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SHORT COMMUNICATION

ENDOGENOUS PHYTOHORMONES AFTER PRUNING IN THREE MANGO CULTIVARS PLANTED UNDER HIGH DENSITY

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In both biennial and regular bearing mango cultivars, there was significant effect of pruning on the level of endogenous hormones in shoot buds. The higher IAA, ABA and lower GAs were found in 'on' year of mango shoots. The IAA was highest in Mallika (4.57, 4.62 µg g^{-1} fw) while highest ABA (0.52, 0.55 µg g^{-1} fw) and lowest IAA (0.297, 0.310 µg g^{-1} fw) were recorded in Amrapali. Shoot-tip pruning removes the source of apical dominance and stimulate axillary bud development. Moderately pruned trees did show highest IAA and moderately pruned Mallika had the highest (4.63, 4.70 µg g^{-1} fw) IAA while lowest was in un-pruned Amrapali trees. The lowest ABA (0.14, 0.09 µg g^{-1} fw) and highest GA like substances (1.36, 1.31 µg g^{-1} fw) were found in the buds of Dashehari while lowest level of GA-like substances (0.78, 0.75 µg g^{-1} fw) were estimated in Amrapali. The control trees had the lowest IAA and the highest GA-like substances while lowest level of GA-like substances was in moderately pruned trees. The un-pruned Dashehari trees had the highest GA-like substances while lowest was found in light pruned Mallika. Light pruned Amrapali showed higher ABA while the lowest was estimated in moderately pruned Dashehari.

***Key words*: ABA, GA, IAA, mango, pruning**

The pruning is an unavoidable necessity of virtually all arboreal fruit crops and adopted to maintain proper physiological balance between growth and fruiting. In mango (*Mangifera indica* L.) it is more important (in subtropics and tropics) due to its tendency for giving frequent flushes, especially in humid tropics. Pruning also prevents trees from getting large and annual pruning regulates flower management programme, reshape to intermediate size of tree or completely rejuvenate large trees that are no longer productive due to their size and height (Davenport 2006). The pruning is an old age orchard practice in deciduous and temperature fruit crops, *viz*. apple, pear, peach, plum etc. and in sub-tropical species, *viz.* grapes, fig and *phalsa* (Rao and Shanmugavelu 1975). The high density orcharding (HDO) in mango has been successful in some cultivars, *viz*. Amrapali (2.5 m x 2.5 m), Mallika (6 m x 6 m) and Dashehari (3.0 m x 2.5 m) with the help of pruning and also supplemented with paclobutrazol (Mazumder *et al*. 1982, Ram and Sirohi 1988, Ram *et al*. 1997). Nevertheless, above cultivars show sharp decline in yield and quality after some years of fruiting owing to overlapping/intermingling of branches, poor light interception, poor photosynthetic rate, high relative humidity and proneness to diseases and pests etc. This condition changes the physiology of shoots or whole tree in such a way that it is not economically productive in long run. Therefore, the time and severity of pruning not only alter physiological make up of the trees but also change the biochemical behaviour, which is the indicator

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of flowering, bearing behaviour and yield patterns. Pruning reduces apical dominance and both growth promoting and inhibiting substance are found in fruiting shoots/ fruits of different stages (Singh and Singh 1972). Pruning and thinning or thinning alone enhanced the leaf ethylene level and the percentage of flowering in mango (Saida *et al.* 1983). The diffusible IAA decreased to a low level in shoot tips and ABA increased dramatically during early flower bud formation. At the same time total cytokinin-like activity increased in the xylem sap, reaching a maximum level at full bloom (Wen Saw Chen 1987). The GA_3-like substance in mango are high (1.5-1.7 µg equivalent g^{-1} fw) at the beginning of dormant period, but declined (about 50%) prior to inflorescence emergence. The change in ABA-like substance is comparatively less during the dormant period and negatively correlated with GA-like substance (Pongsomboon *et al.* 1997). Floral malformation in mango is also associated with higher level of ABA, GA and zeatin but with low level of IAA (Singh 2000). While pruning, it is useful to consider the activity of the growth promoters (gibberellins and cytokinins) and inhibitors (auxins and abscisic acid). In this article, results are presented to illustrate the effect of pruning on endogenous hormonal activities in some mango cultivars (Amrapali, Mallika and Dashehari) growing under high density.

