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Antioxidative enzymes and biochemical changes in paclobutrazol induced flowering in mango (*M. indica*) cultivars

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Abstract

A study was conducted to examine the level of nonstructural carbohydrate, protein concentration and the activity of antioxidative enzymes *viz.* catalase and peroxidase in buds of different stages (Stages : I-before flower bud differentiation, II- flower bud differentiation, III-bud burst, IV- panicle elongation) and their adjacent leaves of biennial (Chausa, Dashehari, Langra) and the regular (Amrapali) cultivars. In the present study, Amrapali being the regular cultivar, contained higher levels of total and reducing sugar (4.49 to 12.67 mg g^{-1} f.wt.) and protein content (1.90 to 6.78 mg g^{-1}) in all the developmental stages of flowering as compared to biennial cultivars. However, in leaves gradual reduction in sugar and protein content was noticed in the advance stages of flowering. Paclobutrazol (2-8 g.a.i.), a flower inducing chemical, enhanced the catalase and peroxidase activities over the untreated control and maximum enhancement was recorded at 8 g.a.i. On the other hand, decreasing trend of protein with paclobutrazol treatment was recorded in adjacent leaves of flower buds. The results implicated the possible role of catalase and peroxidase and other associated biochemical changes in paclobutrazol induced flowering in mango.

Key words

Antioxidative enzymes, Biochemical parameters, Flower bud differentiation, Mango cultivar, Paclobutrazol

Introduction

The biennial bearing habit of mango (*Mangifera indica* L.) is one of the major hurdles for its commercial spread. This cropping problem is entirely an inherent problem of the flowering physiology which is exclusively different from the problems of unfruitfulness. The flowering and fruiting behaviour of mango trees differs markedly among the varieties and even within the different units of the same trees (Babu *et al.,* 2006). Thus, even in bearing trees there are bearing and non- bearing units which could be attributed to the differential potentiality of the shoots to form flower buds (Davenport, 2009). It was reported that the potential shoots of regular bearing mango have narrow variation in their carbon assimilation component as compared to the wide variation in biennial cultivars (Singh *et al.,* 2009). The exhaustion of trees during the period of crop load and vigorous vegetative growth with high gibberellin content at the time of flower bud differentiation and imbalance in C/N ratio have been considered the causes of bienniality in mango which has been attributed as the possible primary mechanism by which paclobutrazol restricts the vegetative growth and promotes flowering. Plant growth retardant induced manipulation in physiological activity, has been considered important determinant of productivity enhancement in fruit crops. Among them, paclobutrazol is considered as one of the important plant growth retardants which restricts vegetative growth and induces flowering in many fruit species including mango (Davenport, 2007). Beneficial effects of paclobutrazol in induction of flowering in different mango cultivars have been reported (Yadava and Singh, 1998; Singh and Singh 2003). Inhibition in gibberellins activity following a check in the conversion of ent-Kaurene to ent-Kaurenoic acid in the gibberellins biosynthesis pathway

Carbohydrate and protein content creates a conducive condition for flowering and oxidative enzymes such as catalase and peroxidase scavenges harmful radicals (Singh *et al.,* 2004) generated during stress, which is generally experienced by mango trees during flowering. Excess inhibitory compounds

formed within cells, during the reproductive period can provoke oxidation and modification of different cellular and molecular compounds which may hamper the flowering process. These enzymes may provide defensive mechanism against inhibitory compounds generated during this period (Mondal *et al.,* 2009; Salekjalali *et al.,* 2011 and Zahra *et al.,* 2009). The relation of these oxidative enzymes with senescence has been reported in several crops (Sairam *et al.,* 2003). In light of the above, present investigation was undertaken to study the biochemical changes occurring in leaves and flower buds during different flower bud differentiation stages, in regular (Amrapali) and biennial (Chausa, Dashehari, and Langra) cultivars. Effect of paclobutrazol on these enzymes activity and protein content was also investigated with an objective to elucidate their role on mango flowering.

Materials and Methods

The present investigations were carried out on 15 year old full bearing trees of 'Chausa', 'Dashehari', 'Langra' (biennial bearing), and 'Amrapali' (regular bearing) cultivars of mango during year 2008-2009 and 2009-2010 at Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow located at 26.54°N latitude, 80.45°E longitude and 127 m above mean sea level. Fifty potential shoots from 5 trees of each cultivars were tagged from each direction and recently matured leaf samples (6-7 month old), adjacent to apical meristem were collected from each direction for the biochemical study. Sampling of buds and leaves was done at four flowering stages (I-before flower bud differentiation, II-flower bud differentiation, III-bud burst, IV-panicle elongation) as suggested by Rajan *et al.* (2011). For enzyme assays single and double distilled water washed leaves were stored in liquid nitrogen to prevent proteolytic degradation of proteins for further analysis, while other biochemical estimations were done in fresh leaf samples. The experiment was performed in a complete randomized design with four replication and repeated thrice.

