NMS system in chilli is stable and commercially used in India and in many other countries.

Pochard (8) identified a male sterility gene *mc509* from a mutant population of bell pepper. The gene was subsequently re-designated as *ms10* (Wang and Bosland, 10). Singh and Kaur (9) transferred *ms10* gene from bell pepper through the conventional backcross method to a multiple disease resistant chilli cv. Punjab Lal. The new NMS line designated as 'MS-12' was utilized to develop commercial hybrids 'CH-1', 'CH- 3' and 'CH-27'.

In recent years, molecular markers have been used to improve efficiency of crop breeding programmes. The linked markers can distinguish the heterozygous fertile from the homozygous fertile plants in segregating generations, thus, facilitating the development of new NMS lines through marker-assisted backcrossing. We used AVRDC-PP12 SSR marker to facilitate transfer of *ms10* gene from 'MS-12' into an array of elite chilli breeding lines through marker-assisted selection.

*Corresponding author's E-mail: msdhaliwal696@gmail.com

The NMS line 'MS-12' was used as a donor parent for the male sterility gene *ms10*. Four elite inbred lines 'VR-16', 'S-217621', 'Selection Dev', and 'DCL-524' were used as the recurrent parents to receive *ms10* gene. Four BC_2F_2 populations were generated from four crosses involving male sterile × male fertile parents. Male sterile and male fertile plants were assessed visually by observing the anther colour of freshly opened flowers and pollen formation. The male sterile plants (*ms10ms10*) showed purple colour anthers without pollen grains sticking to the anthers. The male fertile plants, genetically either *Ms10Ms10* or *Ms10ms10* showed green colour anthers with pollen grains sticking to the anthers. Pollen presence was further confirmed by touching anthers of freshly opened flowers on thumbnail or piece of black paper. Presence of a whitish/ creamy powdery mass (pollen) indicated that the plant is male fertile, while its absence indicated that the plant is male sterile. The genomic DNA of the selected BC_2F_2 plants along with their respective parents were amplified by using the SSR primer pair AVRDC-PP12 (Table 1) linked to the male sterility gene *ms10* (Aulakh *et al*., 1). Frequency of male sterile and male fertile plants obtained genotypically was compared with the frequency of the two classes obtained phenotypically, and the results were interpreted.

$$\text{The recombinant frequency (\%)} = \frac{\text{No. of recombinant events occurred}}{\text{Plant population screened}} \times 100$$

The NMS gene *ms10* has been utilized for hybrids development in chilli. Due to the recessive gene control, its transfer through the conventional backcross method to other genetic backgrounds

Table 1. Characteristics of SSR marker used for screening of segregating populations of chilli derived from the male sterile × male fertile crosses.

Marker	Primer sequence (5'-3')	Motif	Fragment size (bp)	PCR Tm	Source
AVRDC- PP12	(F)TCCTAACTCTTCCCACCACC (R)GGAGAAGTGTAGCTCCAGCC	(TC)10	132	55	AVRDC, Taiwan

is tedious and time consuming. In recent year, molecular markers have been used to improve efficiency of crop breeding programmes. Aulakh *et al.* (1) developed a SSR marker AVRDC-PP12 linked to the male sterility gene *ms10* in chilli. The marker distinguished the heterozygous (*Ms10ms10*) fertile from the homozyguous (*Ms10Ms10*) fertile plants in segregating generations, thus facilitating the development of new NMS lines through marker-assisted backcrossing. The marker has been screened in four breeding populations generated to transfer *ms10* gene into the elite breeding lines, *viz*. 'VR-16, 'S-217621', 'Selection Dev' and 'DCL-524'. Efficiency of the marker-assisted backcrossing was compared on basis of the phenotypic and the genotypic observations.

To test efficiency of *ms10*-linked marker AVRDC-PP12, genomic DNA of individual BC_2F_2 plants of four backcross populations along with their respective parental DNA was amplified with the marker and resolved in 6% polyacrylamide gel. The results showed that the marker AVRDC-PP12 co-segregated with *ms10* gene in three BC_2F_2 populations derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev' into three genotypic classes as expected in one gene segregation, *i.e.* homozygous recessive (*ms10ms10*), homozygous dominant (*Ms10Ms10*) and heterozygote (*Ms10ms10*). The results showed that the marker can efficiently be used for marker-assisted selection (MAS) in progenies derived from the crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev'. The marker failed to differentiate the parents in the cross 'MS-12 × DCL- 524' and hence was not used for further genotyping of the population.

In the backcross progenies, 62 plants of 'MS-12 × VR-16', 72 plants of 'MS-12 × S-217621', and 61 plants of 'MS-12 × Selection Dev' were analyzed for phenotypic and genotypic segregation. The frequency of recombinant events for the marker AVRDC-PP12 was recorded as 3.22, 4.16 and 3.27% in BC_2F_2 populations of crosses 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev', respectively (Table 2). Since the cross-over values are less than 5%, the marker can efficiently be used in MAS for the transfer of male sterility gene *ms10* into the three breeding lines. This would save the precious time and resources required to raise self or test cross progenies in the conventional backcross method.

Earlier, a SCAR marker named MS1-SCAR linked to *ms1* gene (Lee *et al*., 5), a CAPS marker GMS3-CAPS linked to *ms3* gene (Lee *et al*., 6), a GMSK-CAPS marker linked to *msk* gene (Lee *et al*., 4), two SCAR markers SCAR_P2 and SCAR_V17 linked to *ms8* gene (Bartoszewski *et al*., 2) and a high resolution melting marker (HRM) named GMSE linked to an undesignated gene were developed. The markers co-segregated with the linked genes, thus showing their potential use for MAS to transfer the linked genes and strengthening the heterosis breeding programme in peppers.

It is concluded that the markers AVRDC-PP12 distinguished homozygous dominant (*Ms10Ms10*) and heterozygous (*Ms10ms10*) genotypes in three out of four segregating populations incorporated with the *ms10* gene. Thus, the marker proved its applicability in MAS for the development of new NMS lines derived from the crosses between 'MS-12 × VR-16', 'MS-12 × S-217621' and 'MS-12 × Selection Dev'. The marker-assisted backcrossing would facilitate breeding for NMS lines incorporated with *ms10* gene and help to save the precious time and the resources.

Table 2. Segregation analysis of the marker AVRDC-PP12 for male fertility in the BC_2F_2 populations.

Cross	Total plants	Genotypic segregation			Recombinant frequency (%)
		Ms10Ms10	*Ms10ms10*	*ms10ms10*	
MS-12 × VR-16	62	18	34	10	3.22
MS-12 × S-217621	72	30	27	15	4.16
MS-12 × Selection Dev	61	25	31	5	3.27

ACKNOWLEDGEMENTS

This research was supported by a grant (No. BT/PR2055/PBD/16/932/2011) from the Department of Biotechnology, Government of India. Authors are thankful to Dr Roland Schafleitner, Head of Molecular Genetics, AVRDC- The World Vegetable Center, Taiwan for providing information about the SSR markers used in this research.

REFERENCES

1. Aulakh, P.S., Dhaliwal, M.S., Jindal, S.K., Schafleitner, R. and Singh, K. 2016. Mapping of male sterility gene *ms10* in chilli pepper (*Capsicum annuum* L.). *Plant Breed.* doi:10.1111/pbr.12389

2. Bartoszewski, G., Waszczak, C., Gawronski, P., Stepien, I., Bolibok-Bragoszewska, H., Palloix, A., Lefebvre, V., Korzeniewska, A. and Niemirowicz-Szczytt, K. 2012. Mapping of the *ms8* male sterility gene in sweet pepper (*Capsicum annuum* L.) on the chromosome P4 using PCR-based markers useful for breeding programmes. *Euphytica*,**186**: 453-61.

3. Dhaliwal, M.S. and Jindal, S.K. 2014. Induction and exploitation of nuclear and cytoplasmic male sterility in pepper (*Capsicum* spp.): A review. *J. Hort. Sci. Biotech.* **89**: 471-79.

4. Lee, J., Han, J.H., An, C.G., Lee, W.P. and Yoon, J.B. 2010a. A CAPS marker linked to a genic male-sterile gene in the colored sweet pepper, 'Paprika' (*Capsicum annuum* L). *Breed. Sci.* **60**: 93-98.

5. Lee, J., Yoon, J.B., Han, J.H., Lee, W.P., Do, J.W., Ryu, H., Kim, S.H. and Park, H.G. 2010b. A codominant SCAR marker linked to the genic male sterility gene (*ms*1) in chili pepper (*Capsicum annum*). *Plant Breed.* **129**: 35-38.

6. Lee, J., Yoon, J.B., Han, J.H., Lee, W.P., Kim, S.H. and Park, H.G. 2010c. Three AFLP markers tightly linked to the genic male sterility *ms3* gene in chili pepper (*Capsicum annuum* L.) and conversion to a CAPS marker. *Euphytica*, **173**: 55-61.

7. Martin, J. and Crawford, J.H. 1951. Several types of sterility in *Capsicum frutescens. Proc. Amer. Soc. Hort. Sci.* **57**: 335-38.

8. Pochard, E. 1970. Induction of three new male-sterility mutants in pepper (*Capsicum annuum* L.) by mutagenic treatment of monoploid material. **In**: *Proc. EUCARPIA Meeting on Male Sterility in Plants* (*Horticulture*), Versailles, France, pp. 93-95.

9. Singh, J. and Kaur, S. 1986. Present status of hot pepper breeding for multiple disease resistance in Punjab. **In**: *Proc. VI EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant.* Zaragoza, Spain, pp. 111-14.

10. Wang, D. and Bosland, P.W. 2006. The genes of *Capsicum*. *HortSci.* **41**: 1169-87.

11. Yang, C.J., Chen, B.J., Liu, J.G. and Li, S. 2008. Study on the superiority of hybrid breeding by male sterility in hot pepper. *J. China Capsicum*, **1**: 36-38.

Received : May, 2016; Revised : July, 2017;
Accepted : September, 2017

DOI : 10.5958/0974-0112.2017.00119.0

Indian J. Hort. 74(4), December 2017: 615-617

Short communication

Transferability of sponge gourd EST-SSR markers for genetic diversity assessment of *Luffa* species

Nangsol D. Bhutia, A.K. Sureja, Lalit Arya[**], **A.D. Munshi and Manjusha Verma**[*]
Division of Vegetable Science, ICAR-Indian Agricultural Research Institute, New Delhi 110012

ABSTRACT

Genetic diversity was studied in 47 *Luffa* genotypes with 17 EST-SSR primers, which generated 34 alleles, ranging 1-2 loci per primer. Forty seven genotypes were broadly classified into two different clusters. Cluster I comprised of genotypes DRG-98, Utkal Tripti, DRG-6, Sel-102, Pusa Nasdar, Pusa Nutan, Arka Sujat, DRG-73, DRG-61, DRG-42 and DRG-50, while Cluster II consisted of 36 genotypes, respectively. EST-SSR primer C90830_G3 was found to be highly informative with PIC value of 0.3750. The variability in the species could be credited to introgression and selection as a result of long history of cultivation under varied climatic conditions. The present data provide adequate evidence of the applicability of EST-SSR markers for diversity analyses, cultivar identification and characterization of the *Luffa* germplasm.

Key words: EST-SSR, *Luffa acutangula*, *Lufa hermaphrodita*.

Luffa (2n = 26) is a member of the Cucurbitaceae family and originates from India (Islam *et al*., 3). Out of nine species found in the world, seven *Luffa* species are found in India of which, three species *L. acutangula*, *L. cylindrica*, and *L. hermaphrodita* are edible species. The genus *Luffa* derives its name from the product 'loofah', which is used as bathing sponges, scrubber pads, doormats, pillows, and mattresses and also for cleaning utensils. Apart from important underutilized vegetable cucurbits, *L. acutangula* and *L. hermaphrodita* have great potential as biodiesel crop in future. Ridge gourd is characterised by monoecious sex form and solitary long fruits of 15-30 cm in length with prominent ribbed and rough fruit skin. Satputia has hermaphrodite sex form and produces small fruits in cluster. Its fruits have faint line instead of prominent ridge. As a result of the long history of cultivation of *Luffa* in India under varied climatic conditions, a large numbers of variants have been developed from the cultivars through introgression and selection. These genetic resources harbour valuable genes or adaptation to diverse agro-ecological zones, and resistance to diseases, pests and stress environments (Arora and Nayar, 1). Knowledge of the genetic variation between genotypes of the crop gene pools is an important consideration for the classification and utilization of plant genetic resources in crop improvement. There are many approaches used to quantify the diversity at intra- as well as inter-species level, however, molecular markers are considered to have enormous potential to explore genetic diversity by detecting polymorphisms at DNA level (Singh *et al*., 5). The use of various molecular marker methods, which are independent of environmental conditions. Microsatellites sequences (EST-SSR) are especially suited to distinguish closely related genotypes because of their high degree of variability and they are therefore favoured in population studies (Smith and Devey, 6). Considering the importance of *Luffa*, it is necessary to understand the molecular diversity among *Luffa* genotypes and its subsequent utilization in genetic enhancement of *Luffa* sp.

The experimental material comprised of 47 genotypes of *Luffa* species collected from various parts of India and maintained at Division of Vegetable Science, ICAR-IARI, New Delhi. Healthy leaf samples were collected from young plants. The leaves were bulked from five plants, and DNA was isolated using standard method. The leaves were ground to a fine powder in liquid nitrogen and resuspended in CTAB extraction buffer. The supernatant was extracted with chloroform-isoamyl alcohol (24:1), precipitated in absolute ethanol and the pellet resuspended in Tris-EDTA buffer and purified with 10 mg/ ml RNase. The extracted DNA was quantified using a Nanodrop® spectrophotometer (Thermofisher Scientific), diluted to a concentration of 50 ng/ml in 1 × TE buffer, and the diluted samples were kept at 20°C. A total of 191 EST-SSR primers, derived from *L. cylindrica,* were screened using five samples of different *Luffa* species for optimum amplification and an initial polymorphism survey. Of these, 17 EST-SSR primers were selected for profiling 47 *Luffa* genotypes. PCR was carried out in 25 µl reaction volumes with 100 ng genomic DNA,

*Corresponding author's E-mail: manjusha.verma@icar.gov.in
**ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012

1 U *Taq* polymerase, 1 × *Taq* buffer,0.4 µM primer, 20 µM of dNTPs mix, with sterile distilled water to make up the volume. For PCR cycling - Bioer XP cycler was programmed as follows: initial cycle at 94°C for 5 min., 40 cycles at 94°C for 20 s, 48-65°C (depending on primer used) for 30 s, and 71°C for 1 min., followed by a final extension at 72°C for 5 min. PCR product was resolved using 2% agarose gels in 1 × TBE (Tris-borate EDTA) buffer for 4 h at 120 V and visualized with ethidium bromide. A 200 bp ladder (Fermentas) was used as molecular weight marker.