The field experiments were conducted at the Main Orchard, Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi, during 2005-07. Three mango cultivars, *viz*. Amrapali (V_1), Mallika (V_2) and Dashehari (V_3) planted under high density at 2.5 m x 2.5 m, 4.0 m x 3.0 m and 3.0 m x 2.0 m, respectively were selected and maintained under uniform cultural practices. Pruning was done in mid August, 2005 as follows: I_0 (control) un-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. Each variety had three replications with three trees/ treatment. The experiment was conducted under factorial randomized block design. The balanced pruning was performed in all directions of the canopy, which were dense and over-crowded. The control trees were left as such without pruning. As a result of pruning, the trees did show mild-flowering/ fruiting in 2005-06 and was referred as 'off' year and following year (2006-07) as 'on' year. For estimation of hormone level, initially apical shoot buds (apices) were periodically collected [at flower bud differentiation (FBD)stage] in cool ice box, rinsed (3-4 times) with double distilled water and preserved in ethanol (80% v/v) and stored in deep freeze (-20^{0}C). The method of Takahasi and Yamaguchi (1986) was followed for extraction, purification and quantification of endogenous phytohormones, *viz.* IAA, GAs and abscisic acid with slight modification to check the phenolic interference. Five gram of the sample was ground in cold 10 ml absolute alcohol (99%) with pestle and mortar. The total volume was then made up to 50 ml with the absolute alcohol (99%). This was left for 24 hours at 0^{0}C for the extraction of phytohormones. The extracts were filtered through filter paper (Whatman No. 42). All the filtrates were combined and taken for further analysis while, the residue was discarded. The alcohol extract was evaporated to dryness under vacuum on a water bath (35^{0}C). The aqueous phase was added with distilled water and filtered and residue was discarded. The pH of the filtered aqueous phase was adjusted to 8.6 with 1% NaOH. This extract was then treated 3 times with equal volume of ethyl acetate. The resulting treatment separated the extract in two fractions, *viz*. upper ethyl acetate fraction and lower aqueous fraction. The upper ethyl acetate fraction was separated by micro-pipette in another tube. The lower aqueous fraction was adjusted to pH 2.8 with 1% HCl and again extracted 3 times with equal volume of ethyl acetate and allowed to separate in two fractions. The upper ethyl acetate fraction was separated and evaporated to dry under vacuum and dissolved in 1 ml of methanol (HPLC grade) for further quantitative analysis using reverse phase high performance liquid chromatography. The instrument was a Water Associate Model-244 Liquid Chromatograph (Waters Associates, Milford, MA, USA) equipped with 6000 p.s.i. pumps, model-660 solvent programmer and model 46K universal injector. The chromatographic conditions were: (i) mobile phase (water: methanol); IAA (70: 30); ABA (60: 40); GA (70: 30). (ii) flow rate: 1 ml min^{-1}(iii) λ_{max}, (a) indole-3-acetic acid (IAA) = 282 nm, (b) abscisic acid (ABA) = 254 nm, (c) gibberellic acid (GA) = 203 nm, (iv) injection volume: 20 µl (v), column and solvent temperature : ambient (25-28^{0}C). Retention time of different phytohormones was: indole-3-acetic acid (IAA): 7.84, abscisic acid (ABA): 8.66, gibberellic acid (GA): 11.30. For the quantification of amount of

hormones, the respective area of individual hormone at its designated retention time was recorded by integrator and the amount was calculated with the help of standard peak area. The quantity of the hormone was expressed as µg g^{-1} on fresh weight basis. The experimental data were subjected to statistical analysis in Randomized Block Design (Gomez and Gomez, 1984) and the interpretation of results was based on 'F' test. The critical difference (CD) at p = 0.05 was worked out for comparing the means.

Growth and developmental changes do take place due to interplay of endogenous auxins, gibberellins and abscisic acid or the ratio of growth promoter: inhibitors. Manipulating tree growth with pruning stimulates excessive vegetative growth and diminishes flower bud induction if such operation is brought about beyond a limit. Pruning treatments increased the inhibitors level which may be ethylene mediated since ethylene production following injury by pruning treatments was increased.

Data presented in Table 1, showed the highest IAA in Mallika while lowest in Amrapali, which is more or less in conformity with the finding of Chacko *et al.* (1972), that shoots of regular bearing varieties (Totapari Red Small) contain less growth promoting substance than the shoots of Dashehari on trees because their leaves are capable of synthesizing continuously every year. The pruning intensities had marked influence on IAA level in off as well as in 'on' year of experiment because high levels of auxins are necessary for flower bud differentiation in mango. The highest level of IAA was found in moderately pruned trees (I_2) [4.03 ('off' year); 4.05 µg g^{-1} fw ('on' year)] followed by severely pruned trees (I_3) whereas, lowest amount of IAA was recorded in un-pruned one (I_0) (2.09, 2.13 µg g^{-1} fw). The 'on' year had higher amount of IAA than in 'off' year is similar to the finding of Lal and Ram (1977). Auxins thus appears to play a major role in the induction of flowering. The highest IAA level was found in V_2I_2 (moderately pruned Mallika) treatment [5.28 ('off' year); 5.36 µg g^{-1} fw ('on' year)] while lowest in un-pruned Amrapali (V_1I_0) (0.218, 0.237 µg g^{-1} fw).

