Indian J. Plant Physiol., Vol. 10, No. 1, (N.S.) pp. 9-13 (Jan.-Mar., 2005)

PACLOBUTRAZOL INDUCED GROWTH RETARDATION OF MANGO SEEDLINGS

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Received on 3 June, 2003, Revised on 14 March, 2005

SUMMARY

Mango seedlings of polyembryonic cv. Bappakai were given soil-drenching treatment of paclobutrazol (PBZ) at 0, 0.34, 0.85 or 1.71 mM. Observations were recorded on morphological characters at weekly interval and on leaf water potential (Ψ_w) and levels of chlorophyll, total phenols and endogenous plant growth substances [ABA, cytokinins (*t*-ZR and DHZR) and IAA] at a monthly interval one month after PBZ treatment. There was reduction in seedling vigour after 2 months of PBZ treatment, the effect being more prominent at 1.71 mM. The fresh weights of root, leaves and stem also showed reduction. The Ψ_w was less negative and total phenols and chlorophyll content showed increase in response to PBZ application. The contents of leaf ABA and cytokinins (*t*-ZR and DHZR) were higher in treated seedlings. Thus it was suggested that PBZ induced growth retardation in mango seedlings is associated with increased levels of ABA and cytokinins together with higher Ψ_w and phenols in leaves.

Key words: **Leaf water potential, mango seedlings, paclobutrazol, phenols, plant growth substances, seedling vigour**

INTRODUCTION

Paclobutrazol (PBZ) is an important triazole derived plant growth retardant known to regulate tree size and induce flowering in a number of fruit crops including mango (Davies *et al.* 1988, Davies and Curry 1991, Fletcher *et al.* 2000). The soil drenching treatment of PBZ prior to bud differentiation has been shown to suppress vegetative growth and enhance flowering. The growth regulatory activity of PBZ is reported to be associated with its potential to inhibit the biosynthesis of growth promotory substance, namely the gibberellins (Graebe 1987), which are synthesized in plants through the isoprenoid pathway. This pathway is also involved in the synthesis of other plant growth substances (PGSs) like abscisic acid (ABA) and cytokinins. As plant growth and development are controlled by an interaction among different PGSs, the possibility of involvement of these PGSs as well as paclobutrazol-mediated changes in determining the growth response has been examined. Limited information is available on the role of PGSs like ABA, cytokinins and indole-3-acetic acid (IAA) in PBZ mediated growth suppression in mango. Changes in the level of PGSs, biochemical parameters, chlorophyll and total phenols and physiological variables, leaf water potential (Ψ_w) in elucidating their role in the inhibition of growth due to PBZ treatment has been investigated in mango seedlings.

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MATERIALS AND METHODS

Experiments were conducted at the research farm of Indian Institute of Horticultural Research, Hessaraghatta, Bangalore during August-December, 2001. Seedlings of polyembryonic mango cv. Bappakai were raised in sand mixed soil beds (3X5'). After 30 days of germination, healthy and uniformly grown seedlings were transplanted to earthen pots containing farmyard manure and garden soil (1:2). After 45 days of transplantation, the seedlings were given soil-drenching treatment of PBZ (0, 0.34, 0.85 or 1.71 mM). Sixteen seedlings were maintained under each treatment. The experiment was conducted employing completely randomized design. During the experimentation, all pots received uniform volume of water.

Observations were recorded on morphological characters such as plant height, leaf number, and stem girth at 7 days interval till 30 days and data on these parameters at 30 and 60 days after PBZ treatments have been presented. For measurements on root length and weights of plant parts, 4 seedlings were uprooted gently at 30 and 60 days after PBZ treatment and roots were washed thoroughly. After drying the root system between pads of blotting paper, root length was recorded and plant parts were separated for recording of fresh weights of leaves, stem and roots. Leaf samples from another 4 seedlings were drawn at 30 and 60 days of PBZ treatment for Ψ_w and for determining the contents of chlorophyll, total phenol and endogenous PGSs (ABA, cytokinins, *t*-zeatin riboside (*t*-ZR) and dihydrozeatin riboside (DHZR) and IAA). For Ψ_w, leaf discs of uniform size were cut and the measurements were taken on Dew Point Micro Voltmeter (model HR-33T, Wescor, USA). The values of Ψ_w were expressed as –MPa. Leaf chlorophyll content was estimated according to Hiscox and Israelstam (1979). For the determination of total phenols, leaf sample (2.0 g) was extracted with 80% ethanol and the extract was boiled for 3 min. After filtering the cold extract, the filtrate was extracted thrice with petroleum ether (40-60 °C). The aqueous ethanolic extract was used for colour development with Folin Ciocalteu's reagent (Bray and Thorpe 1954) and the absorption was recorded at 660nm after 1 hr. Quantification of phenols was carried out using chlorogenic acid as standard. The endogenous PGSs in leaves namely ABA (Weiler 1982), cytokinins, *t*-ZR and DHZR (Barthe and Stewart 1985) and IAA (Weiler *et al.* 1981) were analysed by ELISA using laboratory raised polyclonal antibodies. Statistical analyses were carried out according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Morphological characters and fresh weights of plant parts

