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Effects of pruning intensity on the biochemical status of shoot buds in three mango (*Mangifera indica* L.) cultivars planted at high density

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SUMMARY
Mango (*Mangifera indica* L.) trees grown at high density show a decline in flowering and fruiting after good fruiting years as a result of various factors. Annual pruning can restore production and productivity in such trees. Chlorophyll, total sugars (TS), total phenolics (TP), and proline contents as well as polyphenol oxidase (PPO) activities, were measured in the 2005–2006 and 2006–2007 seasons in shoot buds with a few leaves in three mango cultivars ('Amrapali', 'Mallika', and 'Dashehari'). Trees were grown at high density in an orchard and the aforesaid parameters were measured 1 month after different degrees of pruning (Stage I) and after subsequent fruit bud differentiation (FBD; Stage II). Severely-pruned mango trees had the highest contents of chlorophyll *a*, while chlorophyll *b* and total chlorophyll contents were found to be highest in moderately-pruned trees. Lightly-pruned trees had the highest contents of reducing sugars (RS), whereas TS contents were highest in severely-pruned trees. The contents of RS and TS increased in shoot buds during the FBD stage. A moderate intensity of pruning significantly increased TP contents, while the lowest TP contents were recorded in non-pruned trees. 'Off'-year shoots had higher TP contents than 'on'-year shoots. Irrespective of pruning intensity, shoot buds of 'Mallika' trees had the highest PPO activities, with lower levels in 'Amrapali' and 'Dashehari' shoot buds. PPO activities were reduced at the FBD stage in 'on'-year shoots. Severely-pruned trees had the highest PPO activities, while the lowest PPO activities were recorded in lightly-pruned trees. Shoot bud proline contents were found to be highest in non-pruned trees, and decreased with increasing pruning intensity. Thus moderate pruning can be adopted in high density orchards to obtain sustainable production with improved maintenance of canopy architecture.

Mango (*Mangifera indica* L.) is the most important fruit crop cultivated in sub-tropical and tropical regions of the World. There is ample scope to enhance fruit production and productivity by adopting high density plantings, coupled with judicious pruning. Pruning is generally practiced in deciduous, temperate fruit crops (e.g., apple, pear, peach, plum), and in some sub-tropical fruits such as grapevine, fig, and phalsa (*Grewia subinaequalis* D.C.; Rao and Shanmugavelu, 1975). However, some researchers have reported that several evergreen fruit trees, including mango, also responded positively to pruning (Fivaz and Stassan, 1996; Oosthuyse, 1997). Accordingly, the technique of high-density orcharding (HDO) has been shown to be successful for some mango cultivars such as 'Amrapali' (planted at a tree-spacing of 2.5 m × 2.5 m), 'Mallika' (at 4.0 m × 3.0 m), and 'Dashehari' (at 3.0 m × 2.5 m) when assisted by pruning (Majumder *et al*., 1982), or by pruning followed by the application of paclobutrazol (Ram and Sirohi, 1988; Ram *et al*., 1997). Nevertheless, when planted at high density, all three mango cultivars showed a decline in fruit yield and quality after 10 – 12 years of fruiting due to canopy factors such as overlapping or intermingling of branches, poor light interception, low photosynthetic rates, and/or high relative humidity leading to an increased susceptibility to pests and/or diseases. These conditions modify the physiology of shoots, or of whole trees in such a way that they are no longer sustainably productive in succeeding years.

The timing and severity of pruning not only alters the physiological status of trees, but also changes their biochemical behaviour, as indicated by their patterns of flowering, fruit bearing, and yield. Leaf chlorophyll contents were higher in non-pruned mango trees than in pruned trees (Sharma and Singh, 2006) especially at the fruit bud differentiation (FBD) stage (September-December; Pandey and Tyagi, 1999). All pruning techniques which induce changes in the partitioning of metabolic reserves tend to reduce excessive vegetative growth, at least in the short-term. Pruning, like other stresses, induces the hydrolysis of some reserves and an accumulation of certain metabolites (Maczulaitys *et al*.,

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1999). Top-pruning of young Summer shoots increased chlorophyll, total soluble protein, RNA, and starch contents and ribulose 1,5 bisphosphate carboxylase (RuBPCase) activities in mulberry leaves (Yamashita and Fuzino, 1994). Carbohydrates play a dominant role in flower bud formation and their levels can be directly correlated with flowering in fruit trees (Pongsomboon *et al*., 1997). It has also been proposed that pruning removes carbon pools and that the shoot promotes new leaf growth in order to increase carbohydrate reserves for the next flowering season (Sauco, 1996). The total sugars (TS) and sucrose contents of leaves increased after Winter pruning in apricot, but starch contents were always higher in non-pruned trees (Kuden and Son, 2000). Polyphenol oxidase (PPO) activities, and the contents of phenolic compounds, have a direct relationship with plant responses to many types of stress, including floral malformation in mango (Sharma *et al*., 2001; Sharma and Singh, 2006).

High density planting of evergreen fruit crops such as mango is being adopted by growers; however, after 10 – 12 years this can result in a decline in flowering and fruiting, thus rendering such orchards less productive. Therefore, it is important to be able to restore productivity in such trees by pruning. Furthermore, it is pertinent to understand what severity of pruning to adopt, and its effects on vegetative growth and reproductive behaviour. Accordingly, the present investigation was undertaken to examine flowering and fruit bearing behaviour in regular- and irregular-bearing mango varieties grown in a high density orchard and to note the biochemical parameters that were affected as a result of various severities of pruning.

MATERIALS AND METHODS

Study site and experimental materials

A field experiment was conducted during the 2005–2006 and 2006–2007 seasons at the Main Orchard, Division of Fruits and Horticultural Technology, IARI, New Delhi. Three mango cultivars, 'Amrapali' (23-year-old trees), 'Mallika' (24-year-old trees), and 'Dashehari' (26-year-old trees) were selected for this study. All three cultivars were planted at high densities: 'Amrapali' (V_1) at a spacing of 2.5 m × 2.5 m; 'Mallika' (V_2) at 4.0 m × 3.0 m, and 'Dashehari' (V_3) at 3.0 m × 2.0 m. The experimental trees (12 trees of each cultivar) were maintained under uniform cultural practices.

Treatments

Pruning was applied in mid-August 2005, and four intensities of pruning were adopted: I_0 (control) non-pruned; I_1 (light) 30 cm from the apex; I_2 (moderate) 60 cm from the apex; and I_3 (severe) 90 cm from the apex. There were 12 trees of each cultivar (three for each pruning intensity) with a single tree taken as one unit (replication). Thus, the total number of trees was 36 (12 trees of each cultivar in each block). The experimental trees were tagged in such a manner as to avoid border effects and each individual tree was surrounded by four trees of each cultivar. Uniform pruning was performed in all directions in each canopy by removing both the inner and a few peripheral branches, which were dense and overcrowded. Control trees received no pruning. As a result of each pruning, all trees showed low flowering and fruiting during 2005–2006, which was referred to as the 'off'-year, while 2006–2007 was called the 'on'-year.