Catalase (CAT) activity was estimated following the method described by Braber *et al.* (1980). One unit of enzyme activity was taken as the amount of enzyme decomposing 1.0 mmole of hydrogen peroxide per minute in 0.1M phosphate buffer (pH 7.0) at 25°C, with initial H_2O_2 concentration of 0.2M.

Peroxidase (POX) activity was estimated by the method of Kunwar and Khan (1982). Enzymatic activity was determined with 100 mM phosphate buffer at pH 7.0, 0.1 N of pyrogallol, 0.02% of H_2O_2 and 2 ml of enzyme extract with final volume of 5 ml assay mixture. Purpurogallin concentration was measured at absorbance of 430 nm. A unit of peroxidase is equal to 0.1 of absorbance (Kar *et al.,* 1976) and both the enzymes activity were done only in Amrapali being regular cultivar and compared with Langra as biennial cultivar.

Total soluble sugar and reducing sugar was estimated following the method of Alcoverro *et al.* (1999), which was based on the standard methods of Yemm and Willis (1954) and Folin and Wu (1919) for soluble and reducing sugar, respectively. Sugar content of extracts was determined spectrophotometrically (double beam UV-VIS spectrophotometer, DB-1646) using an anthrone assay for total sugar and DNSA assay for reducing sugar with glucose as standard.

Total protein content in leaves and buds was estimated as described by Lowry *et al.* (1951). A stock solution (100 µg ml^{-1}) of bovine serum albumin was used as standard and unit was expressed as mg g^{-1} f.wt. Three replicates were examined in each case and a regression curve was worked out of various concentrations of the standard solutions against their respective absorbances. For response of paclobutrazol on protein and enzymes activity separate set of experiment was laid out and the paclobutrazol @ 2, 4, 6 and 8 g.a.i. per tree in Amrapali and Langra was applied once in the month of September in root zone at 15 cm depth.

Statistical analysis : Sigma plot and SAS (Statistical Analysis Software) were used for statistical analysis. All the datas' were expressed as means of four replicates ±SE.

Results and Discussion

The data pertaining to non-structural carbohydrate clearly revealed (Table 1and 2) that the level of reducing sugar

Table 1 : Reducing sugar content in developing flower buds and their adjacent leaves of mango during different flower bud development stages

Cultivars	Reducing sugar (mg g^{-1} f.wt.) Stages							
	I		II		III		IV	
	B	L	B	L	B	L	B	L
Amrapali	6.91±0.05	10.69±0.04	7.56±0.03	12.67±0.33	9.84±0.03	7.82±0.01	4.49±0.08(PD=20.08%)	7.61±0.12
Chausa	3.58±0.03	9.58±0.02	6.26±0.04	13.69±0.13	8.67±0.01	5.73±0.004	1.43±0.04(PD=60.05%)	5.08±0.08
Dashehari	9.71±0.08	12.91±0.06	4.82±0.02	11.41±0.09	5.73±0.02	1.69±0.004	5.47±0.09(PD=43.66%)	1.21±0.06
Langra	3.72±0.01	7.15±0.03	9.64±0.11	7.04±0.05	2.82±0.008	5.20±0.002	2.39±0.11(PD=80.41%)	2.01±0.02
CD (0.05)	**1.47**	**2.27**	**0.56**	**1.50**	**1.47**	**0.43**	**0.15**	**0.18**

(L = Leaves, B = Buds), Stages; I-Before flower bud differentiation, II- flower bud differentiation, III-bud burst, IV- panicle elongation). PD= percent decline