All amplicons were scored based on presence/ absence to produce a binary matrix (1=presence/ 0=absence). Only clear and strong bands were recorded and used for further analysis. Polymorphic information content of the primer was calculated. Pairwise genetic similarity coefficient between individuals were calculated by shared allele distance using PowerMarker v3.25 software.

Total 191 EST-SSR markers derived from *L. cyclindrica* were tested on five genotypes each of *L. acutangula* and *L. hermaphrodita* to assess the transferability of primers across the species. Out of which, 131 numbers of primers amplified in the tested species. These markers were further used for polymorphic profiling across the species and 17 markers gave satisfactory results. Sum total of 17 SSR could amplify 34 alleles, ranging 1-2 loci per primer pair. A high degree of molecular polymorphism was exhibited by all the markers studied. The polymorphic information content ranged from 0.1124 for C87552_ G3 and 0.3750 for C90830_G3. The major allele frequency, gene diversity, heterozygosity and PIC data is presented in the Table 1. Higher the PIC value more will be the usefulness of primer, hence, primer C90830_G3 was found to be highly informative as more number of genotypes can be differentiated by using this primer. Forty seven genotypes were broadly classified into two different clusters as shown in Fig. 1. Cluster I comprised of genotypes DRG-98, Utkal Tripti, DRG-6, Sel-102, Pusa Nasdar, Pusa Nutan, Arka Sujat, DRG-73, DRG-61, DRG-42 and DRG-50. Cluster II comprised of 36 genotypes belonging to *Luffa hermaphrodita*, respectively. The similarity indices among the genotypes within the cluster II were high, suggesting that the genomes of the genotypes do not differ much from each other. The low level of intra-specific diversity could be due to gene flow between them as they are crossable. The narrow genetic diversity within intra-specific varieties has also been reported in *Luffa*. However, all the genotypes clearly differentiated into two clusters, suggesting their distinct taxonomic identity. The diversity at species level could be attributed to its long history of cultivation under varied climatic conditions leading to introgression and selection. Our findings corroborates the findings of Cruz *et al*. (2) and Marr *et al*. (4). Higher the dissimilarity between the genotypes

Table 1. Polymorphic information content of EST-SSR markers used in the analysis.

Marker	Major allele frequency	Gene diversity	Heterozygosity	PIC
C84961_G1	0.6702	0.4421	0.0213	0.3443
C89766_G1	0.7660	0.3585	0.0000	0.2943
C85516_G2	0.6170	0.4726	0.0000	0.3609
C88424_G1	0.7979	0.3225	0.0213	0.2705
C83178_G1	0.8511	0.2535	0.0000	0.2214
C82204_G1	0.7660	0.3585	0.0000	0.2943
C73141_G1	0.5851	0.4855	0.6170	0.3677
C90830_G3	0.5000	0.5000	0.0213	0.3750
C83880_G2	0.7660	0.3585	0.0000	0.2943
C89529_G2	0.9255	0.1378	0.0213	0.1283
C76892_G1	0.8298	0.2825	0.0000	0.2426
C84593_G2	0.7660	0.3585	0.0000	0.2943
C83264_G3	0.9255	0.1378	0.0213	0.1283
C89405_G3	0.5745	0.4889	0.8511	0.3694
C87552_G3	0.9362	0.1195	0.0000	0.1124
C89455_G1	0.7872	0.3350	0.0000	0.2789
C85233_G2	0.5106	0.4998	0.1277	0.3749

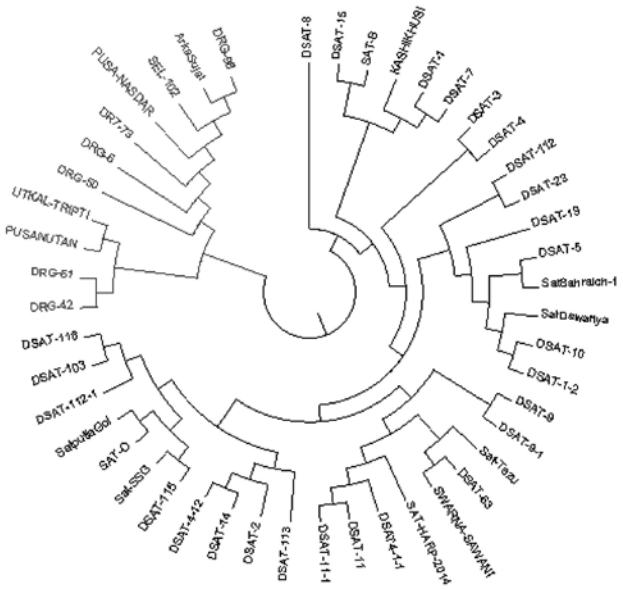


Fig. 1. Cluster diagram of the genotypes of *Luffa* species as obtained from power marker v3.25 software.

better is the scope to include them in a hybridization programme for getting the transgressive segregants. Therefore, to exploit heterosis in *Luffa* the genotypes belonging to different clusters can be used as parent to produce hybrid rather than selecting parents within the cluster. The clustering obtained in this study would be stable even in addition of newer markers and there is less chance of a change in this grouping pattern. The present data provide adequate evidence of the applicability of EST-SSR markers for diversity analyses, cultivar identification and characterization of the *Luffa* germplasm.

REFERENCES

1. Arora, R.K. and Nayar, E.R. 1984. *Wild Relatives of Crop Plants in India*, *NBPGR Monograph No. 7*, 9 p.
2. Cruz, V.M.V., Tolentino, M.I.S., Altoveros, N.C., Villavicencio, M.L.H., Siopongco, L.B., DelaVina, A.C. and Laude, R.P. 1997. Correlations among accessions of Southeast Asian *Luffa* genetic resources and variability estimated by morphological and biochemical methods. *J. Crop. Sci.* **22**: 13-40.
3. Islam, S., Munshi, A.D., Mandal, B., Kumar, R. and Behera, T.K. 2010. Genetics of resistance in *Luffa cylindrica* Roem. against *Tomato leaf curl New Delhi virus*. *Euphytica*, **174**: 83-89.
4. Marr, K.L., Xia, Y.M and Bhattarai, N.K. 2005. Al-lozymic, morphological, and phenological diversity in cultivated *Luffa acutangula* (Cucurbitaceae) from China, Laos and Nepal, and allozyme divergence between *L. acutangula* and *L. aegyptiaca***.** *Econ. Bot*. **59**: 145-65.
5. Singh, D.K., Tewari, R., Singh, N.K. and Singh, S.S. 2016. Genetic diversity in cucumber using Inter Simple Sequence Repeats (ISSR). *Transcriptomics*, **4**: 129.
6. Smith, N.D. and Devey, M.E. 1994. Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genomics*, **37**: 977-83.

Received : January, 2017; Revised : October, 2017; Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00120.7

Indian J. Hort. 74(4), December 2017: 618-622

Short communication

Phosphorus efficient potato cultivars for Nilgiris

Manorama K.*, Govindakrishnan P. and S.S. Lal****

Central Potato Research Station, Muthorai, the Nilgiris 643004, Tamil Nadu

ABSTRACT

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

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

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

Lee *et al*. (7) reported that the cultivar adaptation to low-P stress growing conditions depends on various traits, such as mobilization of insoluble phosphates, utilization of limited bioavailable P sources, and P-uptake efficiency. An elite genotype that can adapt to P-limiting growing conditions needs to be excellent in each of the above traits. If such cultivars are identified and their mechanism is known then it becomes easier to breed varieties with higher P efficiency through improved biotechnological tools. Potato is widely grown in Nilgiris with large doses of P application under lateritic soil conditions. If P efficient cultivars are identified and recommended, it can avoid soil build up of P, thereby eutrophication of water bodies. Hence, the present investigation was carried out.

A field experiment was conducted at Central Potato Research Station, Muthorai, the Nilgiris, Tamil Nadu during 2010 to 2012 for three years by planting seven different potato cultivars under four different levels of P (0, 50, 100 and 150 Kg P_2O_5 per hectare) application. The seven cultivars tried were Kufri Swarna, K. Jyoti, K. Neelima, K. Girdhari, K. Shailja, K. Giriraj and K. Himalini, which differ in their maturity periods. The trials were initiated during summer season (April to August) under rainfed conditions as Nilgiris receive good amount of (800 mm) rainfall during South-West monsoon. The plot size adopted was 2.4 × 2.0 m with four rows of potato having 10 plants in each row (at 60 × 20 cm spacing). Standard cultural practices were followed for interculture and harvesting by cutting the haulms 15 day before harvest. The soil type in experimental plot was sandy loam with high available N and P and medium at K. The soils of Nilgiris are rich in P but the availability is very less because of the transformation of P to Fe and Al phosphates.

Per cent emergence of different cultivars under four levels of P application was estimated after one month of planting. At 45 days, plant height, shoot number and number of leaves per plant were recorded by selecting five plants per plot at random. Shoot weight, root length and root biomass were estimated at 90 days after planting. Tuber number and yield

*ICAR-Indian Institute of Oil palm Research, Pedavegi, Andhra Pradesh; E-mail : kmano1000@yahoo.com

**ICAR-Central Potato Research Institute, Shimla

was estimated in different size grades (<25, 26-50, 51-75 and >75 g) after separation. Tuber yields were recorded net plot wise in all the three years in four different size grades. The total biomass of plants were recorded at 90 days and the tuber yield was at 120 days. Harvest index was calculated in all the varieties at different P levels. P uptake was estimated in different plant parts at 90 days after planting by drawing samples from five plants in each plot. Phosphorus content in tubers was estimated at harvest. Soil samples were analysed for nutrient status using standard procedures before and after the conduct of the experiment. Different nutrient efficiency indices were estimated, *viz.* tuber yield efficiency index, tuber harvest index, agronomic use efficiency (AUE), and phosphorus uptake efficiency (PUE). The pooled data of three years was used to fit quadratic models for yield estimation in seven cultivars and the P_{max} was estimated for standard cv. Kufri Jyoti (Govindakrishnan *et al.*, 4). The yields of different varieties at P_{max} were estimated using the quadratic equations developed.

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

The yield produced by K. Neelima and K. Swarna without P application was higher than all other cultivars even at their highest levels of P application except for K. Girdhari at 100 kg P that too was higher than K. Swarna at zero level of P application. This indicates that these two cultivars are highly native P efficient under Nilgiri conditions. The cultivars K. Swarna (31.3 t/ha) and K. Neelima (32.9 t/ha) produced very high yields under no application of P and the cultivars K. Jyoti, K. Himalini and K. Girdhari responded very well to the application of P at different levels (Tables 1, 2 & 3). Efficiency of the above two cultivars in utilization of soil available P could result in increased tuber yield even at zero level of P application under acidic soil conditions of Nilgiris.

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

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

Cultivar	Pl. ht. (cm)	No. of shoots	No. of leaves	Yield/ net plot (kg)	Tuber No./ net plot	P content in tubers (%)	Plant P conc. (%)	P uptake in stems (kg/ ha)	Stem DMP (t/ ha)	Tuber DMP (t/ ha)	AUE
K. Jyoti	22.76	2.58	17.69	4.49	62	0.2868	0.14	2.02	1.44	4.65	150.13
K. Swarna	33.53	3.05	23.12	6.64	65	0.2988	0.10	2.21	2.21	6.91	222.98
K. Girdhari	24.97	2.87	18.31	4.83	65	0.2955	0.13	2.02	1.56	5.03	162.13
K. Shailja	12.47	1.45	10.85	1.16	19	0.2867	0.14	0.51	0.36	1.21	38.90
K. Himalini	22.73	2.41	15.20	3.58	68	0.2965	0.14	1.57	1.12	3.73	120.39
K. Giriraj	18.01	2.26	14.43	2.78	42	0.2977	0.10	0.90	0.90	2.90	93.42
K. Neelima	31.19	2.69	23.03	6.79	80	0.2913	0.12	2.80	2.33	7.07	228.06
$LSD_{0.05}$	2.711	0.31	2.356	0.607	7	0.0046					
P0	20.31	2.26	15.31	3.59	50	0.2755	0.12	1.39	1.16	3.74	120.77
P50	24.99	2.64	18.43	4.59	61	0.2932	0.13	1.99	1.53	4.78	154.19
P100	25.15	2.58	18.59	4.72	60	0.2993	0.13	2.05	1.57	4.92	158.71
P150	24.21	2.40	17.75	4.37	58	0.3052	0.14	2.10	1.50	4.55	146.84
$LSD_{0.05}$	2.050	0.235	1.781	0.462	5	0.0035					

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

Cultivar	P_0	P_{50}	P_{100}	P_{150}
K. Jyoti	3.3783	4.6433	4.8133	5.0367
K. Swarna	6.0200	6.6583	6.7517	7.1100
K. Girdhari	3.4783	5.0267	6.2700	4.5283
K. Shailja	0.9483	1.4167	1.2467	1.0150
K. Himalini	2.5667	4.1450	3.8367	3.7850
K. Giriraj	2.4383	3.1733	3.0183	2.4900
K. Neelima	6.3250	7.0633	7.1317	6.6283

were intermediate and P responsive. This shows that the cvs. K. Neelima and K. Swarna are highly effective in utilizing the native P.

Harvest index represents conversion efficiency of vegetative source to economical part. The cultivars K. Neelima and K. Swarna recorded the highest values for harvest index indicating that they are the most P efficient and the HI increased with increase in P level upto 150 kg per hectare (Fig. 1). That means these two cultivars are more efficient in converting source into economical parts. Cultivars K. Neelima (228) and K. Swarna (222) had higher agronomic use efficiency (AUE) compared to K. Girdhari (162), K. Jyoti (150) and K. Himalini (120), which showed moderate values. The least AUE values were recorded for K. Shailja (38) and K. Giriraj (93) (Table 1). Earlier, Trehan and Sharma (10) reported that Kufri Pukhraj was the most N, P and K efficient cultivar. The P uptake efficiency indices recorded higher values at no P

Table 3. ANOVA for tuber yield in potato.

Source	df	Type III SS	Mean square	F value	Pr>F
Rep (year)	3	2.4639107	0.8213036	0.73	0.5380
Year	2	154.4015512	77.2007756	68.23	<0.0001
Variety	6	591.4661988	98.5776998	87.12	<0.0001
P level	3	32.1779833	10.7259944	9.48	<0.0001

application in K. Neelima and K. Swarna indicating their efficiency to convert native P to available forms. Other genotypes showed a very low uptake efficiency at no application and the values were lower at higher levels of P application (Fig. 1). The variation in phosphorus efficiency of different potato cultivars was due to both their capability to use absorbed P to produce potato tubers (PUE) and to their capacity to take up more P per unit soil. Trehan and Singh (12) reported that K. Pushkar was more P efficient than K. Pukhraj.

Quadratic models were developed for all the cultivars and from them the optimum P dose was estimated (economic optimum). The tuber yield at economic optimum was 37.7 t/ ha in K. Neelima and 35.0 t/ ha in K. Swarna. The cultivars next in order were K. Girdhari (30.9), K. Jyoti (27.4), K. Himalini, K. Giriraj (16.3) and K. Shailja (6.9) (Table 4). The P_{Max} for standard variety K. Jyoti (135 kg/ha) is estimated using the technique developed by Govindakrishnan *et al.* (4). The yields at P_{Max} and at no P also followed similar trend.