Assessment of the biochemical status of shoot buds

Chlorophylls *a* and *b*, reducing sugars RS, TS, total phenolics (TP) compounds, and proline contents, as well as polyphenol oxidase (PPO) activities in mango shoots consisting of a shoot bud with few leaf primordia, were measured 1 month after pruning (Stage I), and just after FBD (Stage II; November–December in northern India).

The chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), and total chlorophyll (TC) contents of fully-mature, opened leaves from shoot tips were analysed following the method of Barnase *et al*. (1992). Exactly 0.5 g of each clean leaf tissue sample was immersed in 10 ml dimethyl sulphoxide (DMSO; AR grade; SRL Chemicals, Mumbai, India). After incubation at 70ºC for 4 h, the sample was removed and a 1.0 ml aliquot was diluted to 5 ml with 100% (v/v) DMSO then read in a spectrophotometer (MiniSpec SL-171; Elico, Hyderabad, India) at 638 nm, 645 nm, 663 nm, and 480 nm using 100% (v/v) DMSO as the blank.

Reducing sugar (RS) contents were estimated in samples consisting of shoot buds following the method outlined by Nelson and Somogyi in Thimmaiah (2004). Exactly 100 mg of each sample was macerated and extracted twice with 5 ml 80% (v/v) hot ethanol (40ºC). The supernatant was collected and evaporated to dryness in a water bath at 80ºC. Thereafter, 10 ml of distilled water was added to dissolve the sugars. From this, a 0.2 ml aliquot was pipetted into a test tube and the volume increased to 2.0 ml by adding autoclaved double-distilled water. One ml of alkaline copper tartarate solution was added and the sample was kept in a water bath at 100ºC for 10 min. After cooling to 25ºC, 1.0 ml of arseno-molybolic acid reagent was added to each tube and the volume made up to 10 ml with double-distilled water. After 10 min, the absorbance (blue colour) was read using a spectrophotometer at 620 nm. The total amount of RS present in each sample was then calculated from a standard curve.

The TS contents of shoot buds were estimated by the anthrone method (Thimmaiah, 2004). Exactly 100 mg of young leaf tissue from each tree was hydrolysed with 5.0 ml 2.5 M HCl in a boiling water bath for 3 h, then neutralised with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 2,000 × *g* (Sorvall MTX 150 Micro-Ultracentrifuge; Thermo Fisher Scientific, Waltham, MA, USA) for 15 min at ambient temperature. The supernatant was collected and a 1.0 ml sample used for analysis. Four ml of anthrone reagent was added to this aliquot and heated to 90ºC in a water bath for 10 min. The sample was cooled rapidly and the green to dark-green colour that formed was measured at 630 nm in a spectrophotometer, as above.

To measure TP, shoot buds (Mallik and Singh, 1980) were used. Each sample (approx. 500 mg) was homogenised in a cooled mortar and pestle by adding 80% (v/v) ethanol, then centrifuged at 8,000 × *g* for 20 min. The supernatant was filtered (Whatman No. 42 filter paper) and the residue was re-extracted five-times