increased till IIIrd stage (3rd week of December) of flower buds development but declined sharply at IVth stage in all the cultivars, however the decline was less (20.08%) in Amrapali as compared to biennial bearing cultivars (35.71-80.41%). However, the cultivar Chausa and Langra, which have very strict biennial bearing habit, showed maximum reduction (60.05 and 80.41%) in reducing sugar content at IVth (panicle elongation of flower development) stage. Similar decreasing trend in reducing sugar at different flower bud development stage with low magnitude was also found in Dashehari, (43.66%) On the other hand in Langra gradual increment (7.04 mg g^{-1} FW) in sugar content was found up to stage IInd, thereafter significant decrease (2.82 mg g^{-1} f.wt.) was recorded. This may be due to most carbohydrate reserve may got exhausted during flowering process (Urban *et al.,* 2004). The changes in the level of total sugar content might be due to increasing level of reducing sugar and conversion of insoluble fraction of carbohydrate into soluble fraction during flower bud differentiation. On the other hand, sharp decrease was reported in reducing sugar of leaves, at one stage prior to flower bud differentiation which may be due to its higher mobilization towards sink (active site of flower bud differentiation) (Singh *et al.,* 2009). Association of non-structural carbohydrates particularly reducing and total sugar with flowering has been documented in other mango cultivars (Marquis *et al.,* 1997). A marked increase in sugar levels (from 7.56 to 9.84 in Amrapali, from 6.26 to 8.67 in Chausa and from 4.82 to 5.73 in Dashehari) during IIIrd stage (bud burst) was probably a high energy requirement of the strong sinks created by dividing cells for initiation of flower buds.

The data depicted in Table 3 revealed that the protein content in flower bud (stage I-IV) was in the range of (1.90-4.41mg g^{-1} f.wt.) in Amrapali, 0.58-4.94 mg g^{-1} f.wt. in Chausa, 1.84-4.64 mg g^{-1} f.wt. in Dashehari, 1.16-5.5 mg g^{-1} f.wt. in Langra and in Mallika it was 2.21-4.94 mg g^{-1} f.wt. Its level was maximum in Amrapali even before flower bud differentiation and minimum in Dashehari followed by Mallika (Table 5). The protein content was gradually decreased upto IIIrd stage, whereas during IVth stage *i.e.,* at panicle elongation its level significantly increased and maximum increase (20.56 %) was recorded in case of Amrapali and minimum (2.34 %) in Langra. Except in Langra all the cultivars had maximum protein content at stage IV when the panicle start elongating, however its content reduced drastically at IInd and IIIrd stage which may be due to its mobilization for advancing the flowering. A higher accumulation of protein, nitrogen along with carbohydrate during floral initiation has been reported earlier (Urban *et al.,* 2004), which may create conducive condition for flowering. However, leaf protein increased till Ist week of November (II stage) and then decreased. It may be due to hydrolysis of protein during the advance stage of flower bud development. Protein content in leaves adjacent to flower bud was found lower due to its higher sink activity demanding increased protein mobilization to the developing inflorescence. This finding support the earlier report that free amino acid increased appreciably in the leaves of different cultivars of mango during reproductive phases. Maximum protein content was found in Amrapali at Ist bud stage (4.26 mg g^{-1} f.wt.). Its content gradually decreased in the subsequent stages of flower development in

Table 2 : Changes in total sugar content in developing flower buds and leaves of mango during different flower bud development stages

Cultivars	Total Sugar (mg g^{-1} f. wt.)						
	Before flower bud differentiation		Flower bud differentiation		Bud burst		Panicle elongation
	Buds	Leaves	Buds	Leaves	Buds	Leaves	Buds
Amrapali	14.41±0.11	28.32±0.19	17.28±0.18	14.90±0.09	13.83±0.19	17.53±0.11	17.41±0.11
Chausa	14.32±0.22	32.02±0.09	26.04±0.11	26.43±0.11	15.31±0.08	14.32±0.09	16.39±0.22
Dashehari	30.29±0.09	19.87±0.12	19.28±0.14	23.19±0.21	10.63±0.06	18.92±0.15	11.85±0.08
Langra	14.86±0.23	28.61±0.15	38.55±0.22	28.16±0.22	11.29±0.04	20.81±0.14	11.56±0.07
CD(0.05)	**2.59**	**4.61**	**2.28**	**1.16**	**2.68**	**1.83**	**0.69**

Table 3 : Changes in protein content in flower buds and leaves of mango cultivars during different developmental stage of flower bud

Cultivars	Protein (mg g^{-1} f.wt.)							
	Before flower bud differentiation		Flower bud differentiation		Bud burst		Panicle elongation	
	Buds	Leaves	Buds	Leaves	Buds	Leaves	Buds	Leaves
Amrapali	4.26±0.08	1.20±0.01	1.90±0.1	4.74±0.08	2.28±0.11	2.80±0.04	4.44±0.07	6.78±0.14
Chausa	3.96±0.01	1.88±0.05	2.00±0.08	4.80±0.09	0.58±0.08	4.56±0.04	4.16±0.05	3.60±0.02
Dashehari	1.84±0.04	1.50±0.06	2.04±0.07	3.98±0.04	1.74±0.04	4.20±0.01	4.64±0.06	3.92±0.04
Langra	3.34±0.02	4.70±0.04	1.16±0.11	6.64±0.30	5.54±0.02	4.24±0.02	3.56±0.11	2.78±0.07
CD(0.05)	**2.41**	**1.5**	**2.66**	**4.66**	**4.76**	**3.98**	**4.94**	**6.64**