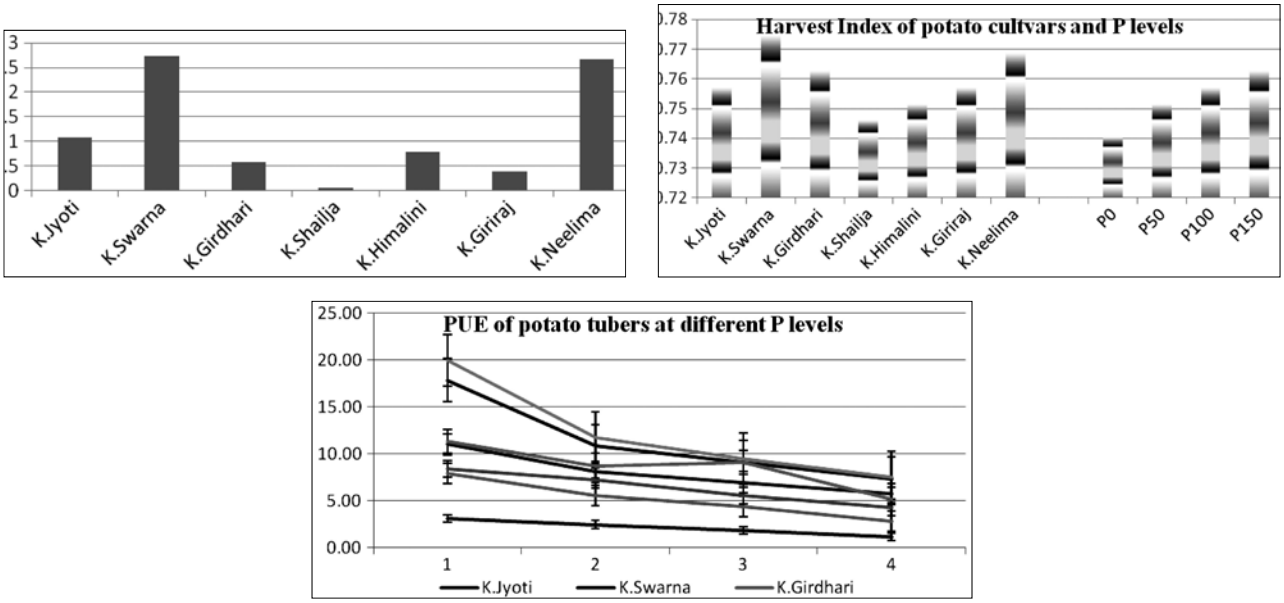


Fig. 1. Tuber yield efficiency, tuber harvest and phosphorus uptake efficiency indices in different potato cultivars.

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

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

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

The root biomass (dry) produced in K. Neelima (3.14 g/ plant) and K. Swarna (3.07 g/ plant) were significantly higher on an average at all the levels of P application substantiating their efficiency in utilizing soil available and applied P resources. Further, these two genotypes are resistant to PCN infection, which makes them maintain healthier roots without any cysts when compared with other cultivars. This could also have been contributed for their better P use efficiency. Lee *et al.* (7) reported that 'Harley Blackwell' and 'Satina' cultivars to show greater P mobilization ability in soils without supplemental P. The ability to uptake more P from soil available level made the cultivars K. Neelima and K. Swarna more efficient in producing better tuber yields in comparison with other inefficient cultivars. Nutrient efficient plants are defined as those plants, which produce higher yields per unit of nutrient, supplied or absorbed than other plants (standards) under similar agro-ecological conditions (Trehan and Singh, 12). The main properties that affect the uptake of nutrients from soil are kinetics of ion absorption by roots, the size of root system and morphological root properties as reported by Jungk and Claassen (6). Gahoonia (3) also reported that phosphate availability could be influenced by root induced changes of soil pH. Investigations are required to confirm the actual reasons for P efficiency.

Different indices were estimated to evaluate their efficiency and it is concluded that among the seven cultivars tested Kufri Neelima and Kufri Swarna are the most P efficient cultivars under Nilgiri conditions where the soils are lateritic with more Fe and Al phosphates. The P efficient cultivars maintained higher vegetative biomass from the initial stages itself and higher root biomass in comparison with others. The higher root volume of these cultivars has contributed for better uptake and thereby enhanced yields even under P deficient (No P) conditions. These cultivars possess resistance to PCN, which is also a positive factor for their higher root activity and there by better utilization of available P. Mineral P resources are dwindling very fast and use of P efficient cultivars is an alternative. Potato tubers are highly correlated with P fertilization. Further studies are required to understand the root morphology and the mechanism of P efficient varieties in utilizing P in a better way for producing more tuber yield. Further, the cultivars K. Girdhari, K. Jyoti and K. Himalini responded greatly to the application of P and proved better P responsive.

REFERENCES

1. Anuradha, M. and Narayanan, A. 1991. Promotion of root elongation by phosphorus deficiency. *Plant Soil*, **136**: 272-75.

2. Fist, A.J. 1987. Regulation of phosphorus transport in tropical grain legumes. Ph.D. thesis, University of Queensland, St. Lucia, Australia.

3. Gahoonia, T.S. 1987. Phosphate availability as influenced by root induced changes of soil pH. Ph.D. thesis. University of Goethingen, Germany.

4. Govindakrishnan, P.M., Sharma R.C., Khan, I.A. and Sharma, H.C. 1999. A technique for selecting potato (*Solanum tuberosum*) genotypes with low nitrogen requirement. *Indian J. Agric. Sci.* **69**: 350-54.

5. Jungk, A., Asher, C.J., Edwards, D.G. and Meyer, D. 1990. Influence of phosphate status on phosphate uptake kinetics of maize and soybean. *Plant Soil*, **124**: 175-82.

6. Jungk, A. and Claassen, N.1997. Ion diffusion in the soil-root system. *Adv. Agron*. **61**: 53-110.

7. Lee, W.C., Guodong, L. and Ashok, K. Alva. 2013. Potato cultivar evaluation for phosphorus-use efficiency. *J. Crop Improv.* **27**: 617-26.

8. Miyasaka, S.C. and Habte, M. 2001. Plant mechanisms and mycorrhizal symbioses to increase phosphorus uptake efficiency. *Comm. Soil Sci. Plant Anal*. **32**: 1101-47.

9. Smil, V. 2000. Phosphorus in the environment: Natural flows and human interferences *Ann. Rev. Energ. Env*. **25**: 53-88.

10. Trehan, S.P. and Sharma, R.C. 2003. Root-shoot ratio as an indicator of zinc uptake efficiency of different potato cultivars. *Comm. Soil Sci. Pl. Anal.* **34**: 919-32.

11. Trehan, S.P. and Sharma, R.C. 2000. Phosphorus and zinc uptake efficiency of potato (*Solanum tuberosum*) in comparison to wheat (*Triticum aestivum*), maize (*Zea mays*) and sunflower (*Helianthus annuus*). *Indian J. Agric. Sci.* **70**: 840-45.

12. Trehan, S.P. and Singh, B.P. 2013 Nutrient efficiency of different crop species and potato varieties - in retrospect and prospect. *Potato J*. **40**: 1-21.

Received : October, 2016; Revised : November, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00121.9

Indian J. Hort. 74(4), December 2017: 623-626

Short communication

Energy requirements for attainment of different phenological stages in broccoli inbreds

S.K. Dhankhar* and Chandanshive Aniket V.

Department of Vegetable Science, CCS Haryana Agricultural University, Hisar 125004

ABSTRACT

The field experiment comprised of three broccoli inbreds was carried out to assess the requirement of thermal energy, helio-thermal units, photo-thermal units, relative temperature, disparity and thermal use efficiency for days to 50% head initiation, days to 50% head maturity and duration of head maturity at CCS Haryana Agricultural University, Hisar during winter seasons of 2013 and 2014. The results revealed that broccoli crop in general required 813.7, 168.1 and 981.8 (°C day h) thermal units, 5161.1, 705.4 and 5866.5 (°C day h) heilo-thermal units, 8600.7, 1713.9 and 10314.5 (°C day h) photo-thermal units and 4042.2, 1996.6 and 6038.8 (°C day h) relative temperature disparity for head initiation, duration of head maturity and head maturity. Minimum thermal use efficiency (14.3°C day h) was measured for days to 50% head maturity followed by days to 50% head initiation (17.30°C day h) and duration of head maturity (83.44°C day h). Genotypic differences also observed for requirement of thermal energy in attainment of different phenological traits in broccoli. Marketable curd yield expressed significant positive correlation with heat unit consumed in both years. The R^2 values of regression equation could explain the variation between 72-81% of the total variation in production of curd in broccoli.

Key words: Broccoli, heat unit, inbred, thermal use efficiency, thermal requirement.

Broccoli (*Brassica oleracea* L. var. *italica* Plenck), belongs to the family Cruciferae, closely resemble to cauliflower bears large flower head of green colour is generally used as a cooked vegetable. It needs 12-16°C for seed germination, 18-23°C for plant growth and 12-18°C for head development. A minimum of six hours of sunlight needed for its successful cultivation. Varieties roughly divided into early, mid, season and late season groups, respectively. Early maturing varieties are more sensitive to low temperature than the late maturing varieties. Long days and hot weather conditions during summer cause the broccoli to bolt. (Wang, 14) reported that the duration of a particular stage of growth were directly related to temperature, which could be predicted for a particular species by using sum of daily air temperature or cumulative thermal requirement, known as growing degree-days (GDD) or heat units (HU). Growing degree days are a measure of heat accumulation used to predict phenological development rates such as the date that a flower will open or a crop will reach maturity and further to explore the agro-climatic potential of a region (Pandey and Shekh, 10). Thus, air temperature based on agro-meteorological indices like growing degree-days, helio-thermal units, photo-thermal units and the thermal or heat use efficiency (Hundal *et al*., 6) were successfully used for describing phenological behaviour and growth parameters. Gouri *et al*. (5) found that the crop growth response largely influenced by crop microclimate environment, which varies from top of the canopy to the soil surface and affects crop development and yield. The environmental factors influencing growth are interception of photo-synthetically active radiation and temperature but light plays a key role in influencing crop production. The occurrence of different phenological events during the crop growth period in relation to temperature estimated by using accumulated heat units or growing degree-days. Thermal time is an independent variable to describe plant development (Dwyer and Stewart, 3), which used as a tool for characterizing thermal responses in different crops. Knowledge of accumulated growing degree-days can provide an estimate of harvest date as well as crop development stage (Bonhomme, 1). Hence, the present field experiment was conducted to study energy requirement for initiation of head and its maturity/marketable crud yield.

The experimental material consisted of three inbred broccoli lines, *viz*., GH-1, PH-1 and DPH-1. The experiment was conducted during winter seasons of 2013 and 2014 at Research Farm, Department of Vegetable Science, CCS HAU, Hisar situated 29°10'N; 75°46'E and 215 above sea level to study the thermal requirement for days to 50% head initiation, days to 50% head maturity and duration of head maturity. Seedlings of three inbred lines were transplanted on 20th October of 2013 and 2014 in

*Corresponding author's E-mail: dhankharsk@gmail.com

randomized block design with four replications at spacing of 50 cm between rows and 50 cm between plants. All the agronomical practices were adopted to raise a healthy crop. The observations were taken on three phenological stages (traits), *viz*., days to 50% curd initiation (number of days taken from date of planting to dates when 50% plant shows head/curd initiation in each inbred), days to 50% curd maturity (number of days taken from date of planting to dates when days 50% plants show marketable head/ curd in each inbred) and duration of head maturity (number of days taken from 50% curd initiation to 50% head maturity) were recorded in both the years. The yield of marketable curd was also recorded (q/ ha) for each inbred used for correlation study with weather variables. The data pooled over the year 2013 and 2014 for statistical analysis. The daily agro-meteorological data recorded at CCS HAU, Hisar was used for the calculation of thermal requirement for days taken to 50% head initiation, days to 50% head maturity and duration of head maturity in broccoli. Different agro-meteorological indices like growing degree-days (GDD), helio-thermal units (HTU), photo-thermal units (PTU), relative temperature disparity (RTD) and thermal use efficiency (HUE) were calculated on daily basis and summations were made for different stages separately from sowing to 50% harvesting of heads.

Thermal units or growing degree days (GDD) were calculated by taking the average of daily maximum and minimum temperature and subtracting a base temperature, T_{base} (usually 5°C for winter season crops) as suggested by (Monteith, 9):

$$GDD = \frac{T_{max} + T_{min}}{2} - T_{base}$$

Similarly, helio-thermal units, photo-thermal units and relative temperature disparity were calculated using the following procedures:

HTU (°C day h) = GDD × Duration of bright sunshine hours (Rajput, 11)

PTU (°C day h) = GDD × Day length (h) (Major *et al*., 8)

$$RTD\ (°C\ day\ h) = \frac{(T_{max} - T_{min})}{T_{max}} \times 100 \quad \text{(Rajput, 11)}$$

The thermal use efficiency was calculated (kg/ ha per degree) to compare the relative performance of different genotypes with respect to utilization of heat by using the formula given below:

$$\text{Thermal use efficiency (TUE)} = \frac{\text{Head fresh wt. (kg /ha)}}{\text{GDD (°C day)}}$$

The correlation and predictive regression equation were also work out between thermal indices and curd yield of different inbred of broccoli.

Average thermal requirements of three inbred of broccoli for days to 50% head initiation (Table 1), days to 50% head maturity (Table 2) and duration of head maturity presented in (Table 3), respectively. Thermal units required to attain different stages varied with inbred lines. Among all the inbred lines, the maximum mean thermal units were needed by inbred GH-1 (819.2 day°C) followed by PH-1 (813.6 day°C) and DPH-1 (808.4 day°C) for reaching a stage of 50% head initiation (Table 1). The inbred DPH-1 used the lowest accumulated thermal units of 970.6 day°C for days to 50% head maturity, whereas, the inbred GH-1 required the highest amount of accumulated thermal units (992.5 day°C) followed by PH-1 (982.4 day°C) for this trait (Table 2). Similar trend were recorded for the duration of head maturity among all the inbreds. This might due to genotypic differences for consumption of thermal units to a trait attainment as Dhankhar and Singh (3) reported in okra.

Helio-thermal units (HTU) required for different stages presented as the inbred GH-1 utilized the highest amount of accumulated helio-thermal units for all the character studied *i.e*., days to 50% head initiation (5195.5 day°C h) (Table 1), days to 50% head maturity (5936.1°C day h) (Table 2) and duration of head maturity (740.6°C day) (Table 3). The lowest helio-thermal unit utilization value was observed in inbred DPH-1 for all three traits. All inbreds required less helio-thermal units for duration of head maturity followed by days to 50% head initiation and days to 50% head maturity. This might be due to the more number of days and growing degree-days taken by the respective traits to complete stage. The results

Table 1. Average requirement of agro-meteorological indices for different growth stages of broccoli inbreds.