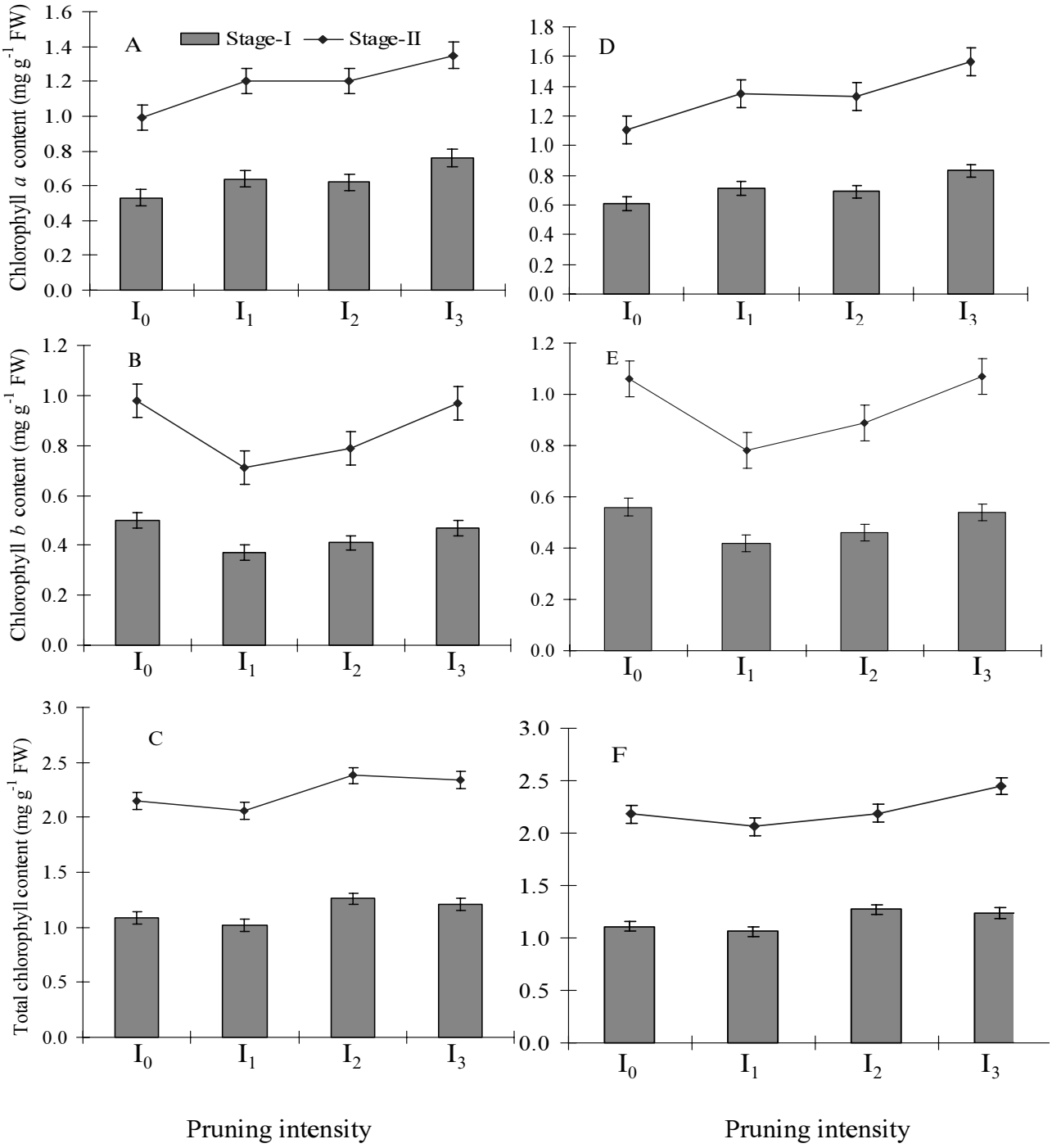

FIG. 1
Effect of pruning intensity (I_0 – I_3) on the chlorophyll *a* (Panels A, D), chlorophyll *b* (Panels B, E), and total chlorophyll (Panels C, F) contents of young leaves on three mango cultivars planted at high density. Data were recorded in 2005–2006 (Panels A–C) and in 2006–2007 (Panels D–F). Stage-I, 1 month after pruning. Stage-II, just after fruit bud differentiation (FBD; November – December). Vertical bars represent ±SE of the means for three replicates (one tree per replicate).

with 80% (v/v) ethanol. The supernatants collected were pooled and evaporated to dryness in a water bath (60ºC). Each residue was dissolved in 5.0 ml distilled water, from which 0.2 ml was sampled and made up to a total volume of 3.0 ml with distilled water. Exactly 0.5 ml of fresh Folin-Ciocalteau reagent (Glaxo Chemicals, Mumbai, India) was added to this. After 3 min, 2.0 ml 20% (w/v) Na_2CO_3 solution was added to each tube, mixed thoroughly, and placed in a water bath at 58ºC for exactly 1 min. The tube was then cooled to room temperature and its absorbance was measured at 650 nm in a spectrophotometer using Folin-Ciocalteau reagent as a blank.

Polyphenol oxidase (PPO) activity (catecholase and cresolase fractions) was measured in shoot buds according to the procedures suggested by Sanchez-Ferrer *et al*. (1988) and Valero *et al*. (1989). Catecholase activity was determined using 30 mM 4-methyl catechol (4-MC) as substrate in 10.0 mM sodium acetate buffer (pH 4.5). Four ml of 100 mM phosphate buffer (pH 7.3) was added to 0.5 ml of each crude shoot-bud extract. At time zero, 0.5 ml of 30 mM 4-MC

in 10 mM sodium acetate buffer (pH 4.5) was added to this mixture. The increase in absorbance at 400 nm due to the appearance of the corresponding 4-methyl-*p*-benzoquinone at 30ºC was measured spectrophotometrically. The optical density was recorded after 8 min. PPO activity was expressed as ΔA_{400} g^{-1} fresh weight (FW) min^{-1}. To measure cresolase activity, 0.5 mM 4-methylphenol (*p*-cresol) in 10.0 mM phosphate buffer pH 7.0 was used as substrate, as reported by Sanchez-Ferrer *et al*. (1988).

A rapid colorimetric method (Bates *et al*., 1973) was employed to estimate proline contents. Approx. 500 mg of fresh shoot tip with a few small leaves was homogenised in 10 ml of 3% (w/v) sulphosalicylic acid and filtered through a Whatman No. 2 filter paper. Two ml of the filtrate was reacted with 2.0 ml acid ninhydrin reagent and 2.0 ml glacial acetic acid in a test-tube for 1 h in a water bath at 100ºC. The reaction was terminated by placing the test-tube in an ice bath. Four ml of 100% (v/v) toluene was then added and mixed for 15 – 20 s using a vortex mixer. The chromophore-containing toluene fraction was aspirated from the aqueous phase using a micropipette, warmed to room temperature (25ºC), and its absorbance was read at 520 nm using toluene as the blank. The proline concentration in each sample was determined from a standard curve using analytical grade proline (SRL Chemicals) and calculated on a g^{-1} FW basis.

Statistical analysis

The experiment was arranged as a randomised block design with a factorial treatment structure (four pruning intensities and three mango cultivars). Each tree in a block was treated as an experimental unit, and randomly assigned for pruning. All treatment combinations (12) were replicated three times. The data were subjected to statistical analysis using two-factor ANOVA (Gomez and Gomez, 1984) and interpretation of the results was based on the F-test. Percentage data were subjected to arcsine √% transformation before analysis to provide a normal distribution. The critical difference (CD) at $P = 0.05$ was used to compare means.

RESULTS AND DISCUSSION

Chlorophyll contents of mango leaves

Chlorophyll contents varied during the vegetative and reproductive stages. Mango tree malformation has been attributed to a four-fold decrease in the chlorophyll levels (Singh and Rathore, 1983), particularly in the chlorophyll *a* contents in the leaves, which decreased with an increase in the number of malformed shoots in 'Dashehari' mango trees under sub-tropical conditions, as in New Delhi. Severe pruning led to the highest leaf chlorophyll *a* content (Figure 1A,C), while moderate pruning enhanced chlorophyll *b* and TC levels in the 'on'-year. Severe pruning resulted in a higher number of young leaves which contained higher levels of chlorophyll *a* and TC (Majumder and Chatterjee, 1972) than in non-pruned trees which may have resulted in a substantial reduction in floral malformation (Singh, 2007) after pruning. Leaf chlorophyll contents increased during the phase of active growth and declined thereafter, with increasing leaf maturity. Non-pruned trees had higher chlorophyll *b* contents during the 'off'-year (the vegetative phase); while, during the 'on'-year, they had the same maximum value as in moderately-pruned trees (Figure 1B, E) because of the decrease in light intensity (Kappel and Flora, 1983). Furthermore, chlorophyll *a* and TC contents were highest in severely-pruned 'Dashehari' trees; while chlorophyll *b* contents were highest in moderately-pruned 'Mallika' trees (Table I). Total chlorophyll contents also increased after moderate-pruning (Bhanu Pratap, 2002) compared to light or non-pruned mango trees (Figure 1C, F). Chlorophyll *a* contents decreased at the pre-flowering stage compared to the vegetative stage (Suryanarayana, 1981). However, the intensity of pruning did not significantly improve TC contents immediately after FBD (Stage II) during the 'off'-year. At Stage-I, the total chlorophyll contents were higher compared to Stage-II in both years of the experiment.