Stages-Oct to Jan (20 days interval)

Table 4 : Response of paclobutrazol on catalase and peroxidase activity in mango cultivars

Paclobutrazol concentration	Amrapali (Unit mg^{-1} protein)		Langra (Unit mg^{-1} protein)	
	Catalase	Peroxidase	Catalase	Peroxidase
Control	10.85±0.43	18.36±0.24	3.11±0.12	16.00±0.90
2 g.a.i.	12.47±0.32	27.30±0.72	4.01±0.04	16.50±0.43
4 g.a.i.	13.65±0.98	33.76±0.31	6.58±0.23	27.86±0.39
6 g.a.i.	16.30±0.82	47.37±0.71	7.95±0.30	37.50±0.79
8 g.a.i.	19.33±0.92	65.06±0.78	12.84±0.14	50.99±1.07
CD(0.05)	**1.59**	**4.52**	**1.38**	**9.68**

(g.a.i: gram active ingredient)

Table 5 : Response of paclobutrazol on protein concentration in mango cultivars

Paclobutrazol concentration	Amrapali (mg g^{-1} f.wt.)	Langra (mg g^{-1} f.wt.)
Control	7.12±0.012	5.37±0.11
2 g.a.i.	6.22±0.08	4.44±0.12
4 g.a.i.	5.25±0.13	3.54±0.09
6 g.a.i.	4.71±0.07	3.42±0.07
8 g.a.i.	4.30±0.09	2.74±0.04
CD(0.05)	**1.43**	**1.29**

leaves (Table 3), however in flower bud increasing trend of these attributes was recorded (Table 3). Later on its content gradually decreased in leaves in contrast to its increasing trend in flower buds of different developmental stages. In Amrapali at IVth stage, maximum protein content (6.78 mg g^{-1} f.wt.) was found than other cultivars which may be due to greater activity of source to support sink in flower developmental phases in order to give strength and building blocks to developing buds. On the other hand, in Chausa (3.60 mg g^{-1} f.wt.), Dashehari (3.92 mg g^{-1} f.wt.) and Langra (2.78 mg g^{-1} f.wt.) the protein level significantly reduced in leaves when it reached stage IVth (panicle emergence). This might be one of the reasons for their erratic behavior of flowering in successive year of mango cropping. The results confirmed massive protein mobilization from leaves to flower bud terminal at bud burst stage in order to sustain high protein demand during flowering.

The total soluble protein content in leaves decreased as a result of paclobutrazol treatment, without any noticeable changes in untreated controls. The total protein content of leaf gradually decreased with increasing concentration of paclobutrazol (Fig. 2) in leaves at FBD stage which may be due to its accelerated mobilization towards flower buds (sink). The maximum decrease was noticed at 8 g.a.i. treatment in both the cultivars as compared to control. Decrease in protein content in Amrapali was from 7.0 mg g^{-1}f.wt. to 5.0 mg g^{-1} f.wt., whereas in Langra protein content decreased from 5.5 mg g^{-1} f.wt. to 3.0 mg g^{-1} f.wt. In regular bearing mango more number of low molecular weight proteins (3.0 to 20.1 KD) in paclobutrazol treated trees was reported (Singh *et al.,* 2011; 2005). The targeting and channelization of low molecular weight protein from source (leaves) to sink (fruits) is known to be easier than its higher molecular weight (Singh *et al.,* 2011). Thus increasing mobilization of low molecular weight proteins may be one of the aid of paclobutrazol for enhancing flowering in mango.

CAT and POD activities in the leaves were found higher in Amrapali (CAT= 10.58 unit mg^{-1} protein, POX= 18.36 unit mg^{-1} protein) as compared to Langra (CAT= 3.11 unit mg^{-1} protein, POX= 16.00 unit mg^{-1} protein). Activity of both the oxidative enzymes increased with increasing concentration of paclobutrazol (Fig. 3). However, the rate of enhancement in POD activity was noticed more as compared to CAT activity in both the cultivars. A significant and linear relationship (Fig. 2) of enzymes activity with concentration of paclobutrazol was observed (R^2=0.97 and 0.95). Increased activity of these two antioxidative enzymes may be useful to metabolize and detoxify the inhibitory compound generated during flower development and provide protection against internal stress (Kraus *et al.,*1995) for normal metabolism during the important reproductive stage of mango.