Inbred	Days to 50% head initiation				
	GDD (°C day)	HTU (°C day h)	PTU (°C day h)	RTD (°C day h)	HUE
DPH-1	808.4	5129.3	8557.3	3946.0	19.59
PH-1	813.6	5158.5	8579.5	4042.8	18.15
GH-1	819.2	5195.5	8665.3	4137.9	14.18
Mean	813.7	5161.1	8600.7	4042.2	17.30
CD at 5%	4.95	8.14	5.75	9.31	2.73

Table 2. Average requirement of agro-meteorological indices for different growth stages of broccoli inbreds.

Inbred	Days to 50% head maturity				
	GDD (°C day)	HTU (°C day h)	PTU (°C day h)	RTD (°C day h)	HUE
DPH-1	970.6	5797.6	10214.9	5876.3	16.30
PH-1	982.4	5865.7	10287.1	6034.9	14.83
GH-1	992.5	5936.1	10441.5	6205.2	11.81
Mean	981.8	5866.5	10314.5	6038.8	14.31
CD at 5%	7.10	7.41	5.54	7.03	2.87

Table 3. Average requirement of agro-meteorological indices for different growth stages of broccoli inbreds.

Inbred	Duration of head maturity				
	GDD (°C day)	HTU (°C day h)	PTU (°C day h)	RTD (°C day h)	HUE
DPH-1	162.2	668.4	1657.6	1930.3	93.36
PH-1	168.9	707.2	1707.6	1992.1	86.26
GH-1	173.3	740.6	1776.2	2067.4	70.71
Mean	168.1	705.4	1713.9	1996.6	83.44
CD at 5%	4.22	7.65	8.79	8.27	6.23

are in line with the findings of Kumar *et al*. (7) in stover. The maximum their photo-thermal units were required by the inbred line GH-1 for attaining the stages of days to 50% head initiation (8665.3°C day h) (Table 1), duration of head maturity (1776.2°C day h) (Table 3) and days to 50% head maturity (10441.5°C day h) (Table 2). The minimum thermal units were accumulated by the inbred line DPH-1 for all the traits studied. The present results are in conformity with the results of Singh *et al*. (13) and Ram *et al*. (12) who observed that the photoperiodic and its interactions directly affect the phenological stages in wheat.

Relative temperature disparity value is dependent on maximum and minimum temperature during the different phenological stages of inbred lines. Among all the inbreds, maximum value of relative temperature disparity was utilized by inbred GH-1 for all the traits like days to 50% head initiation (4137.9°C day h) (Table 1), duration of head maturity (2067.4°C day h) (Table 3) and days to 50% head maturity (6205.2°C day h) (Table 2). The highest value of relative temperature disparity was used by the trait days to 50% head maturity (6038.8°C day h) (Table 2) followed by days to 50% head initiation (4042.2°C day h) (Table 1) and duration of head maturity (1996.6°C day h) (Table 3) to reach their stages. This might be due to the number of days taken by the respective traits to attain their stage in indices of white clover (Fyffe *et al*., 4; Kumar *et al*., 7).

Among these three inbreds, the maximum thermal use efficiency was used by inbred DPH-1 also followed by PH-1 and GH-1 for the all the traits. Minimum thermal use efficiency utilized by the trait days to 50% head maturity (14.31) (Table 2) followed by days to 50% head initiation (17.30) (Table 1) and duration of head maturity (83.44) (Table 3), respectively. The efficiency of thermal energy conversion for curd yield depends upon the genetic makeup of the genotype, time of head initiation and maturity of the inbred for its accomplishment of different stages. Dhankhar and Singh (2) also studied on thermal use efficiency in okra and observed genotypic differences for consumption of thermal units, respectively.

Regression model was developed for curd yield prediction in broccoli using curd yield of the inbreds for every year and pooled data along with accumulated thermal units by inbreds during the period of trial. Thermal units utilized by inbred for the 50% head maturity period were used for simple correlation and regression studies. The thermal units consumed for curd yield in each year expressed significant positive association values (0.88 and 0.77), with curd yield, whereas pooled data indicated 92 percent deviation in curd yield due to accumulated thermal use efficiency. The estimation of regression models (Table 4), which reveal that the model accounted for 81 and 72 percent of total variations as revealed by R^2 values in curd yield for first and second year, respectively, whereas, the pooled data showed 79 percent variability in curd yield due to the utilization of thermal unit.

There was genotypic difference for the requirement of thermal unit, helio-thermal unit, photo-thermal

Table 4. Regression equation for curd yield prediction in broccoli.

Year	Regression equation	R^2 value	SE	Correlation with curd yield
1st	Y = -354.83 + 0.482TU	81	0.05	0.88**
2nd	Y = -364.56 + 0.498TU	72	0.07	0.77**
Pooled	Y = -378.81 + 0.509TU	79	0.04	0.92**

unit, relative temperature disparity and thermal use efficiency for head initiation, head maturity and duration of head maturity. However, broccoli in common require 813.7 and 981.8 day°C h thermal units, 5161.1 and 5866.5 day°C h, helio-thermal units, 8600.7 and 10314.5 day°C h, photo-thermal units 4042.2 and 6038.8 day°C h, relative temperature disparity 17.3 and 14.3 thermal use efficiency for head initiation and head maturity, respectively. Curd production showed the evidence of positive relationship with thermal unit consumption. R^2 values of regression equation of pooled data could explain 79 percent variability of curd yield in broccoli under Hisar conditions. Thus, different agro-meteorological indices requirement and their efficient utilization by broccoli inbreds can be used to predict the performance of a crop to breed or introduce boccoli genotype in a particular region depending upon the thermal environment.

REFERENCES

1. Bonhomme, R. 2000. Basis and limits to using 'degree- days' units. *European J. Agron.* **13**: 1-10.
2. Dhankhar, S.K. and Singh, S. 2013. Thermal requirement for flowering and fruit yield attainment in advance lines of okra. *J. Agrometeor.* **15**: 39-42.
3. Dwyer, L.M. and Stewart, D.W. 1986. Leaf area development in field-grown maize. *Agron. J.* **78**: 334-48.
4. Fyffe, D.C. and Titley, M.E. 1989. Phenology studies and the prediction of harvest dates of broccoli in the Lockyer valley. *Acta Hort.* **247**: 53-58.
5. Gouri, V., Reddy, D.R., Rao, S.B.S.N. and Rao, A.Y. 2005. Thermal requirement of rabi groundnut in southern Telangana zone of Andhra Pradesh. *J. Agrometeor.* **7**: 90-94.
6. Hundal, S.S., Singh, H., Kaur, P. and Dhaliwal, L.K. 2003. Relationship between agro-climatic indices. *Indian J. Agric. Sci.* **73**: 668-70.
7. Kumar, R., Kaundal, M., Vats, S.K. and Kumar, S. 2012. Agrometeorological indices of white clover (*Trifolium repens*) in Western Himalayas. *J. Agrometeor.* **14**: 138-42.
8. Major, D.J., Johnson, D.R., Tanner, J.W. and Anderson, I.C. 1975. Effect of day length and temperature on soybean development. *Crop Sci.* **15**: 174-79.
9. Monteith, J.L. 1984. Consistency and convenience in the choice of units for agriculture science. *Exp. Agric.* **20**: 105-17.
10. Pandey, V. and Shekh, A.M. 1999. Assessment of agroclimatic potentials of north Gujarat agroclimatic zone. *J. Agrometeor.* **1**: 89-92.
11. Rajput, R.P. 1980. Response of soybean crop to climatic and soil environments. Ph.D. thesis, IARI, New Delhi
12. Ram, H., Singh, G., Mavi, G.S. and Sohu, V.S. 2012. Accumulated heat unit requirement and yield of irrigated wheat (*Triticum aestivum* L.) varieties under different crop growing environment in central Punjab. *J. Agrometeor.* **14**: 147-53.
13. Singh, A.K., Mishra, A.N. and Tripathi, P. 2012. Thermal regime requirement and plant responses of chickpea cultivars under variable weather conditions. *J. Agrometeor.* **14**: 67-69.
14. Wang, J.Y. 1960. A critique of the heat units approach to plant response studies. *Ecol.* **40**: 785-90.

Received : December, 2016; Revised : August, 2017;
Accepted : September, 2017

DOI : 10.5958/0974-0112.2017.00122.0

Indian J. Hort. 74(4), December 2017: 627-630

Short communication

Influence of particle size on rheological properties of mango peel powder

Manoj Mahawar*, Kirti Jalgaonkar, V.S. Meena, V. Eyarkai Nambi, Bharat Bhushan and Pankaj K. Kannaujia**

Horticultural Crop Processing Division, ICAR-Central Institute of Postharvest Engineering and Technology, Abohar 141004, Punjab

ABSTRACT

This study was envisaged to investigate the effect of particle size on the rheological characteristics of mango cv. Bangalora peel powder under ambient conditions using a rotational rheometer. The consequences of sample concentration, *i.e.*, powder: water (1:4, 1:5, 1:6) was also evaluated. Sieve analysis using screens (420, 355, 250, 125 microns) was done to achieve the desired variation in particle size. Shear stress-shear rate data was adequately fitted to Herschel-Bulkey, Power law and Casson law and the rheological properties were represented graphically by rheograms for 0 to 100 s^{-1} shear rate. Relative parameters and regression analysis against each model determined the flow characteristics of the samples. Selected three models showed well representation of the rheological data, with high regression coefficients. Model parameters (consistency coefficient, flow behaviour index and R^2) validated the deviation in rheological characteristics with particle size and sample concentration. Shear stress (τ) increases steeply with sample concentration for equivalent particle size, however, for the same sample concentration, negative correlation of particle size with shear stress was reported. The identification of suitable particle size and sample concentration might be useful in understanding of rheological properties of the peel powder of selected cultivar.

Key words: Mango, particle size, peel powder, rheological properties.

Mango (*Mangifera indica* L.) occupies prominent position in the world market, being among the main fruits of immense commercial importance. Mango peel which constitutes about 15-20% of the fruit (Beerh *et al*., 5) is rich in dietary fibre, pectin, carotenoids, polyphenols, antioxidants and enzymes (Ajila *et al*., 3; Kim *et al*., 9; Koubala *et al*., 10). To explore the possibility of using mango peel in food processing operations, it becomes quite neccesary to understand its rheological properties with respect to particle size. The rheological behaviour of any material may vary as a function of concentration, temperature, particle size, concentration of solute, molecular weight, pressure, suspended matter, processing conditions and the addition of additives *etc*. (Bourne, 6; Kumar *et al*., 11). Flow behaviour of liquid food samples can be very well described using different rheological models (Power law, Casson law and Power law with yield stress (Herschel-Bulkley) depending upon its characteristics (Vandresan *et al*., 16). Despite of its importance for food quality, any systematic study does not correlates with the relationship of particle size distribution and rheology of fruit peel powder suspensions. Hence, this study was envisaged to investigate the effect of particle size and sample concentration on the rheological properties of mango cv. Bangalora peel powder.

*Corresponding author's E-mail: manojmahawar362@gmail.com.
**Transfer of Technology Division, ICAR-CIPHET, Ludhiana (Punjab).

Fresh and fully ripened fruits of mango cv. Bangalora were obtained from the orchard of Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Fruits were manually peeled with a knife and further peels were washed to remove the pulp and dirt substance adhered. Thereafter, peels were subjected to drying using solar dryer initially (maximum temperature: 40 ± 2°C with 70% RH) to remove significant amount of moisture. Further, these samples were kept in tray dryer (50 ± 3°C for 12 h) until the final moisture content reaches 12-14% (w.b.). Later on, dried peel was grounded into fine powder using laboratory grinder (Phillips India, 750 W).

During powder making of peel, particles having broad variation in size, shape and surface characteristics were formed. Therefore, it becomes quite difficult for better understanding of the rationale behind variation in rheological properties as a function of particle size. Sieve analysis was performed to obtain a uniform size range of particles which helped in demonstrating the particle size dependency on the rheological properties. The powder was sieved using sieve shaker and separated into four different fractions 420 µ (A), 355 µ (B), 250 µ (C) and 125 µ (D). Samples were collected and packed in zip lock covers, wrapped in aluminium foil and kept in refrigerator at 4-6°C. The required sample amount

was taken as per the need. Further, for better understanding of viscosity related properties, test samples with 1:4, 1:5 and 1:6 concentration (powder: water) were prepared in triplicate and subjected for evaluation on rheometer. The moisture content of fresh as well as dried peels was determined using hot air-oven method (130 ± 5°C for 1 h) until it reaches to constant weight. Peel powder (1 g) was mixed with water to make the ratio of powder: water as 1:3, 1:4, 1:5, 1:6, 1:7 and 1:8 and checked for flowability and consistency. Samples (1:4, 1:5 and 1:6) were observed to contain desired flow characteristics, hence, selected for further experimentation in order to evaluate their rheological behaviour.

Rheological measurements were made *via* Rheometer make Antonpaar, model Rheoplus /32 MCR 52 (Probe: PP50, Diameter 49.984 mm, Part No. 79045) parallel plates with the measuring system PP50-SN26252 and measuring cell P-PTD 200/ AIR- SN80967699. All the measurements were taken at 33 ± 1°C. A sample of approximately 10 ml was placed in the sample space of rheometer for measurement. Usually, Power Law is used to indicate pseudoplasticity due to dissolved solids, through the fluid behaviour index *n* (Pelegrine *et al*., 14). The shearing rheological behaviour of a fluid is represented by a straight line in power law (Keshani *et al*., 8). It can be described as τ = Kγn. Where τ is the shear stress (Pa); γ the shear rate (s^{-1}); K the consistency coefficient (Pa s^n) and *n* the flow behaviour index of product. Two more parameters ('a' and 'b') associated with Power law model analysis were referred to be as regression parameters. Casson proposed the (τ)^0.5 = (τoc)^0.5 + Kc (γ)^0.5 following expression which was used to demonstrate the effect of suspended material (Pelegrine *et al*., 14). Herschel-Bulkley expressed τ = τo + Kγ^n equation. τ_o and τ_{oc} are yield stress (Pa) and Casson yield ($Pa^{0.5}$) respectively, γ is shear rate (s^{-1}), K is the consistency coefficient (Pa s^n), Kc is Casson constant (Pa s)$^{0.5}$ and n is flow behaviour index (dimensionless).

The models, *viz*., Herschel-Bulkley, Power and Casson laws were used as a tool for estimation of the relationship between shear stress and shear rate. The estimated values of the model parameters helped in characterisation of the flow behaviour of tested samples. The data obtained during rheological examination was subjected to model analysis and regression coefficients were recorded and compared. Various other parameters like flow behaviour index, consistency coefficient, regression parameters were also observed. Values of 'n' less than unity indicates a shear-thinning behaviour, while values greater than unity suggest a shear-thickening behaviour (Al-Malah *et al*., 4). Model with highest R^2 value was found suitable in depicting the adequate rheological behaviour of samples. Peel (fresh and dried) and peel powder were subjected to moisture analysis so as to determine the moisture percentage prior to and subsequent to drying. The moisture content of fresh peel, dried peel and peel powder samples were found to be 83.83 ± 0.37, 12.70 ± 1.25 and 12.48 ± 0.53% (w.b.), respectively.