Reducing sugar (RS) and total sugar (TS) contents in shoot buds

Current year shoot buds that emerged after severe pruning (I_3) had higher levels of RS (Figure 2A, B) and

TABLE I

Effect of cultivar and pruning intensity on chlorophyll a, b *and total chlorophyll contents in young leaves of three mango cultivars planted at high density*

	Chlorophyll *a* (mg g^{-1} FW)				Chlorophyll *b* (mg g^{-1} FW)				Total chlorophyll (mg g^{-1} FW)			
	2005–2006*		2006–2007**		2005–2006*		2006–2007**		2005–2006*		2006–2007**	
Treatment	I[#]	II	I	II	I	II	I	II	I	II	I	II
V_1I_0	0.55[$]	1.00	0.63	1.10	0.44	0.92	0.49	0.97	1.02	2.16	1.06	2.08
V_1I_1	0.96	1.70	1.03	1.92	0.27	0.47	0.33	0.48	1.10	2.16	1.16	2.14
V_1I_2	0.39	0.81	0.45	0.85	0.32	0.68	0.39	0.75	0.93	1.74	0.92	1.72
V_1I_3	0.50	0.99	0.56	1.11	0.37	0.78	0.44	0.86	0.94	1.83	0.97	1.99
V_2I_0	0.55	1.03	0.62	1.13	0.54	0.99	0.60	1.06	1.10	2.05	1.11	2.12
V_2I_1	0.54	1.04	0.61	1.21	0.53	1.06	0.58	1.16	1.11	2.42	1.18	2.44
V_2I_2	0.63	1.14	0.67	1.27	0.69	1.35	0.75	1.50	1.46	2.70	1.40	2.83
V_2I_3	0.62	1.02	0.68	1.24	0.55	1.14	0.64	1.29	1.21	2.43	1.27	2.54
V_3I_0	0.48	0.96	0.57	1.11	0.52	1.02	0.50	1.16	1.15	2.23	1.18	2.36
V_3I_1	0.42	0.78	0.49	0.93	0.31	0.61	0.35	0.69	0.86	1.60	0.84	1.62
V_3I_2	0.85	1.65	0.95	1.87	0.22	0.34	0.24	0.43	1.39	2.68	1.41	2.04
V_3I_3	1.17	2.04	1.25	2.35	0.48	0.97	0.55	1.06	1.50	2.77	1.47	2.83
P^a (V)	0.09	0.15	0.09	0.17	0.08	0.13	0.08	0.14	0.15	0.25	0.14	0.20
P^b (I)	0.11	0.18	0.11	0.19	0.09	0.15	0.09	0.16	0.18	n.s	0.17	0.32
P^c (V × I)	0.19	0.31	0.18	0.34	0.16	0.26	0.16	0.28	0.31	0.50	0.29	0.56

* 'off'-year, ** 'on'-year. Data are the means of three trees (replicates). [$]Values are means ± SE (n = 3).
[a,b,c]Significant at $P \leq 0.05$ after factorial analysis of the variance. P = LSD. [a]Cultivar (V); [b]Pruning intensity (I); [c](V × I).
[#]Stage I: 1 month after pruning; [#]Stage II: just after fruit bud differentiation (FBD; November – December).
V_1 = 'Amrapali'; V_2 = 'Mallika'; V_3 = 'Dashehari'; I_0 = non-pruned (control), I_1 = lightly-pruned, I_2 = moderately-pruned, I_3 = severely-pruned.

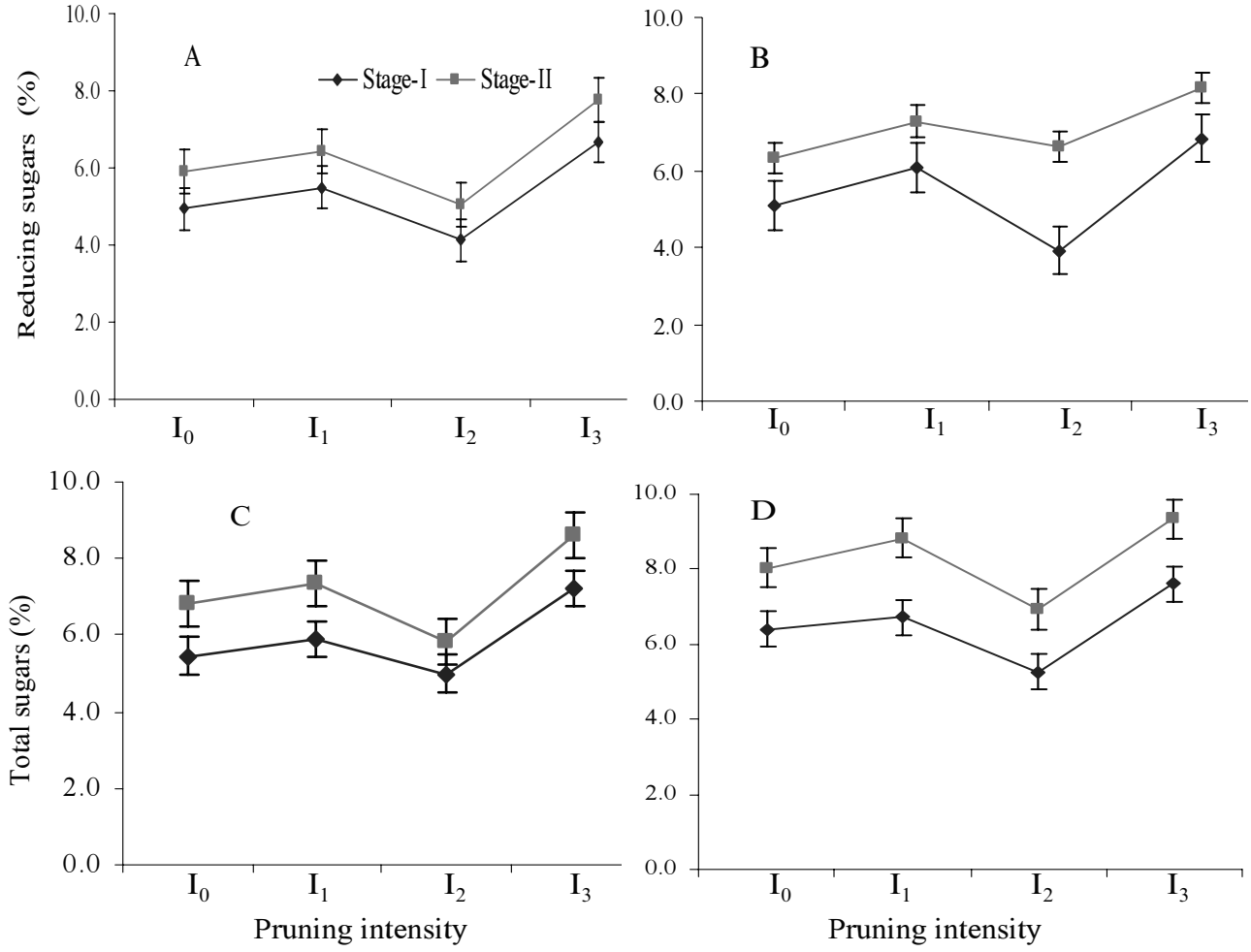

FIG. 2
Effect of pruning intensity (I_0 – I_3) on reducing sugar (Panels A, B) and total sugar (Panels C, D) contents in shoot buds in three mango cultivars planted at high density in 2005–2006 (Panels A, C) and 2006–2007 (Panels B, D). Stage-I, 1 month after pruning. Stage-II, just after fruit bud differentiation (FBD; November – December). Vertical bars represent ±SE of the means for three replicates (one tree per replicate).