Thus, it is clear from the present data that sugar and protein content are directly or indirectly associated with the flower development in mango. Exogenous application of paclobutrazol notably increased the level of CAT and POD enzymes activity during flowering (Table 4). The increased level particularly of CAT

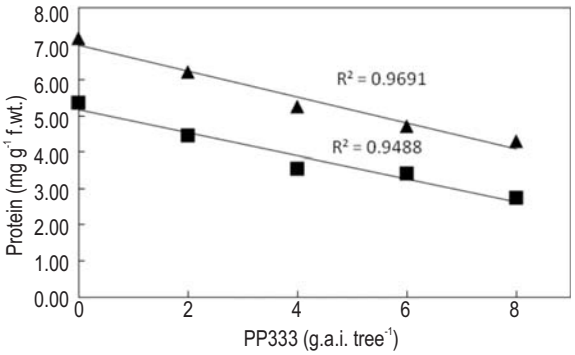

Fig 1 : Effect of paclobutrazol (PP333) on protein concentration in mango cv. Amrapali (▲—▲) and Langra (■—■)

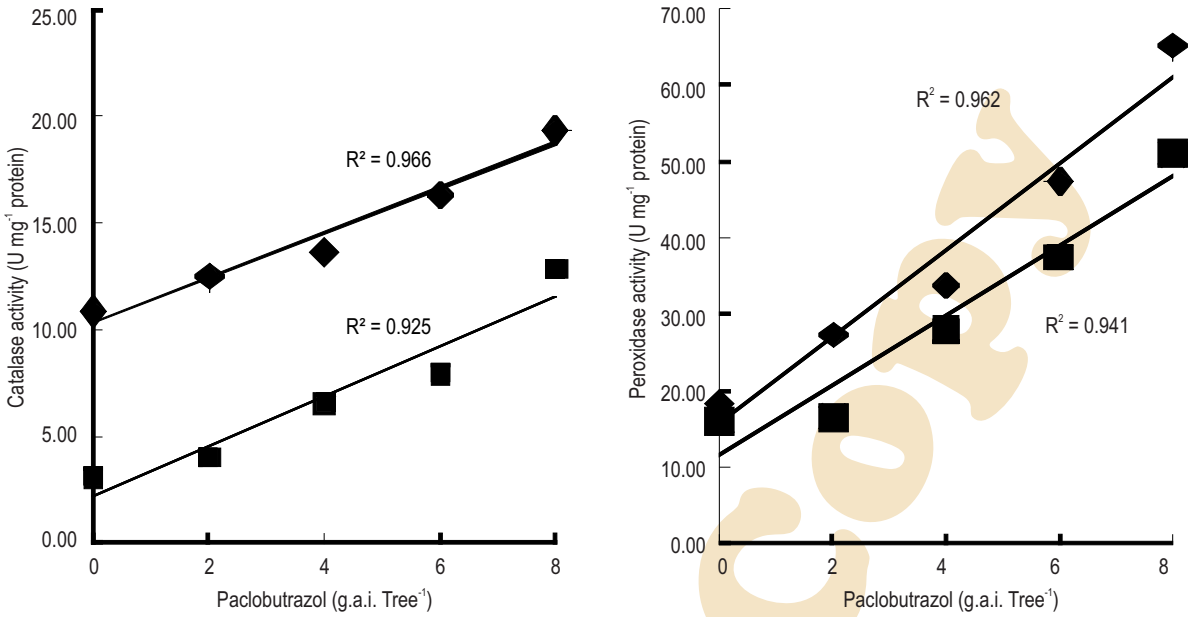

Fig. 2 : Relationship between paclobutrazol concentration and Catalase and Peroxidase activity in leaves of Amrapali (♦—♦) and Langra (■—■)

may provide greater tolerance to oxidative damage occurred by internal stress produced during active state of flowering (Hernandez *et al.,* 2002). Improvement in CAT and POD activity with paclobutrazol was also reported in citrus fruits (Sharma *et al.,* 2011).

The beneficial role of paclobutrazol can be explained, based on its ability to combine with iron containing enzyme (CAT and POX) and to foster their activity. Findings of the present study showed that paclobutrazol has pleiotropic effect, as its primary action is in the inhibition of gibberellic acid biosynthesis and secondary action includes elevation of antioxidative enzyme activities which may inturn promote flower induction.

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