The model parameters and regression analysis (R^2 values) of all three models used were depicted and compared for the sample with desired characteristics. The parameters related to model analysis as well as regression coefficient values after model interpretation are given in Table 1 (Herschel-Bulkey), Table 2 (Power law) and Table 3 (Casson law), respectively. $R^2 \geq 0.90$ was considered as criteria for judging a sample with required rheological features. Overall, it has been observed that the shear stress (τ) values were positively correlated with sample concentration and the same has been substantiated by model analysis.

The rheological behaviour of suspensions is affected by the sample particle size along with its distribution. Mango peel contains around 10-15% pectin on dry basis (Beerh *et al*., 5) and pectin acts as a thickener for food materials (Mesbahi *et al*., 12). Due to particle size reduction of peel powder, availability of soluble pectin increases and that might be the reason for significant increase in viscosity of the finer particle size samples. This phenomenon contributed for higher shear stress values at higher sample concentration.

Table 1. Model fitting, parameters and regression analysis through Herschel-Bulkey law.

Sample	τ (Pa)	K (Pa s^n)	n	R^2
A16	90 ± 1.90	9.86 ± 1.59	0.33	0.972
A15	2036 ± 8.65	-	0.01	0.572
A14	4937.50 ± 17.34	-	0.01	0.701
B16	64.71 ± 1.77	8.57 ± 1.42	0.57	0.996
B15	190.10 ± 1.16	9.76 ± 2.18	0.70	0.999
B14	543.82 ± 4.50	10.43 ± 4.17	0.92	0.995
C16	48.08 ± 0.34	11.68 ± 4.69	0.46	0.999
C15	115.43 ± 0.48	14.46 ± 1.65	0.57	0.999
C14	345.41 ± 3.95	60.07 ± 6.38	0.62	0.997
D16	25.47 ± 0.21	28.55 ± 7.37	0.44	0.999
D15	49.08 ± 0.27	37.44 ± 9.0	0.55	0.999
D14	101.41 ± 3.62	69.07 ± 12.86	0.64	0.997

All values are average calculated from 3 replications of each sample.

Table 2. Model fitting, parameters and regression analysis through Power law.

Sample	a	b	R^2	SD
A16	80.33 ± 23.27	0.18 ± 0.07	0.966	2.30
A15	391.27 ± 15.17	-0.01 ± 0.014	0.579	8.58
A14	738.04 ± 7.54	-0.05 ± 0.081	0.715	16.93
B16	48.98 ± 1.03	0.32 ± 0.008	0.992	2.55
B15	118.79 ± 4.13	0.27 ±0.009	0.985	6.64
B14	361.81 ± 25.07	0.16 ±0.010	0.936	17.22
C16	37.98 ± 4.83	0.35 ± 0.016	0.995	1.79
C15	76.77 ± 6.74	0.33 ± 0.005	0.993	4.19
C14	302.89 ± 36.42	0.22 ± 0.016	0.993	7.20
D16	42.88 ± 6.68	0.39 ± 0.011	0.999	0.85
D15	43.46 ± 7.68	0.37 ± 0.009	0.997	2.14
D14	133.20 ± 8.40	0.32 ± 0.028	0.996	5.03

Where, 'a' and 'b' are the regression parameters related to Power law model.
SD values are related to shear stress (τ).
All values are average calculated from 3 replications of each sample.

Table 3. Model fitting, parameters and regression analysis through Casson law.

Sample	τ (Pa)	a	B	R^2
A16	100.53 ± 2.36	9.96 ±1.16	0.34 ± 0.12	0.962
A15	354.65 ± 9.18	18.83 ± 0.19	-0.14 ± 0.04	0.515
A14	635.17 ± 19.43	25.20 ± 0.04	-0.27 ± 0.04	0.789
B16	71.09 ± 1.53	8.43 ± 0.08	0.63 ± 0.02	0.996
B15	166.09 ± 1.41	12.89 ± 0.18	0.68 ± 0.03	0.999
B14	451.29 ± 9.32	21.24 ± 0.58	0.78 ± 0.03	0.980
C16	64.73 ± 1.23	7.47 ± 0.46	0.64 ± 0.03	0.998
C15	112.65 ± 1.04	10.60 ± 0.48	0.84 ± 0.01	0.999
C14	404.47 ± 6.20	20.08 ± 1.06	0.91 ± 0.04	0.995
D16	55.95 ± 2.74	8.01 ± 0.61	0.76 ± 0.02	0.994
D15	64.33 ± 1.89	8.02 ± 0.33	0.85 ± 0.01	0.998
D14	193.89 ± 8.11	13.92 ± 0.33	1.08 ± 0.11	0.991

All values are average calculated from 3 replications of each sample.

Reduction in the average particle size of peel powder samples having similar sample concentration, decline in shear stress (τ) was reported. This may be due to the fact that particle size reduction attributed towards an increase in surface area, which helped in binding up of water molecules during rehydration. This helped in making the resultant sample homogeneously viscous and thus shear stress values decreased. Similar results were reported for apple puree (Espinosa *et al*., 7), for chest nut flour (Moreira *et al*., 13; Ahmed *et al*., 2). On the contrary, disruption of steady state properties was observed in the samples with larger particles. This was probably because of the sedimentation promoted by the instability of the internal network due to shear as also reported by Sato *et al*. (16) for jaboticaba pulp. The detailed model analysis has been represented in the forthcoming section.

Shear stress gains positively with increasing sample concentration with values ranged between 25.47 ± 0.21 to 4937.50 ± 17.34 Pa (Table 1). This trend was due to the fact that force per unit area requirement will be more for coarser particle size samples as compared to finer and homogeneously viscous samples. On the other hand, 'τ' reduces with decrease in particle size, which may be because; lesser force was required to disrupt sample structure having finer particle size. As flow behaviour index 'n' values ranged between 0.01 and 0.92, shear thinning and pseudoplastic nature for all peel powder samples was indicated. The results are in conformity with previous results obtained by Pelegrine *et al*. (14) for pineapple and mango pulps and Ahmed (1) for ginger paste.

Results showed reasonably good fitting to power law model with R^2 values 0.936 to 0.999 except for samples of 420 microns sieve. Value of regression parameter 'a' was within 37.98 and 738.04, while for 'b' it was between -0.05 to 0.39. Shear thinning flow behaviour was depicted for all samples, where n<1. Higher regression coefficient values ($R^2 \geq 0.962$) were obtained for all samples except for undersize of 420 microns sieve and this showed reasonably good fitting of data pertaining to this model. From the model interpretation, it was clear that peel powder samples have rheological characteristics over the whole sample concentration and particle size range selected. Peel powder except, which got passed through IS 420 microns, rest of the samples justified for the rheological characteristics.

The present study indicated that mango peel powder possesses rheological characteristics which differs with selected combination of sample concentration and particle size. Herschel-Bulkley, Power law and Casson models were suitable in representing the flow behaviour of the tested samples. Shear stress (τ) values were positively correlated with sample concentration and the same was indicated by model analysis. Both consistency indices and flow indices (Herschel-Bulkley law) increased with increase in sample concentration. Regression parameter 'a' and regression parameter 'b' pertaining to Power law and Casson law increased and decreased with sample concentration, respectively. With the decrease in average particle

size of peel powder samples (with similar sample concentration), reduction in shear stress (τ) values was reported. As evident from model simulation and regression analysis, peel powder samples of size 420 microns were not found appropriate for further possible rheological related studies.

ACKNOWLEDGMENTS

The first author is thankful to Director, ICAR-CIPHET, Ludhiana and Department of Food & Agricultural Process Engineering, Tamil Nadu Agricultural University, Coimbatore for facilities.

REFERENCES

1. Ahmed, J. 2004. Rheological behaviour and colour changes of ginger paste during storage. *Int. J. Food Sci. Tech.* **39**: 325-30.

2. Ahmed, J., Attar, H. and Arfat, Y.A. 2016. Effect of particle size on compositional, functional, pasting and rheological properties of commercial water chestnut flour. *Food Hydrocolloids*, **52**: 888-95.

3. Ajila, C.M., Naidu, K.A., Bhat, S.G. and Prasada Rao, U.J.S. 2007. Bioactive compounds and antioxidant potential of mango peel extract. *Food Chem*. **105**: 982-88.

4. Al-Malah, K.I., Azzam, M.O.J. and Abu-Jdayil, B. 2000. Effect of glucose concentration on the rheological properties of wheat-starch dispersions. *Food Hydrocolloids*, **14**: 491-96.

5. Beerh, O.P., Raghuramaiah, B. and Krishnamurthy, G.V. 1976. Utilizaan of mango waste: Peel as a source of pectin. *J. Food Sci. Tech.* **13**: 96-97.

6. Bourne, M.C. 2002. *Food texture and Viscosity: Concept and Measurement*, Academic Press, London.

7. Espinosa, L., Toa, N., Symoneaux, Renard, C.N.G.C., Biau, N. and Cuveliera, G. 2011. Effect of processing on rheological, structural and sensory properties of apple puree. *Procedia Food Sci*. **1**: 513-20.

8. Keshani, S., Chuah, A.L. and Russly, A.R. 2012. Effect of temperature and concentration on rheological properties pomelo juice concentrates. *Int. Food Res. J.* **19**: 553-62.

9. Kim, H., Moon, J.Y., Kim, H., Lee, D.S., Cho, M., Choi, H.K., Kim, Y.S., Mosaddik, A. and Cho, S.K. 2010. Antioxidant and antiproliferative activities of mango (*Mangifera indica L.*) flesh and peel. *Food Chem*. **121**: 429-36.

10. Koubala, B.B., Kansci, G., Mborne, L.I., Crepeau, M.J., Thibault, J.F. and Ralet, M.C. 2008. Effect of extraction conditions on some physicochemical characteristics of pectins from 'Amellioree' and 'Mango' mango peels. *Food Hydrocolloids*, **22**: 1345-51.

11. Kumar, R., Manjunatha, S., Kathiravan, T., Vijayalakshmi, S., Nadanasabapathi, S. and Raju, P.S. 2015. Rheological characteristics of inulin solution at low concentrations: Effect of temperature and solid content. *J. Food Sci. Tech.* **52**: 5611-20.

12. Mesbahi, G., Jamaliana, J. and Farahnaky, A. 2005. A comparative study on functional properties of beet and citrus pectins in food systems. *Food Hydrocolloids*, **19**: 731-38.

13. Moreira, R., Chenlo, F., Torres, M.D. and Prieto, D.M. 2010. Influence of the particle size on the rheological behaviour of chestnut flour doughs. *J. Food Engg*. **100**: 270-77.

14. Pelegrine, D.H., Silva, F.C. and Gasparetto, C.A. 2002. Rheological behaviour of pineapple and mango pulps. *Lebensm.-Wiss. u. Tech.* **35**: 645-48.

15. Sato, A.C.K. and Cunha, R.L. 2009. Effect of particle size on rheological properties of jaboticaba pulp. *J. Food Engg.* **91**: 566-70.

16. Vandresen, S., Quadri, M.G.N., Souza, J.A.R. and Hotza, D. 2009. Temperature effect on the rheological behaviour of carrot juice. *J. Food Engg*. **92**: 269-74.

Received : July, 2017; Revised : November, 2017;
Accepted : November, 2017

DOI : 10.5958/0974-0112.2017.00123.2

Indian J. Hort. 74(4), December 2017: 631-634

Short communication

Optimization of *aonla*-blended juice based on antioxidants and sensory qualities using response surface methodology

V.S. Meena*, V.S. Yadav, R.S. Meena**, P.C. Sharma, Kirti Jalgaonkar, Manoj and V.E. Nambi**

ICAR- Central Institute of Post-Harvest Engineering and Technology, Abohar, Punjab

ABSTRACT

Present research investigation was aimed to determine the optimum combination of *aonla* (*Emblica officinalis* Gaertn.), pomegranate and aloe vera juices, which were mixed together in various proportions to obtain a suitable blend. Box Behnken design was adopted with three factors at five levels of each. The responses were analysed to fit a polynomial model by least square technique. Optimized blend was observed to contain *aonla* juice (71.6%), pomegranate juice (15.6%) and aloe vera (12.7%). Resulted with the responses of TSS 8.91°Brix, acidity 0.40%, ascorbic acid 80.1 mg/ 100 ml, colour *L* 23.55 and overall sensory score of 7.9. Regression models for TSS, ascorbic acid and sensory were found significant and the coefficients of determination R^2 were found in the range up to 0.93 for these dependent variables. Results showed that the optimized blend of *aonla*-pomegranate-aloe vera juice is acceptable for further development of premium beverages and can be preserved for 6 months with good quality attributes.

Key words: *Aonla*, juice blending, optimization, vitamin C.

Among fruits, *aonla* commonly known as Indian gooseberry (*Emblica officinalis* Gaertn syn. *Phyllanthus emblica* L.) finds a special place in India as it has got tremendous medicinal values, rich source of vitamins C (400-600 mg/ 100 g), pectins & tannins. Although, consumption of raw *aonla* fruit is considered to be good or human health, but because of the inherent high astringency it has little table value (Jain and Khurdiya, 5). *Aonla* juice is the preferred product, due to easily digestible, highly refreshing, thirst-quenching, appetizing and nutritionally far superior than much synthetic and aerated drink (Nayak *et al*., 6). Various workers have reported that two or more fruit juices/ pulps may be blended in various proportions for preparation of RTS, nectar and other beverages (Bhardwaj and Mukherjee, 3; Deka *et al*., 4; Ram *et al*., 7). Sasi Kumar *et al.* (7) reported that RTS beverage developed by blending aloe vera, *aonla* and ginger in ratio of 70:15:15 recorded the highest sensory scores, taste, flavour and overall acceptability. Optimization of composition of blend juice ingredients is essential to obtain a product with good taste, flavour and colour. The purpose of present work was to study the effect of blending ratio of various fruit juices on quality and optimization to obtain higher quality juice with improved physico-chemical and sensory characteristics.

The experiment was conducted during 2014-15 at ICAR-CIPHET, Abohar, Punjab. The fully matured, well-developed and uniform sized *aonla* fruits of cv. Chakaiya, aloe vera leaf (*Aloe barbadensis*) and pomegranate cv. Mridula were harvested from orchard of the institute. Selected fruits were thoroughly washed in running tap water to remove dirt, dust particles and insecticidal residues. *Aonla* fruit was first shredded with the help of *aonla* shredder machine; juice was obtained by hydraulic press and stored in controlled storage conditions (4 ± 2°C). Selected aloe vera leaves were subjected for pre-processing (cut of tip and edge), gel was extracted and juice was filtered through muslin cloth. The fully ripe fruits of pomegranate were cut and arils were separated. These arils were passed through juicer to extract the juice. The juices so obtained were kept for 24 h in refrigerator (4 ± 2°C) for sedimentation. The calculated juice blends as per design were filled in pre-sterilized 200 ml opaque white glass bottles. The bottles were sealed air tight and pasteurized in hot water at 85°C for 15 min. and cooled. Juice blends were stored in two lots at room temperature (28 ± 4°C) and refrigerators (4 ± 2°C) and evaluated periodically for physico-chemical, and sensory characteristics.