TS (Figure 2C, D) than in previous year shoots. Increased RS and TS levels may have some positive effects on floral induction, or there may be an intimate relationship between synthesis of the flowering hormone, if any, and the accumulation of total carbohydrates (Sen *et al*., 1965; Bagchi *et al*., 2008). In this study, the numbers of non-flowering shoots were higher in severely-pruned trees than in moderately-pruned trees (Devi and Tyagi, 1991). Due to the V × I interaction (Table II), higher RS and TS levels were recorded in severely-pruned 'Amrapali' trees (9.83% and 11.16%, respectively) in the 'off'-year. Pruning did not have a significant effect on RS contents after FBD in the 'on'-year. It was noteworthy that the RS and TS contents were higher at FBD (Stage II) than at the vegetative growth stage (Stage I). Such variations in sugar content may be due to a root:shoot imbalance and the smaller canopy volume (Jyothi *et al*., 2000; Rawash *et al*., 1984; Pathak and Pandey, 1978; Ravi Shankar and Rao, 1982; Singh *et al*., 2009a).

Total phenolics (TP) contents and polyphenol oxidase (PPO) activities in shoot buds

Among the three cultivars, 'Amrapali' trees had the highest TP contents due to their dwarf stature (Chandrababu *et al*., 1985) compared to the more vigorous 'Dashehari' trees. The shoot tissues that emerged in the 'off'-year had higher levels of TP than in the 'on'-year (Mishra and Dhillon, 1981). Moderate (I_2) and light-pruning (I_1) of mango trees significantly increased the TP contents of shoot buds (to 7.60 and 7.35 mg g^{-1} FW, respectively) compared to non-pruned control trees (6.59 mg g^{-1} FW; Figure 3A, B). The former also showed normal FBD as their TP levels increased at the vegetative stage (Patil *et al*., 1992a). Likewise, moderate-pruning in 'Amrapali' trees (V_1I_2) resulted in an accumulation of TP in shoot buds, while non-pruned 'Mallika' trees had the lowest TP contents (Table III). Thus, TP contents decreased with an increase in pruning intensity in mango. Pruned trees also had higher levels of indoleacetic acid (IAA) and lower IAA-oxidase activities, which may have resulted in higher accumulations of TP in shoot buds during the 'off'-year than the 'on'-year (Rawash *et al*., 1984; Murti and Upreti, 2003; Singh *et al*., 2009b).

PPO activities (catecholase and cresolase fractions) declined at FBD (Stage II) during the 'on'-year. Annual-bearing cultivars such as 'Amrapali' exhibited lower PPO activities and showed a higher incidence of malformation (Singh, 2007) compared to biennial-bearing cultivars.

TABLE II
Effect of cultivar and pruning intensity on reducing sugars and total sugars contents in shoot buds of three mango cultivars planted at high density

	Reducing sugar (%)				Total sugars (%)			
	2005–2006*		2006–2007**		2005–2006*		2006–2007**	
Treatment	I[#]	II	I	II	I	II	I	II
V_1I_0	3.90[$]	4.86	3.76	5.10	4.0	5.86	5.16	7.10
V_1I_1	9.06	10.20	10.06	12.16	8.93	11.30	10.10	12.83
V_1I_2	1.56	2.00	1.36	3.40	1.73	2.96	2.72	4.23
V_1I_3	9.83	11.16	10.03	11.50	9.96	12.00	11.16	13.16
V_2I_0	8.86	9.83	9.50	10.07	9.33	10.63	10.21	11.71
V_2I_1	4.50	5.21	4.30	5.86	5.20	6.20	5.00	7.53
V_2I_2	6.73	7.60	5.73	7.73	5.00	8.30	7.96	9.40
V_2I_3	4.62	5.16	4.51	5.05	5.00	6.06	4.05	6.58
V_3I_0	2.03	3.00	2.07	3.24	3.03	3.96	3.83	5.27
V_3I_1	2.93	3.85	3.33	4.11	3.56	4.60	4.20	6.10
V_3I_2	4.08	5.50	4.70	5.76	6.66	7.80	6.70	8.30
V_3I_3	5.00	6.81	6.0	7.15	6.66	7.80	6.70	8.30
P^a (V)	0.67	0.67	0.73	1.62	0.75	0.68	0.99	0.96
P^b (I)	0.77	0.77	0.85	n.s.	0.87	0.91	1.14	1.10
P^c (V × I)	1.33	1.34	1.47	3.25	1.51	1.37	1.97	1.92

*'off'-year, **'on'-year. Data are the means of three trees (replicates).
[$]Values are means ± SE (n = 3). [a,b,c]Significant at $P \leq 0.05$ after factorial analysis of the variance.
LSD ($P \leq 0.05$) represented as P. [a]Cultivar (V); [b]Pruning intensity (I); [c](V × I).
[#]Stage I: 1 month after pruning; [#]Stage II: just after fruit bud differentiation (FBD; November – December).
V_1= 'Amrapali'; V_2 = 'Mallika'; V_3 = 'Dashehari'; I_0 = non-pruned (control), I_1 = lightly-pruned, I_2 = moderately-pruned, I_3 = severely-pruned.

Thus, PPO activity was inversely related both to flowering and the incidence of malformation, and declined during FBD in the 'on'-year (Sharma *et al*., 2001). PPO activities were found to be highest in severely-pruned trees and lowest (0.470, 0.467; 0.419, 0.401 ΔA_{400} g^{-1} min^{-1} for catecholase at Stage I and Stage II, and for cresolase at Stage I and Stage II, respectively) in lightly-pruned trees in an 'on'-year (2005–2006); thus justifying an increase in pruning intensity, which resulted in higher PPO activities (Table III). After pruning, mango plants may induce a self-defense mechanism by increasing the activities of both PPO enzyme fractions (Bagchi *et al*. 2008). Thus, increased severity of pruning not only minimised the incidence of malformation (Singh, 2007), but also increased PPO activities. TP contents and PPO activities decreased from the vegetative to the reproductive stages, and also from 'off'-year shoots to 'on'-year shoots, with a negative correlation found between PPO activity and the number of flowering panicles (Singh, 2007).