The GAs delay the initiation of bud break but does not determine whether the resulting flush of growth is vegetative or reproductive and showed more activities in 'off' year than in 'on' year. The level of GAs in shoot buds were also affected by different pruning intensities (Table 1) especially in 'off' year of experiment. The maximum GAs estimated in un-pruned trees (I_0) (1.236 µg g^{-1} fw) (Pratap 2002) and much reduced (1.15 µg g^{-1} fw) in moderately pruned trees (I_2) (1.027 µg g^{-1} fw) with higher concentration in 'off' year than in 'on' year. It is also presumed that in mature green leaves of un-pruned trees contained low level of GAs than at the leaf differentiation stage (Wen-Shaw Chen 1987) which is contradictory to the above finding. However, in 'on' year GAs decreased than in 'off' year is similar to finding of Pal and Ram (1978) that the endogenous level of gibberellins were much higher in 'off' year than in 'on' year (shoot tips). Further, the combination of cultivars and pruning intensities affected level of GAs in such a manner that un-pruned Dashehari (V_3I_0) showed highest activity [1.77 ('off' year); 1.70 µg g^{-1} fw ('on' year)] and lowest in light pruned Mallika (V_2I_1) because former had more malformed panicle, less floriferous than later (V_2I_1). Mishra and Dhillon (1979) found that an increase in the level of endogenous gibberellins will account for the production of solely male flowers, continuous vegetative growth and persistence of the malformed panicles on the tree.

The abscisic acid (ABA) a growth inhibitor checks vegetative growth of mango thereby providing conditions suitable for flower bud initiation. In the present investigation (Table 1) the maximum ABA activities were found in Amrapali [0.525 ('off' year); 0.552 µg g^{-1} fw ('on' year)] while minimum in Dashehari [0.140 ('off' year); 0.098 µg g^{-1} fw ('on' year)], as the former is a regular bearer. This is in conformity with the results obtained by Jogdande and Chaudhari (2001) that Neelum (Regular bearer) showed the highest level of ABA at all stages of shoot development than Alphonso (Alternate bearer). The ABA level was higher in 'on' year than in 'off' year. The pruning intensities did not have significant effect on ABA level in 'off' as well as in 'on' year due to new leaves produced growth promoter in such amount that would have balanced the ABA in old mango shoots. On the other hand, the ABA level was highest in Amrapali coupled with light pruning (0.646, 0.663 µg g^{-1} fw) while lowest ABA concentration was observed in moderately pruned Dashehari, since in former,

Table 1. Effect of cultivar and pruning intensity on endogenous hormone level in different mango cultivars planted under high density

Treatments†	Hormone level (µg g^{-1} fw) in shoot bud at FBD stage					
	IAA		GA-like substances		ABA	
	2005-06*	2006-07**	2005-06*	2006-07**	2005-06*	2006-07**
(V_1)	0.297	0.310	0.786	0.758	0.525	0.552
(V_2)	4.57	4.62	1.253	1.101	0.145	0.158
(V_3)	2.83	3.37	1.369	1.316	0.140	0.098
SEm±	0.143	0.150	0.040	0.030	0.041	0.010
$CD_{(0.05)}$	0.410	0.432	0.116	0.110	0.119	0.029
I_0	2.09	2.13	1.236	1.150	0.241	0.257
I_1	2.64	2.68	1.140	1.074	0.267	0.279
I_2	4.03	4.05	1.027	0.973	0.230	0.261
I_3	3.51	3.59	1.142	1.036	0.354	0.277
SEm±	0.165	0.174	0.047	0.044	0.048	0.011
$CD_{(0.05}$	0.474	0.499	0.135	NS	NS	NS
V_1I_0	0.218	0.237	0.696	0.695	0.363	0.380
V_1I_1	0.384	0.399	0.821	0.741	0.646	0.663
V_1I_2	0.276	0.287	0.822	0.799	0.520	0.547
V_1I_3	0.309	0.317	0.822	0.797	0.570	0.579
V_2I_0	4.36	4.50	1.23	1.05	0.217	0.230
V_2I_1	4.63	4.70	0.93	0.87	0.100	0.110
V_2I_2	5.28	5.36	1.20	1.06	0.130	0.143
V_2I_3	4.0	4.60	1.64	1.41	0.133	0.147
V_3I_0	1.60	1.94	1.77	1.70	0.142	0.161
V_3I_1	2.61	2.90	1.68	1.60	0.053	0.065
V_3I_2	3.20	3.70	1.05	1.05	0.039	0.060
V_3I_3	4.20	4.88	0.950	0.870	0.085	0.105
SEm±	0.286	0.301	0.081	0.076	0.083	0.080
$CD_{(0.05)}$	0.821	0.860	0.233	0.220	0.239	0.507

*'off' year, †the details of treatment are given in the text.
** 'on' year

combination fruiting shoots were more in number than in latter which is similar to results obtained by Singh and Singh (1972). The ABA concentration which was higher in 'on' year than in 'off' year is in conformity with the finding of Saida *et al.* (1983) who recorded ethylene (a growth inhibitors) level higher in all cultivars in 'on' year than in 'off' year. Thus, higher level of ABA is conducive to the process of flowering in mango and its manipulation by spray of synthetic substance or by pruning at appropriate time may be helpful to induced flowering during off season.

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