The colour of juices was measured using handy colorimeter NR 3000 (Nippon Deshkhon, Japan). The degree of browning was expressed by the changes in the colour (*L*, *a* and *b*) values. The titratable acidity were determined by the standard methods (AOAC and ascorbic acid, 2). The blended beverages were evaluated for sensory qualities on the basis of colour (appearance), taste and aroma, palatability and

*Corresponding author's present address: Officer In-charge, National Bureau of Plant Genetic Resources (ICAR-NBPGR), Experimental Farm, Issapur 110173, New Delhi; E-mail: vjy_meena@yahoo.com

**Rajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan

overall acceptability by a panel of 10 judges on a 9-point Hedonic scale (Amerine *et al.*, 1). Response, surface methodology (RSM) using Design expert package (Statease Inc. Minneapolis, USA) was used to investigate the effect of different juice ratio on its quality and acceptability. Box-Behnken design with 20 treatments (Table 1) including five centre point experiments was applied to establish and evaluate the relationship of responses (TSS, acidity, colour, and overall sensory acceptability) with respect to the independent variables (*aonla*, pomegranate and aloe vera juice).

Corresponding values of various responses of dependent variables are given in Table 1. A wide variation in all the responses was observed for different juice blending proportion combinations, *viz*. colour value L^* (16.2 to 34.8), a^* (8.15 to 28), b^* (2.1 to 9.12), TSS (6.1 to 10.5°Brix), acidity (0.3 to 1.8%) overall sensory (5.5 to 7.9) and ascorbic acid (20 to 210 mg/ 100 ml). Juice percent had significant effects on overall acceptability ($p < 0.01$). The 3D response (Fig.1) was generated for the fitted model to visualize the combined effect of two variables on each dependent variable like TSS, acidity and ascorbic acid, while keeping third variable at its central value. A second order polynomial fitted for all the data performed, regression coefficients for each response and the fitted equations are given in eq.1-4.

Ascorbic acid ranged from 20 to 210 mg/ 100 g (Fig. 1) reveals that independent variable (X_1) affected the ascorbic acid content significantly. The interaction effect (*aonla* × aloe vera juice) and (*aonla* × pomegranate juice) were negatively related. The changes in ascorbic acid with respect to the juice ratios could be predicted using the quadratic model as given in eq.1. TSS varied from 6.1° to 10.5°Brix for different blends. The *aonla* juice and pomegranate juice had their significant effects on TSS ($p < 0.01$) of the final blend. The results revealed that out of all independent factors, pomegranate juice plays a major role in deciding the TSS of final product since it contains higher amount of sugars. The positive coefficients on linear term between *aonla* and pomegranate juice indicated that TSS increased with the increase in pomegranate juice (Fig.1). Similar results were obtained by Ram *et al.* (7). While, negative coefficients for aloe vera indicated that TSS decreased with increase in aloe vera juice. There was also significant interaction ($p < 0.01$) between *aonla* and pomegranate variable, while TSS was negatively

Table 1. Experimental design and responses with respect to *aonla*-pomegranate-aloe vera juice blends.

Run	Uncoded variable			Response						
	X_1	X_2	X_3	*L*	*a*	*b*	TSS	Acidity	Sensory	AA
1	50	10	10	23.09	11.96	3.95	9.2	0.91	6.9	105
2	100	0	0	18.59	9.05	3.52	6.1	1.12	6.1	145
3	0	20	0	22.71	22.39	2.75	10.2	0.52	6.2	60
4	0	0	0	21.96	13.81	4.09	9	0.56	6.3	52
5	50	10	0	22	12	5.56	10.5	0.92	7.12	95
6	0	0	20	19	11.97	4.09	6.2	0.52	6.1	49
7	50	20	10	22.49	20.33	3.1	9.5	1.12	6.3	110
8	0	10	10	26.42	13.49	3.24	6.4	0.3	5.5	10
9	50	10	10	27.94	23.52	9.59	9.5	0.98	6.5	62
10	50	10	10	22	8.17	11.27	9.6	1	6.5	63
11	100	20	20	31	8.15	10.2	9.6	1.22	7.5	150
12	50	10	10	29.2	28	17.25	10.4	1.02	7	104
13	100	0	20	32.66	25	8.02	9.1	1.4	7.8	140
14	0	20	20	22.79	17.5	2.1	8.1	0.6	6.9	68
15	50	10	20	34.88	23.22	15.98	6.2	0.96	6.7	110
16	100	20	0	16.2	13.36	7.8	9.9	1.5	7.9	160
17	50	0	10	17.07	14.58	14.25	8.9	0.8	6.8	100
18	100	10	10	26	16	19.12	9.6	1.8	7	210
19	50	10	10	20.18	13.7	11.3	9.7	1.2	6.6	65
20	50	10	10	17.83	13.6	3.6	9.6	1.18	6.4	64

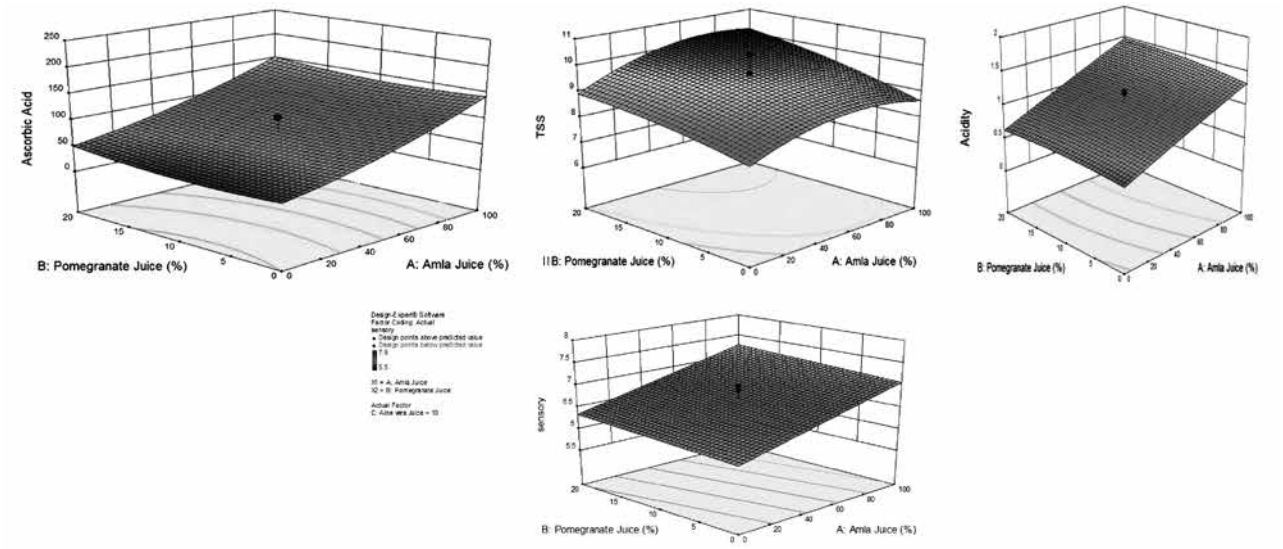


Fig. 1. 3D Response surface plots of with respect to change in pomegranate: *aonla* juice ratio, a. ascorbic acid; b. TSS; c. acidity, and d. overall sensory

influenced with aloe vera. The TSS content increased rapidly with increase in pomegranate juice per cent and while less during aloe vera juice per cent. The P values indicated that concentration of pomegranate juice was the most influencing factor followed by *aonla* juice and aloe vera was least effective over TSS gain. As it can explain for TSS, pomegranate juice has an effect on the total carbohydrates content that could increase total solids in blended juice. Second-order polynomial equation for TSS seems quite adequate to fit model (Equation 2).

$$\textit{Ascorbic acid} = 77.31 + 51.43X_1 + 5.04X_2 + 1.11X_3 - 0.25\,X_1X_2 - 2.50\,X_1X_3 + 0.75\,X_2X_3 + 10.64X_1^2 + 8.88X_2^2 + 7.99X_3^2\ (R^2 = 0.93) \quad (\ldots 1)$$

$$\text{TSS} = 9.66 + 0.48X_1 + 0.62X_2 - 0.69X_3 + 0.15\,X_1X_2 + 0.95\,X_1X_3 - 0.32X_2X_3 - 0.57X_1^2 - 0.042 - 0.15X_2^2 - 0.049 - 0.45X_3^2\ (R^2{=}0.84) \quad (\ldots 2)$$

$$\textit{Acidity} = 1.05 + 0.41X_1 + 0.057X_2 + 0.0078X_3 + 0.02X_1X_2 - 0.005X_1X_3 - 0.055X_2X_3 - 0.00983X_1^2 - 0.042X_2^2 - 0.049X_3^2\ (R^2 = 0.87) \quad (\ldots 3)$$

$$\textit{Overall Sensory} = 6.64 + 0.46X_1 + 0.100X_2 + 0.080X_3 + 0.10X_1X_2 + 0.10X_1X_3 - 0.15X_2X_3 - 0.081X_1^2 + 0.025X_2^2 + 0.15X_3^2\ (R^2 = 0.87) \quad (\ldots 4)$$

Juice colour is a key sensory attribute in influencing consumer acceptance. The juice percentage had significant effects on colour value ($p<0.01$). There was some homogeneity in colour parameters (L^*, a^* and b^*) among all the blends. Both a^* and b^* values were non-significantly different among all treatments, while L^* value was significantly increased with blending (p-values of 0.04, 0.045, and 0.74). Concentration of pomegranate juice leads to a decrease in the L^* value, while *aonla* and aloe vera juices increased the L^* value. The calculated colour a value ranged from 16.2 to 34.8. The positive coefficient of the linear term of pomegranate juice percentage indicated that as pomegranate juice content increased, a^* value increased proportionally. Similar finds were obtained by Jain and Khurdiya (4) and Seema *et al*. (9).

Acidity of the juice blend ranged 0.30 to 1.80. Concerning acidity, the intention was to incorporate the sufficient quantity of *aonla* juice in the blended juice with higher sensory scores. There was increase in acidity level when *aonla* juice increased. The *aonla* juice and pomegranate juice has significant effects ($p<0.01$) on acidity. The positive coefficients on linear term between *aonla* juices indicated that acidity increased with the increase in *aonla* juice (Fig. 1). Further, it was observed that when aloe vera juice was blended with *aonla* and pomegranate juice acidity was substantially lowered (negative coefficient of aloe vera juice and pomegranate juice). The three process variables were found non-significant in their respective quadratic terms. Moreover, 3D response (Fig. 1) was generated for the fitted model to visualize the combined effect of two variables on acidity, was found non-linear except pomegranate juice and aloe vera juice blending. The acidity content increased rapidly with increase in *aonla* juice per cent and pomegranate juice, while less during aloe vera juice per cent.

The combined effect of all variables was significant at quadratic level ($p = 0.001$) for key responses. Linear terms of all the three variables affected the sensory attributes of juice significantly ($p = 0.001$), whereas, interaction among variables for sensory attributes was not significant. The equation pertaining to overall sensory score of the blend is given in eq. 4. Earlier, Bhardwaj and Mukherjee

(3), Ram *et al.* (7) and Sasi Kumar *et al*. (9) found that the blending of fruit juice gave the best result on the basis of overall sensory quality and vitamin C content. Numerical optimization technique was adopted to find the optimum combination of juice blend ratio. The constraints were set such that the selected variables (X1, X2 and X3) would be minimum from economical point of view for the most important product attribute and close to the optimum for the others. The main criteria for constraints optimization were maximum possible maximum ascorbic acid and targeted TSS and colour were most important quality parameters. The process parameters for juice blending were numerically optimized for desirability function having equal importance (+) to all the three process parameters and equal importance (+++++) to two responses. The goal setting begins at a random starting point and proceeds up the steepest slope on the response surface for a maximum value of ascorbic acid. Table 2 shows the software generated optimum conditions of independent variables with the predicted values of responses. A graphical multi response optimization technique was adapted to determine the workable optimum conditions for the seasonal juice blending. The model equation for the response variables predicted values under the identified optimum conditions, which were experimentally verified to be in general agreement in the model. The 3D plots for all responses were superimposed and regions that best satisfy all the constraints were selected as optimum conditions. The optimized blending ratio was *aonla* juice, 15.6 per cent pomegranate juice and 12.7 per cent aloe vera juice to obtain optimum sensory and other chemical quality factors.

The RSM was effective in optimizing process parameters for juice percent of in the range of *aonla* juice in range of 71.6 per cent, pomegranate juice percent 15.6 per cent and aloe vera juice 12.7 per cent.

Table 2. Goal for optimization and the optimized values for each variables of *aonla*-based juice blend.

Parameter/ Variable	Goal	Optimized value
X1: *Aonla* juice (%)	Maximize	72.2
X2: Pomegranate juice (%)	in range	15.2
X3: Aloe vera juice	in range	12.6
Colour *L*	in range	23.50
Colour *a*	Maximize	-
Ascorbic acid	Maximize	81.5
Acidity	Minimize	0.41
TSS	Maximize	9.0
Colour *b*	Minimize	-
Overall sensory	Maximize	8.0

ACKNOWLEDGEMENT

Authors are thankful to Director, ICAR-CIPHET, Ludhiana, Punjab for facilities.

REFERENCES

1. Amerine, M.A., Pangborn, R.M. and Roessler, E.B. 1965 *Principles of Sensory Evaluation of Food*, Academic Press, New York, USA.
2. A.O.A.C. 2005. Latimer, J.W. and Horwitz, W. (Eds.) *Official Methods of Analysis*, AOAC International Press, Washington, DC.
3. Bhardwaj, R. and Mukherjee, S. 2011. Effects of fruit juice blending ratios on Kinnow juice preservation at ambient storage conditions. *African J. Food Sci.* **5**: 281-86.
4. Deka, B.C., Sethi, V., Parsad, R. and Batra, P. 2001. Application of mixtures methodology for beverages from mixed fruit juice/ pulp. *J. Food Sci. Tech.* **38**: 615-18.
5. Jain, S.K. and Khurdiya, D. 2004. Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry (*Emblica officinalis* Gaertn.) juice. *Plant Foods Human Nutr.* **59**: 63-66.
6. Nayak, P., Bhatt, D.K., Shukla, D.K. and Tandon, D.K. 2011. Evaluation of aonla (*Phyllanthus emblica* G.) segments-in-syrup prepared from stored fruits. *Res. J. Agric. Sci.* **43**: 252-57.
7. Ram, R., Meena, M., Sonkar, P., Lata, R. and Upadhyay, A. 2011. Standardization and evaluation of blended aonla (*Emblica officinalis* Gaertn) and bael (*Aegle marmelos* Correa) RTS beverages. *Plant Arch.* **11**: 205-08.
8. Sasi Kumar, R., Ray, R., Paul, P. and Suresh, C. 2013. Development and storage studies of therapeutic ready to serve (RTS) made from blend of *Aloe vera*, aonla and ginger juice. *J. Food Process. Tech.* **4**: 2.
9. Seema Shekhawat, Rathore, N.S. and Kaushik, R.A. 2014. Advances in processing and product development of aonla (*Emblica officinalis*) in indian context- A review. *Int. J. Food Nutr. Sci.* **3**: 242-48.