Proline contents

Biennial-bearing in mango has a more profound effect on proline contents than the regular pattern of flowering. In the present study, the proline contents of shoot buds with a few leaves were greater in non-pruned trees due to a higher incidence of floral malformation. It is notable that increased pruning intensity decreased proline contents (Table IV; Singh, 2007). The proline contents of malformed, non-pruned seedlings were higher than in healthy seedlings due to a higher rate of biosynthesis in malformed tissues and improved translocation of amino acids from healthy to malformed tissues (Singh and Dhillon, 1994). The new shoots that emerged after

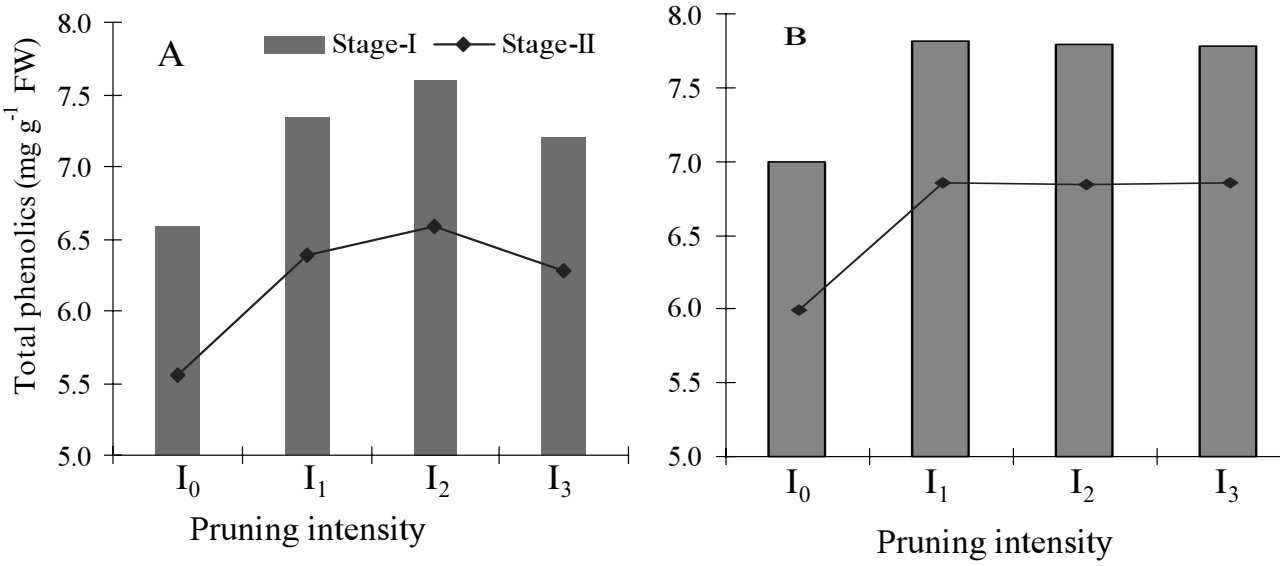

FIG. 3
Effect of pruning intensity (I_0 – I_3) on the total phenolics contents of shoot buds of three mango cultivars planted at high density in 2005–2006 (Panel A) and 2006–2007 (Panel B). Stage-I, 1 month after pruning. Stage-II, just after fruit bud differentiation (FBD; November – December). Vertical bars represent ±SE of the means for three replicates (one tree per replicate).

TABLE III

Effect of cultivar and pruning intensity on total phenolics contents and the activities of polyphenol oxidase in shoot buds of three mango cultivars planted at high density

	Total phenolics (mg g^{-1} FW) in shoot buds				PPO activity (ΔA_{400} g^{-1} min^{-1})							
					Catecholase				Cresolase			
	2005–2006*		2006–2007**		2005–2006*		2006–2007**		2005–2006*		2006–2007**	
Treatment	I[#]	II	I	II	I	II	I	II	I	II	I	II
V_1I_0	8.35$	7.30	8.00	6.98	0.478	0.467	0.476	0.459	0.496	0.481	0.488	0.474
V_1I_1	8.11	7.08	7.75	6.77	0.263	0.261	0.261	0.252	0.334	0.320	0.326	0.314
V_1I_2	8.88	7.93	7.88	6.96	0.541	0.535	0.535	0.526	0.481	0.470	0.466	0.462
V_1I_3	7.60	6.91	7.58	6.70	0.851	0.864	0.857	0.846	0.499	0.468	0.472	0.479
V_2I_0	4.61	3.56	5.18	4.20	0.781	0.766	0.774	0.758	0.484	0.475	0.478	0.462
V_2I_1	7.01	6.00	7.86	6.88	0.815	0.806	0.811	0.778	0.477	0.464	0.465	0.447
V_2I_2	7.01	5.96	7.85	6.20	0.863	0.852	0.856	0.842	0.489	0.457	0.473	0.459
V_2I_3	6.98	6.02	7.93	7.02	0.602	0.556	0.572	0.544	0.534	0.509	0.517	0.502
V_3I_0	6.80	5.85	7.81	6.80	0.379	0.357	0.379	0.349	0.484	0.473	0.476	0.463
V_3I_1	6.90	6.08	7.83	6.72	0.331	0.333	0.330	0.320	0.447	0.420	0.431	0.416
V_3I_2	6.90	5.86	7.65	6.68	0.356	0.355	0.347	0.335	0.449	0.421	0.428	0.411
V_3I_3	7.00	5.92	7.82	6.83	0.372	0.356	0.364	0.347	0.453	0.435	0.450	0.424
P^a (V)	0.202	0.194	0.097	0.085	0.006	0.009	0.007	0.009	0.007	0.009	0.009	0.007
P^b (I)	0.234	0.225	0.112	0.099	0.008	0.010	0.008	0.012	0.009	0.010	0.010	0.009
P^c (V × I)	0.400	0.300	0.194	0.171	0.013	0.016	0.014	0.019	0.105	0.018	0.018	0.015

* 'off'-year, ** 'on'-year. Data are the means of three trees (replicates). $Values are means ± SE (n = 3).
[a,b,c]Significant at $P \leq 0.05$ after factorial analysis of the variance. [a]Cultivar (V); [b]Pruning intensity (I); [c](V × I). LSD ($P \leq 0.05$) is represented as *P*.
[#]Stage I: 1 month after pruning; [#]Stage II: just after fruit bud differentiation (FBD; November – December).
V_1 = 'Amrapali'; V_2 = 'Mallika'; V_3 = 'Dashehari'; I_0 = non-pruned (control), I_1 = lightly-pruned, I_2 = moderately-pruned, I_3 = severely-pruned.

pruning had higher total free amino acid contents due to their requirement for vegetative growth (Bagchi *et al*., 2008). The emergence of new shoots and young leaves with a low incidence of malformation after pruning (Singh, 2007), delayed senescence, but reduced proline contents (Patil *et al*., 1992b; Singh and Dhillon, 1994) in shoot buds. The higher rates of cell division in severely-pruned trees, due to an increase in the rates of kinetin synthesis, lowered the cysteine, leucine and proline contents of mango fruit (Kumar *et al*., 1991). Non-pruned 'Dashehari' trees had the highest proline contents, which decreased after pruning, but no clear trend was noted in the other mango cultivars tested (Table IV). Shoot buds had higher proline contents in the 'on'-year than the 'off'-year, confirming the fact that proline contents were usually higher during FBD (Rao *et al*., 1982).

In conclusion, different intensities of pruning had profound effects on the biochemical status of mango trees which, in turn, had a direct influence on the vegetative growth and flowering parameters in three varieties of mango planted at high densities. The effects varied with the bearing habit of the genotype and with the intensity of pruning. Total chlorophyll and chlorophyll *b* contents were enhanced as a result of moderate pruning. Moderate pruning (60 cm from the shoot tip) in high-density mango orchards can therefore be adopted to restore optimum vegetative growth and fruiting.

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TABLE IV

Effect of cultivar and pruning intensity on proline contents of shoot buds in three mango cultivars planted at high density

	Proline content (µg g^{-1} FW)			
	2005–2006*		2006–2007**	
Treatment	I[#]	II	I	II
V_1I_0	19.63[$]	22.50	18.63	21.66
V_1I_1	22.86	24.30	21.63	23.16
V_1I_2	31.83	33.53	30.00	35.10
V_1I_3	12.33	14.46	11.40	13.46
V_2I_0	25.76	27.20	24.70	27.0
V_2I_1	27.65	28.63	26.06	27.63
V_2I_2	16.16	18.16	14.90	16.66
V_2I_3	22.86	26.03	21.20	23.08
V_3I_0	36.23	38.90	34.78	37.30
V_3I_1	29.68	30.86	28.28	29.43
V_3I_2	31.03	32.03	29.80	30.96
V_3I_3	19.06	21.03	17.90	19.36
P^a (V)	5.37	5.65	5.44	5.79
P^b (I)	2.16	2.27	2.18	2.33
P^c (V × I)	10.75	11.31	14.52	11.59

*'off'-year, **'on'-year. Data are the means of three trees (replicates).
[$]Values are means ± SE (n = 3). [a,b,c]Significant at $P \leq 0.05$ after factorial analysis of the variance.
LSD ($P \leq 0.05$) is represented as *P*. [a]Cultivar (V); [b]Pruning intensity (I); [c](V × I).
[#]Stage I: 1 month after pruning; [#]Stage II: just after fruit bud differentiation (FBD; November – December).
V_1= 'Amrapali'; V_2 = 'Mallika'; V_3 = 'Dashehari'; I_0 = non-pruned (control), I_1 = lightly-pruned, I_2 = moderately-pruned, I_3 = severely-pruned.

REFERENCES

BAGCHI, T. B., SUKUL, P. and GHOSH, B. (2008). Biochemical changes during off-season flowering in guava (*Psidium guajava* L.) induced by bending and pruning. *Journal of Tropical Agriculture*, **46,** 64–66.

BARNASE, J. D., BALAGUAR, L., MAURIGUE, E., ELVIRA, S. and DAVISON, A. W. (1992). A reappraisal of the use of DMSO for the extraction and determination of chlorophyll '*a*' and '*b*' in lichens and higher plants. *Environmental and Experimental Botany*, **32,** 87–99.

BATES, L. S., WALDREN, R. D. and TEARE, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, **39,** 205–207.

BHANU, P. (2002). *Morpho-Physiological and Biochemical Changes Brought About by Pruning in Mango cv. Amrapali Under High Density Orcharding*. Ph.D. Thesis. Post-Graduate School, Indian Agricultural Research Institute, Pusa, New Delhi. India. 94 pp.

CHANDRABABU, R., VIJAYAN, K. P., NATRAJARATNAM, N. and DHARMARAJ, G. (1985). Phenolics and growth habit in mango (*Magnifera indica* L.) varieties. *Current Science*, **4,** 437–438.

DEVI, T. M. and TYAGI, D. N. (1991). Physiology of mango (*Mangifera indica* L.): Fractions of carbohydrates, nitrogen and related enzymes in leaves of flowered and non-flowered shoots of mango. *Indian Journal Plant Physiology*, **34,** 30–36.

FIVAZ, J. and STASSEN, P. J. C. (1996). The effect of training systems, time of pruning together with fruit thinning on tree development, yield and fruit size of 'Sensation' mangoes. *Intigtings Bulletin – Instituut-vir-Tropiese-en-Sub Tropiese-Gewasse*, **285,** 23–38.

GOMEZ, K. A. and GOMEZ, A. A. (1984). *Statistical Procedures for Agricultural Research*. 2nd Edition. John. Wiley and Sons. Inc., New York, NY, USA. 680 pp.

JYOTHI, M. H., SOPPIN, R. and EKBOTE, S. (2000). Biochemical changes in the leaf of bearing and non-bearing trees of some mango varieties. *Advances in Agricultural Research in India*, **10,** 17–23.

KAPPEL, F. and FLORE, J. A. (1983). Effect of shade on photosynthesis, specific leaf weight, leaf chlorophyll content and morphology of young peach trees. *Journal of the American Society for Horticultural Science*, **108,** 541–544.

KUDEN, A. and SON, L. (2000). Pruning affects carbohydrate accumulation in the shoots and leaves of 'Precoce de Tyrinth' apricot. *Journal of Horticultural Science & Biotechnology*, **75,** 539–541.

KUMAR, S., DAS, D. K. and PRASAD, V. S. (1991). Free amino acid composition in fruits of two mango cultivars during maturation and ripening. *Indian Journal of Plant Physiology*, **34,** 1–8.

MACZULAITYS, D. C., DISQUET, I. L. and BORY, G. (1999). Pruning stress: Changes in the tree physiology and their effects on the tree health. *Acta Horticulturae*, **496,** 61–67.

MAJUMDER, B. C. and CHATTERJEE, S. K. (1972). Biochemical studies on leaves with their ageing process in mango (*Mangifera indica* L.) cultivars. II: Changes of chlorophyll contents. *Punjab Horticultural Journal*, **12,** 111–115.

MAJUMDER, P. K., SHARMA, D. K. and SINGH, R. N. (1982). Study on high density orcharding on mango (*Mangifera indica* L.). *Punjab Horticultural Journal*, **22,** 123–127.