Received : June, 2017; Revised : October, 2017; Accepted : November, 2017

Indian J. Hort. 74(4), December 2017: i-vi

Hort News

The Horticultural Society of India, New Delhi organized its ***Platinum Jubilee Foundation Day Lecture***, **Executive Council** and **Annual General Body Meetings** on 16th December, 2017. Brief highlights of the events are given hereunder;

Platinum Jubilee Foundation Day Lecture

The Platinum Jubilee Foundation Day Lecture was held on 6th November, 2017 at Dr B.P. Pal Auditorium, ICAR-IARI, Pusa campus, New Delhi. About 200 invitees and members attended the event. The programme was inaugurated by presentation of invocation song by the Post Graduate students of IARI followed by lighting of the ceremonial lamp by the dignitaries. Thereafter, Dr K.L. Chadha, President, HSI welcomed the dignitaries and guests on the occasion of Platinum Jubilee Foundation Day Lecture. During his remarks he highlighted the journey of the society since its inception in 1942 and its activities and seminars, conferences, brainstorming sessions, *etc.* organised in the past and also the genesis of holding Indian Horticulture Congresses. He further informed about the different Awards and Fellowships conferred to recognise excellence in research and development in different sub-disciplines of Horticulture; and Memorial Lectures and Foundation Day Lectures delivered by different luminaries in the past.

Release of Publication

The society's handbook ***Directory of Life Members of HSI*** covering the complete updated address of the all members was released by the dignitaries to mark the occasion.

Presentation of HSI Awards

During inaugural function, the Society honoured several scientists for their contributions to research and development in the Horticulture. Dr Kirti Singh, Senior Vice President of the society read the citations, while President, HSI and the dignitaries presented the awards.

The *HSI-ShivShakti Lifetime Achievement Award for 2017* was conferred jointly to Dr Pritam Kalia, Former Head, Div. of Vegetable Science, IARI, New Delhi and Prof. R.K. Pathak, Former Director, ICAR-CISH, Lucknow. The other awards conferred were *Shri Girdhari Lal Chadha Memorial Gold Medal* conferred on Dr (Mrs.) N. Vijayakumari, Principal Scientist, ICAR-CCRI, Nagpur, Maharashtra; *Dr Kirti Singh Gold Medal* to Dr Major Singh Dhaliwal, Additional Director of Research (HFS), Punjab Agricultural University; Ludhiana; and *Dr J.C. Anand Gold Medal* on Dr Desh Beer Singh, Director, ICAR-CITH, Srinagar, Jammu & Kashmir. *Dr B.R. Barwale Best Thesis Award in Horticultural Biotechnology-2017* was jointly conferred on Dr Gograj Singh Jat, Scientist, Division of Vegetable Science, ICAR-IARI, Pusa, New Delhi and Dr Shiv Lal, Scientist, ICAR-CITH, Srinagar, Jammu & Kashmir.

Some scientists could not attend the function in person, however their names were announced. These were Dr Rakesh Pandey, Principal Scientist & Head, Microbial Technology & Nematology Department, CSIR-CIMAP, Lucknow, Uttar Pradesh the *Dr Manmohan Attavar Gold Medal in Floriculture*, Dr Prasath Duraisamy, Principal Scientist (Horticulture), ICAR-IISR, Kozhikode, Kerala for *Shri D.P. Ghosh Young Scientist Award*-2017 and the Award for the Best Research Paper published in Indian Journal of Horticulture during 2016 for the team of scientists, namely, Drs Manju Sharma, B.C. Suman and Dharmesh Gupta from Project Directorate on Mushroom Research, Solan, Himachal Pradesh for their research paper entitled "*Development of Agaricus bisporus hybrids and their evaluation for higher yield*" published in *Indian Journal of Horticulture* Vol. No. 73(4): 550-56.

HSI Foundation Day Lecture

The HSI Foundation Day Lecture was delivered by Dr S.K. Pattanayak, Secretary, Ministry of Agriculture & Farmers Welfare, GoI; on the topic '***R&D Initiatives to Address the Emerging Challenge in Horticulture Sector***', while Prof. Ramesh Chand, Member, NITI Aayog, GoI presided over the function and also delivered a special talk on "***Role of Horticulture in transforming Agrarian Economy in India***". Dr A.K. Singh, Director IARI and DDG (Agril. Extn.) ICAR, introduced the speakers. Dr A.K. Singh, Deputy Director General (Horticultural Science), ICAR also graced the occasion.

Dr S.K. Pattanayak, the speaker spoke about his association with the Horticulture Development in different professional capacities. He highlighted several pertinent issues related to Horticulture Development during the last three decades, *i.e.* increase in area, production and availability of different horticultural produce and products, rising exports *etc.* He talked about availability of improved varieties, production technologies, rootsocks and quality planting material, diagnostic kits, biological control, GAP protocols, dearth of processing varieties, *etc.* He emphasized the role of improved cultivars with

high quality production, productivity, resistance to pests and diseases and tolerance to biotic stresses; application of biotechnological tools, disease-free quality planting material, post-harvest management, storage, value-addition, cold chain development, international collaboration for resource conservation, protected cultivation using energy saving techniques, recycling of wastewater *etc*.

Dr Ramesh Chand, Member NITI Aayog also highlighted the journey of Indian Horticulture and role of Horticulture R&D in National economy, through his critical analysis made over a period. He informed that horticulture is not only environmentally safe but fits well with the integrated farming systems; creates livelihood and employment generation opportunities. He also presented the problems of Horticulture production like marketing, storage, value-addition and trade, crop insurance, export and commodity chain. He presented several success stories need for tapping potential of horticulture led development in NE region, promotion of indigenous fruit and vegetable crops for improving nutrition of locals as well as earnings. He urged that HSI should take lead as a Think tank and flagging R&D issues by holding conferences and congresses and sending recommendations to the policy making institutions on relevant issues for consideration.

At the end he applauded the speaker for excellent presentation on the govt. initiatives, policies interventions and schemes for Horticulture sector. He suggested areas for future R&D efforts and approach for meeting the Prime Minister's slogan for doubling farmers' income by 2022. At the end, Foundation Day Speaker and Chairman of the Session and the Guest of Honour were felicitated by the President, HSI.

Dr S.K. Singh, Secretary and Editor-in-Chief, HSI presented the Vote of Thanks.

Executive Council Meeting

The second Executive Council meeting of the society for 2017 was held on 16.12.2017 at 11:30 am in the Conference Room of the Division of Vegetable Science, ICAR-IARI, New Delhi. Dr K.L. Chadha, President, HSI greeted the members. Thereafter, two minute silence was observed to condole the death of Dr Manmohan Attavar, Chairman, Indo American Hybrid Seeds, Bengaluru and patron of the society. Thereafter, different reports by Secretary, Treasurer, and Editor-in-Chief were presented and approval taken. Different activities undertaken by the society were presented for information. The Dr K.L. Chadha, President requested the Election Officer, Dr Jai Prakash, Joint Secretary, HSI to present the results of election. He informed about different members who stand elected for the period 2018-2020, namely, Dr S.P. Ghosh as Vice President (unopposed), Dr Pritam Kalia as Editor-in-Chief (unopposed); and Dr Brahma Singh, Dr R.R. Sharma and Dr K.K. Jindal as Executive Councilor. The house congratulated the elected members. President, HSI also acknowledged the cooperation and support rendered by the outgoing EC members.

Annual General Body Meeting-2017

The Annual General Body meeting of HSI was held on 16th November, 2017, at 3:00 pm in the Auditorium of NRCPB, Pusa Campus, New Delhi. The President, adjourned the meeting due to lack of quorum and the same was resumed after 15 minutes, which was attended by ere about 90 members. Thereafter, the President, HSI informed about the untimely death of Dr Manmohan Attavar, Chairman, M/s Indo-American Hybrid Seeds, Bengaluru on December 12th, 2017. As a mark of respect and remembrance of the departed soul, two-minute silence was observed by the members. Thereafter, he welcomed the members to the meeting and agenda items were presented and proceedings conducted. Confirmation of the proceedings of last AGM, Action Taken Report, Secretary's, Treasurer's and Editors' reports were presented and got confirmed. Different agenda items were also discussed/ informed to the members. Further, different activities and events (First EC meeting and Platinum Jubilee Foundation Day Lecture) organised by the society were highlighted by the President.

Conferment of Fellowship of HSI

During AGM, the Fellowships of the Society for the year 2017 were conferred by Dr K.L. Chadha, President, HSI on the following scientists, *i.e.* Dr Jai Prakash, Pr. Scientist, Division of Fruits & Horticultural Technology, ICAR-IARI, New Delhi; Dr K.K. Srivastava, Principal Scientist, ICAR-CISH, Rehmankhera, Lucknow, Uttar Pradesh; Dr Somkuwar Ramhari, Principal Scientist (Hort.), ICAR-NRC - Grapes, Pune, Maharashtra; Dr A.D. Munshi, Principal Scientist, Division of Vegetable Science, ICAR-IARI, New Delhi; Dr B.S. Tomar, Principal Scientist & Head, Division of Vegetable Science, ICAR-IARI, New Delhi. Dr Kirti Singh, Sr. Vice President, HSI read the citations, while the President inducted the fellows. Four fellow designates, namely, Dr A.K. Singh, PS, ICAR-CHES (ICAR-CIAH), Vejalpur, Godhra, Gujarat; Dr Rajbir Singh, Director, ATARI, PAU Campus, Ludhiana; Dr Anjani Kumar Jha, Principal Scientist & Head, Horticulture Division, ICAR Research Complex for NEH Region, Umiam, Meghalaya and Dr Alka Singh, Associate Professor and Head, Department of Floriculture and Landscape Architecture, ASPEE

College of Horticulture and Forestry, NAU, Navsari, Gujarat could not attend the event *in person*, hence were conferred the fellowship *in absentia*. Thereafter, Treasurer's and Editors' reports were presented and got confirmed; information for holding the *8th Indian Horticulture Congress-2018* was also discussed.

The General Body approved by Voice Vote the unanimous decision taken by the Executive Council in its meeting held on July 15th, 2017 to change the name of *the Horticultural Society of India* as **National Academy of Horticulture Sciences**. The procedure indicated was also approved. At the end, President, HSI thanked the members and wished all for the Christmas and New Year-2018.

The Vote of Thanks delivered by Dr Jai Prakash, Joint Secretary of the society.

Glimpses of the HSI Events

Platinum Jubilee Foundation Day Lecture

Dr K.L. Chadha, President, HSI welcoming the dignitaries and the invitees. (L-R) Dr Kirti Singh, Sr. Vice President; Dr A.K. Singh, Dir. IARI and DDG (Agril. Extn) ICAR, Dr S.K. Pattanayak, Secretary, MA&FW, GoI, and Dr Ramesh Chand, Member NITI Aayog

Presentation of Invocation song by PG students of IARI, New Delhi

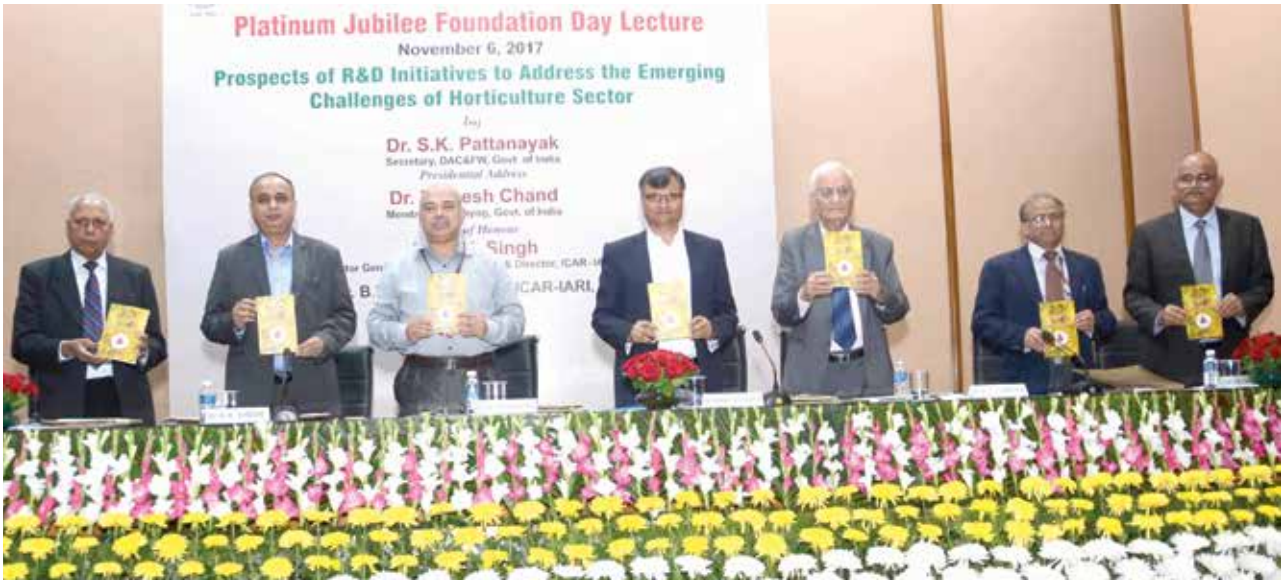
Inauguration of Function by lighting of ceremonial lamp by the dignitaries, namely, Dr S.K. Pattanayak, Secretary, MA&FW, GoI, Dr Ramesh Chand, Member, NITI Aayog, Dr A.K. Singh, Director, IARI and DDG (Agril. Extn.) ICAR

Dr A.K. Singh, introducing the Speaker of the Foundation Day Lecture

Dr S.K. Pattanayak, delivering the Foundation Day Lecture

Dr Ramesh Chand presenting the Special Lecture

Release of Publication



Release of publication '*HSI Directory of Life Members*' by the dignitaries. (L-R) Dr Kirti Singh, Dr A.K. Singh, Dr S.K. Pattanayak, Dr Ramesh Chand, Dr D.L. Chadha, Dr A.K. Singh and Dr S.K. Singh.