MALLIK, C. P. and SINGH, M. B. (1980). *Plants Enzymology and Histoenzymology*. Kalyani Publishers, New Delhi, India. 286 pp.

MISHRA, K. A. and DHILLON, B. S. (1981). Ribonucleic acid, proteins, phenols and amino acids in the leaves in relation to fruit bud differentiation in 'Langra' mango. *Indian Journal of Agricultural Sciences*, **51,** 447–449.

MURTI, G. S. R. and UPRETI, K. K. (2003). Endogenous hormones and phenols in rootstock seedling of mango cultivars and their relationship with seedling vigour. *European Journal of Horticultural Science*, **68,** 2–7.

OOSTHUYSE, S. A. (1997). Some principle pertaining to mango pruning and the adopted practices of pruning mango trees in South Africa. *Acta Horticulturae*, **455,** 413–421.

PANDEY, S. and TYAGI, D. N. (1999). Changes in chlorophyll content and photosynthetic rate of four cultivars of mango during reproductive phase. *Biologia Plantarum*, **42,** 457–461.

PATHAK, R. A. and PANDEY, R. M. (1978). Changes in the chemical composition of mango (*Mangifera indica* L.) leaves cv. Dashehari at different stages of flowering and fruit growth. *Indian Journal of Horticulture*, **35,** 309–313.

PATIL, P. B., RAO, M. M., BASARKAR, P. W., SRINIVASAN, C. N. and NALWADI, U. G. (1992a). Physiological and biochemical factors associated with fruit bud differentiation in 'Alphonso' mango: V – Total free amino acids. *Karnataka Journal of Agricultural Sciences*, **5,** 224–228.

PATIL, P. B., RAO, M. M., SRINIVASAN, C. N. BASARKAR, P. W. and NALWADI, U. G. (1992b). Physiological and biochemical factors associated with fruit bud differentiation in 'Alphonso' mango: VI – Total free phenols and polyphenol oxidase. *Karnataka Journal of Agricultural Sciences*, **5,** 338–342.

PONGSOMBOON, W., SUBHADRABANDHU, S. and STEPHENSON, R. A. (1997). Some aspects of eco-physiology of flowering intensity in mango (*Mangifera indica* L.). cv. Nam Dok Mai in a semi-tropical monsoon Asian climate. *Scientia Horticulturae*, **70,** 45–56.

RAM, S. and SIROHI, S. C. (1988). Studies on high density orcharding in mango cv. Dashehari. *Acta Horticulturae*, **231,** 339–344.

RAM, S., SINGH, C. P. and KUMAR, S. (1997). A success story of high density orcharding in mango. *Acta Horticulturae*, **455,** 375–382.

RAO, M. M., RAVI SHANKAR, H. and BOJAPPA, K. M. (1982). Amino-acid composition of shoots of 'Alphonso' mango at pre and post fruit bud differentiation stage. *South India Horticulture*, **30,** 1–3.

RAO, V. N. M. and SHANMUGAVELU, K. G. (1975). Mango responds to pruning. *Indian Horticulture*, **20,** 5–6.

RAVI SHANKAR, H. and RAO, M. M. (1982). Studies on changes in carbohydrate fraction and minerals in 'Alphonso' mango shoots. *Journal of Maharashtra Agricultural Universities*, **7,** 143–145.

RAWASH, M. A., NABAWY, S. E., HAMMADY, E. A., KHALIFA, A. and MASRY, E. H. (1984). Flowering of mango trees in relation to chemical composition of shoots. *Egyptian Journal of Horticulture*, **11,** 19–30.

SANCHEZ-FERRER, A., BRU, R., CABANES, J. and GARCIA-CARMONA, F. (1988). Characterization of catecholase and cresolase activities of grape polyphenol oxidase. *Phytochemistry*, **27,** 319–320.

SAUCO, V. G. (1996). Horticultural practices of mango. *Acta Horticulturae*, **455,** 391–400.

SEN, P. K., SEN, S. and CHAUDHARY, T. D. (1965). Carbohydrate and nitrogen contents of mango shoots in relation to their fruit bud formation. *Indian Agriculturists*, **9,** 133–140.

SHARMA, R. R. and SINGH, R. (2006). Pruning intensity modifies canopy micro-climate and influences sex ratio, malformation incidence and development of fruited panicles in 'Amrapali' mango (*Mangifera indica* L.). *Scientia Horticulturae*, **109,** 118–122.

SHARMA, R. R., GOSWAMI, A. M., SINGH, C. N., CHHONKAR, O. P. and SINGH, G. (2001). Catecholase and cresolase activities and phenolic content in mango (*Mangifera indica* L.) at panicle initiation. *Scientia Horticulturae*, **87,** 147–151.

SINGH, P. N. and RATHORE, V. S. (1983). Changes in moisture content, dry matter accumulation, chlorophyll and nutrient contents in mango malformation. *Indian Journal of Horticulture*, **40,** 21–25.

SINGH, S. K. (2007). *Effect of Pruning on Growth, Yield and Fruit Quality of Some Mango Cultivars Planted under High Density*. Ph.D. Thesis. Post-Graduate School, Indian Agricultural Research Institute, New Delhi, India. 118 pp.

SINGH, S. K., SINGH, S. K., SHARMA, R. R. and SRIVASTAV, M. (2009a). Effect of pruning on morpho-physiological parameters and micro-climate under high density planting of mango (*Mangifera indica* L.) *Indian Journal of Agricultural Sciences*, **79,** 632–635

SINGH, S. K., SINGH, S. K. and SHARMA, R. R. (2009b). Endogenous phytohormones after pruning in three mango cultivars planted under high density. *Indian Journal of Plant Physiology*, **14,** 392–396

SINGH, Z. and DHILLON, B. S. (1994). Metabolic changes associated with vegetative malformation of mango (*Mangifera indica* L.). *Tropical Agriculture*, **71,** 320–322.

SURYANARAYANA, V. (1981). Influence of growth retardants on respiration, chlorophyll and carotenoids pigments in mango leaves in relation to flowering. *Indian Journal of Horticulture*, **38,** 29–31.

THIMMAIAH, S. K. (2004). *Standard Methods of Biochemical Analysis*. Kalyani Publishers, New Delhi, India. 545 pp.

VALERO, E., SANCHEZ-FERRER, A., VARON, V. and GARCIA-CARMONA, F. (1989). Evolution of grape polyphenol oxidase activity and phenolic content pattern during maturation and vinification. *Vitis*, **28,** 85–95.

YAMASHITA, T. and FUZINO, A. (1994). Effects of pruning of young and old shoots on ribulose bisphosphate carboxylase and other constituents in leaves of the mulberry tree (*Morus alba* L.). *Journal of Experimental Botany*, **37,** 1836–1841.