Felicitation of Dignitaries

Dr K.L. Chadha, President, HSI felicitating Foundation Day Speaker Dr S.K. Pattanayak

Dr K.L. Chadha, President, HSI felicitating the Chairman of the Session Dr Ramesh Chand

Dr K.L. Chadha, President, HSI felicitating Guest of Honour Dr A.K. Singh

Dr S.K. Singh, Secretary, HSI presenting the vote of thanks

Invitees and Members attending the Platinum Jubilee Foundation Day Lecture-2017

Conferment of HSI Awards

Dr Kirti Singh, Sr. Vice President, HSI reading the citations. Dignitaries presenting Annual HSI Awards to Dr Pritam Kalia, Prof. R.K. Pathak, Dr (Mrs.) N. Vijayakumari, Dr M.S. Dhaliwal, Dr Shiv Lal and Dr Gograj Singh Jat.

Local Organizing Team members for the event

Annual General Body Meeting

Annual General Body-2017 meeting of the HSI being chaired by Dr K.L. Chadha, President, HSI. Seen on the dias are Dr Kirti Singh, Sr. Vice President, Dr M.L. Chadha, Vice President, Dr S.K. Singh, Secretary and Dr Jai Prakash, Joint Secretary

Conferment of HSI Fellowships

Conferment of HSI Fellowship by Dr K.L. Chadha, President, HSI to Dr R.G. Somkuwar, Dr B.S. Tomar, Dr A.D. Munshi, Dr Jai Prakash and Dr Dinesh Kumar during the Annual General Body-2017 meeting

List of Referees for Review of Research Papers during 2017 (July to December)

Dr S.E. Apshara, Dakshina Kannada, Karnataka

Dr Ram Asrey, IARI, New Delhi

Dr B.L. Attri, PDMR, Solan

Dr O.P. Awasthi, IARI, New Delhi

Dr Anju Bajpai, CISH, Lucknow

Dr D.R. Biswas, IARI, New Delhi

Dr Reeta Bhatia, IARI-RS, Katrain

Dr Anchal Dass, IARI, New Delhi

Dr S.S. Dey, IARI-RS, Katrain

Dr Ajmer Singh Dhatt, PAU, Ludhiana

Dr W.S. Dhillon, ICAR, New Delhi

Dr M.R. Dinesh, IIHR, Bengaluru

Dr A.K. Dubey, IARI, New Delhi

Dr S.K. Dutta, ICAR CNEHR, Kolasib, Mizoram

Dr Kishor Gaikwad, NRCPB, New Delhi

Dr K.K. Gangopadhaya, NBPGR, New Delhi

Dr P.P.S. Gill, PAU, Ludhiana

Dr Robin Gogoi, IARI, New Delhi

Dr A.K. Goswami, IARI, New Delhi

Dr P.M. Haldankar, KKV, Dapoli

Dr Vinayaka Hegde, CPCRI, Kasaragod

Dr Zakir Hussain, IARI, New Delhi

Dr Gograj S. Jat, IARI, New Delhi

Dr K.J. Jeyabaskaran, Tamilnadu

Dr Pradeep Karmakar, IIVR, Varanasi

Dr Anil Khar, IARI, New Delhi

Dr Arun Kishor, CITH-RS, Mukteshwar

Dr Hare Krishna, CIAH, Bikaner

Dr Ajay Kumar, NRCP, Sholapur

Dr Rajiv Kumar, IIHR, Bengaluru

Dr P. Suresh Kumar, NRCB, Trichy

Dr Sandeep K. Lal, IARI, New Delhi

Dr Shiv Lal, CITH, Srinagar

Dr B.V.C. Mahajan, PAU, Ludhiana

Dr. Vijay Mahajan, PDOG, Pune

Dr L.N. Mahawar, MPUA&T, Udaipur

Dr R.L. Misra, , New Delhi

Dr A. Nagaraja, IARI, New Delhi

Dr Sujatha Nair, IIHR, Bengaluru

Dr Namita, IARI, New Delhi

Dr C.K. Narayana, IIHR, Bengaluru

Dr Vishal Nath, NRCL, Bihar

Dr R.K. Pal, NRCP, Sholapur

Dr Sapna Panwar, IARI, New Delhi

Dr V.B. Patel, IARI, New Delhi

Dr Kalidas Pati, CTCRI-RS, Bhubaneswar

Dr M. Pitchaimuthu, IIHR, Bengaluru

Dr Jai Prakash, IARI, New Delhi

Dr D. Prasath, IISR, Kerala

Dr K.V. Raman, USA

Dr H. Rymbai, ICAR CNEHR, Umiam, Meghalaya

Dr Naved Sabir, IARI, New Delhi

Dr M.S. Saraswathi, Trichy

Dr Indu Sawant, NRCG, Pune

Dr Shruti Sethi, IARI, New Delhi

Dr M. Shareefa, IISR, Kozhikode

Dr Akhilesh Sharma, YSPUH&F, Solan

Dr Nimisha Sharma, IARI, New Delhi

Dr R.M. Sharma, IARI, New Delhi

Dr S.K. Sharma, IARI RS, Pune

Dr A.K. Shukla, IARI RS, Shimla

Dr Anshuman Singh, CSSRI, Karnal

Dr Awtar Singh, IARI, New Delhi

Dr Brahma Singh, New Delhi

Dr Desh Beer Singh, Srinagar

Dr Dinesh Singh, IARI, New Delhi

Dr Kanhaiya Singh, IARI, New Delhi

Dr N.V. Singh, NRCP, Solapur

Dr Rakesh Singh, NBPGR, New Delhi

Dr Zora Singh, Australia

Dr V. Sridhar, IIHR, Bengaluru

Dr Sachin S. Suroshe, IARI, New Delhi

Dr C.K. Thankamani, IISR, Kozhikode

Dr Madhubala Thakre, IARI, New Delhi

Dr Jagesh K. Tiwari, CPRI, Shimla

Dr S. Uma, NRCB, Trichy

Dr B. Varalakshmi, IIHR, Bengaluru

Dr M.K. Verma, IARI, New Delhi

Dr A.K. Yadava, USA

Dr Ashish K. Yadav, NRCO, Gangtok

Dr R.K. Yadav, IARI, New Delhi

Dr S.K. Yadav, IARI, New Delhi

The Editorial Team of the Horticultural Society of India gratefully acknowledges the assistance provided by the above referees in reviewing the articles published in the Indian Journal of Horticulture during 2017.

Editor-in-Chief
Indian Journal of Horticulture

Obituary

Manmohan Attavar
(12.07.1932 to 12.12.2017)

Dr Manmohan Attavar, a renowned horticulturist, an eminent ornamental crop and vegetable breeder and Chairman, Indo American Hybrid Seeds, Bengaluru passed away on December 12th, 2017 at Mangalore on his way to join a family function. He did his B.Sc. and M.Sc., degrees at Universities of California, Missisipi and Michigan, USA. On his return, he worked under the guidance of Dr M.H. Marygowda, and started petunia breeding at Lalbagh Gardens, Bengaluru. He founded the Indo-American Hybrid Seeds (IAHS) in 1965, which later came to be known as leader in private sector investment in scientific research, quality seed production and other farm service to farmers. He had professional interest in ornamentals, olericulture, protected cultivation, Hi-tech nursery, custom services and export and quality assurance. He released the first tomato hybrid Karnataka and capsicum hybrid Bharat, which are still very popular. Under his leadership the, IAHS established the most modern Molecular Biology Laboratory for DNA sequencing and gene transfer, Hi-volume and largest tissue culture laboratory, commercial protected cultivation units and quality seedling raising in protrays. Dr Attavar authored a book titled, "*Floriculture: Technology*, *Trades and Trends*" published by Oxford and IBH. He also published 'VATIKA"-a much circulated and widely read magazine to bring science based technologies for the farmers.

Dr Attavar was a well-wisher of the Horticultural society of India and supported it as patron. He also sponsored an annual award in Floriculture, which was named as *Dr Manmohan Attavar Award in Floriculture*. To mark his contribution to Horticulture R&D, he was conferred the nation's civilian award Padma Shri. His wife Late Mamta Attavar was a pious lady. He is survived by his children Mr. Santosh Attavar and Ms Rashmi Attavar. His demise is a great loss to the Horticulture fraternity. The Horticultural Society of India deeply mourns his death and prays that his soul rests in peace.

ACKNOWLEDGEMENT

The Horticultural Society of India acknowledges the partial Financial Assistance provided by the Indian Council of Agricultural Research, New Delhi for printing of Indian Journal of Horticulture in 2017

The President and the
Executive Council of
the Horticultural Society of India
Wish all its Members a

Happy and Prosperous
New Year-2018

GUIDELINES TO THE CONTRIBUTORS

Indian Journal of Horticulture is the official publication of **the Horticultural Society of India**. It features the original research in all branches of Horticulture and other cognate sciences of sufficient relevance and primary interest to the horticulturists. The publication is generally open to the members the Horticultural Society of India but it also accepts papers from non-members on subjects related to Horticulture. The journal publishes three types of articles, *i.e.*, **Review/ Strategy paper** (exclusively by invitation from the personalities of eminence), **Research paper** and **Short communication**. The manuscripts should be submitted in duplicate in all respect to **the Editor-in-Chief, the Horticultural Society of India, F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi - 110 012, India.** Each manuscript must be typed doubled spaced on one side of a A4 size page. Clearness, brevity and conciseness are essential in form, style, punctuation, spelling and use of English language. Manuscripts should conform to the S.I. system for numerical data and data should be subjected to appropriate statistical analysis. On receipt of an article at the Editorial Office, an acknowledgement giving the manuscript number is sent to the corresponding author. This number should be quoted while making any future enquiry about its status.

***Review/ Strategy paper*:** This article is received through invitation. It should be comprehensive, up-to-date and critical on a recent topic of importance. The maximum page limit is of **16 double-spaced typed pages** including tables and figures. It should cite latest literatures and identify some gaps for future. It should have a specific **Title** followed by the **Name(s) of the author(s)**, **Affiliation**, **Abstract, Key words,** main text with subheadings, **Acknowledgements** (wherever applicable) and **References**.

***Research paper*:** The paper should describe a new and confirmed findings. Should not generally exceed **12 typed pages** including tables/ figures etc. A research paper has the following features.

Title followed by **Author**(s) and **Affiliation:** Address of the institution(s) where the research was undertaken.

Abstract: A concise summary (200 to 300 words) of the entire work done along with the highlights of the findings.

Key words: Maximum of five key words to be indicated.

Introduction: A short introduction of the crop along with the research problem followed by a brief review of literature.

Materials and methods: Describe the materials used in the experiments, year of experimentation, site etc. Describe the methods employed for collection of data in short.

Results and discussion: This segment should focus on the fulfillment of stated objectives as given in the introduction. Should contain the findings presented in the form of tables, figures and photographs. As far as possible, the data should be statistically analyzed following a suitable experimental design. Same data should not be presented in the table and figure form. Avoid use of numerical values in findings, rather mention the trends and discuss with the available literatures. At the end give short conclusion. Insertion of coloured figures as photograph(s) will be charged from the author(s) as applicable and suggested by the printer.

Acknowledgements (wherever applicable).

References: Reference to literature should be arranged alphabetically and numbered according to author's names, should be placed at the end of the article. Each reference should contain the names of the author with initials, the year of the publication, title of the article, the abbreviated title of the publication according to the World List of Scientific Periodicals, volume and page(s). In the text, the reference should be indicated by the author's name, followed by the serial number in brackets. **Maximum of 15 key references to be cited**.

1. Scaffer, B. and Guaye, G.O. 1989. Effects of pruning on light interception, specific leaf density and chlorophyll content of mango. *Scientia Hort.* **41**: 55-61.
2. Laxmi, D.V. 1997. Studies on somatic embryogenesis in mango (*Mangifera indica* L.). Ph.D. thesis, P.G. School, Indian Agricultural Research Institute, New Delhi.
3. Sunderland, N. 1977. Nuclear cytology. **In**: *Plant Cell and Tissue Culture*, Vol. II. H.E. Street (Ed.), University of California Press, Berkeley, California, USA, pp.171-206.
4. Chase, S.S. 1974. Utilization of haploids in plant breeding: breeding diploid species. **In**: *Haploids in Higher Plants*: *Advances and Potential. Proc. Intl. Symp.* 10-14 June, 1974, University of Guelph. K.J. Kasha (Ed.), University of Guelph, Canada, pp. 211-30.
5. Panse, V.G. and Sukhatme, P.V. 1978. *Statistical Methods for Agricultural Workers*, Indian Council of Agricultural Research, New Delhi, 381 p.

***Short communication*:** The text including table(s) and figure(s) **should not exceed five typed pages**. It should have a short **title**; followed by name of **author(s)** and **affiliation**, **Abstract** (100 words), **Key words** (3-5), **Short research paper** and **References (7 max.)**. There should be no sub-headings, *i.e.* Introduction, Materials and Methods, Results & Discussion etc. The manuscript should be in paragraphs mentioning the brief introduction of the of the topic and relevance of the work, followed by a short description of the materials and the methods employed, results and discussion based on the data presented in 1 or 2 table(s)/ figure(s) and a short conclusion at the end.

General instructions

- All the manuscript should be typed double-spaced on one side of A4 size paper with proper margin.
- Generic and specific names should be italicized throughout the manuscript. Similarly, the vernacular names are to italicized.
- Each table should have a heading stating its content clearly and concisely. Place at which a table is to be inserted should be indicated in the text by pencil. Tables should be typed on separate sheets, each with a heading. Tables should be typed with the first letter (T) only capital, table No. in Arabic numerals. All measurements should be in metric units.
- Data to be presented in graphical form should be sent on quality glossy contrast paper without folding. Each illustration must be referred to in the text and Roman numerals should be used in numbering. Photograph(s) of good contract must be mounted on hard paper to avoid folding and a separate sheet must be given for the title for each photograph sent as figure.
- At the bottom of the first page present address of the corresponding author, **E-mail ID** etc. must be specified.
- Revised manuscript is acceptable in duplicate along with a **CD/E-mail**. It is essential to cite related research papers published in Indian Journal of Horticulture and other horticultural journals and update the references while revising a manuscript.
- No reprint of the published article will be supplied to the authors.
- Article forwarded to the Editor-in-Chief for publication is understood to be offered to INDIAN JOURNAL OF HORTICULTURE exclusively. It is also understood that the authors have obtained a prior approval of their Department, Faculty or Institute in case where such approval is a necessary.
- **Acceptance of a manuscript for publication in Indian Journal of Horticulture shall automatically mean transfer of Copyright to the Horticultural Society of India. At the time of acceptance of a manuscript, all the authors should be the annual member of the Society.**
- The Editorial Board takes no responsibility for the fact or the opinion expressed in the Journal.
- Electronic submission of manuscript : www.indianjournals.com; hsi42@rediffmail.com
- **All submissions should strictly follow journal format. Deviation from format and exceeding the page limit are liable for non-starter of the review process and further processing. Up-to-date literature must be cited. Avoid self citation.**

CONTENTS

Published by Dr K.L. Chadha and edited by Dr S.K. Singh for the Horticultural Society of India, F1, National Society's Block, National Agricultural Science Centre Complex, Todapur, Pusa Campus, New Delhi 110 012, India and printed at Malhotra Publishing House, B-6, DSIDC Packaging Complex, Kirti Nagar, New Delhi 110 015, India.