At 30 days, the growth inhibitory effects of PBZ on morphological characters and weights of different parts were not evident (Table 1 and 2). However at 60 days, PBZ treatments distinctly restricted the vigour of mango seedlings, the effect being greater under its higher concentration (1.71 mM). At 1.71 mM PBZ treatment, the mango seedlings experienced 12.7, 27.9, 24.2 and

Table 1. Effect of paclobutrazol on plant height, leaf number, stem girth and root length in mango seedlings cv. Bappakai.

Treatments	Plant height (cm)		Leaf number		Stem girth (cm)		Root length (cm)	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
Control	11.17	32.4	9.5	13.6	1.92	3.30	18.3	47.0
Paclobutrazol								
0.34 mM	19.25	29.6	10.3	12.2	1.67	3.00	18.0	31.9
0.85 mM	18.33	29.0	10.8	12.8	1.58	2.60	20.5	24.8
1.71 mM	19.67	28.3	10.5	9.8	1.83	2.50	19.8	26.4
CD at 5%, p<0.05	NS	3.9	NS	NS	NS	0.41	NS	2.7

Table 2. Effect of paclobutrazol on plant fresh weight (g) in mango seedlings cv. Bappakai.

Treatments	Leaf weight		Stem weight		Root weight		Total weight	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
Control	8.62	14.00	7.28	17.20	8.65	18.32	24.55	49.52
Paclobutrazol								
0.34 mM	8.95	11.40	7.57	10.04	9.03	15.58	25.55	37.02
0.85 mM	8.06	11.30	7.70	9.76	7.66	14.76	23.43	35.82
1.71 mM	8.60	8.66	7.61	9.28	9.70	13.38	25.92	31.32
CD at 5%, p<0.05	NS	2.6	NS	3.0	NS	3.2	NS	7.6

43.8 % reduction in plant height, leaf number, stem girth and root length, respectively as compared to controls. Similar growth inhibitory response of PBZ in mango (other than roots) has been reported earlier in fully-grown trees (Murti *et al*. 2001). The effect of PBZ on root growth is similar to that of pea (Wang and Lin, 1982), maize and soybean (Barnes *et al.* 1989). There was also significant reduction in the fresh weight of all plant parts following 60 days of PBZ treatment (Table 2).

Leaf water potential (Ψ_w)

Distinct changes were recorded in leaf Ψ_w following PBZ treatments. The PBZ treated plants especially at 1.71 mM had less negative Ψ_w values (-2.5 & -2.1 MPa) both at 30 and 60 days of treatment as compared to those of respective untreated ones (-3.2 & -2.8 MPa) (Table 3). There is lack of information on the role of plant water relations in growth restriction response of PBZ in tree crops. Our earlier investigation showing that the seedlings of polyembryonic mango cultivars with lesser vigour have less negative Ψ_w are in line with PBZ induced changes in Ψ_w (Murti and Upreti 2003). Davies *et al.* (1988) reported that the triazole treated plants characteristically use less water. Further investigations are required on plant water relations in respect to PBZ treatment.

Total phenols

Total phenol content in the leaves of seedlings registered significant increase as a result of PBZ treatments, the increase being more marked at higher dosage of PBZ (Table 3). Murti and Upreti (2003) reported that total phenols are inversely related to seedlings vigour of polyembryonic mango cultivars. Kurian *et al.* (1994) reported that higher phenol content was associated with the dwarfing habit of mango cultivars. It is thus possible that the vigour inhibitory response induced by PBZ in the mango seedlings could be a consequence of higher phenol content.

Table 3. Effect of paclobutrazol on leaf water potential and total phenol content in mango seedlings cv. Bappakai.

Treatments	Water potential (-MPa)		Total phenol content* (mg/g fw)	
	30 days	60 days	30 days	60 days
Control	3.19	2.77	39.60	19.80
Paclobutrazol				
0.34 mM	3.02	2.84	38.42	22.46
0.85 mM	2.72	2.38	41.37	26.30
1.71 mM	2.51	2.12	49.64	39.30
CD at 5%, p<0.05	0.23	0.14	2.86	1.54

*Chlorogenic acid equivalent

Chlorophyll content

There was an increase in the content of chlorophyll a (40.0 and 55.0%), chlorophyll b (63.6 and 50.0%) and total chlorophyll (44.4 and 54.8%) after 30 and 60 days of PBZ application; the increase being significant at 1.71 mM (Table 4). However, no discernible changes were witnessed with regards to the ratio of chlorophyll a to chlorophyll b. Increase in the chlorophyll content with PBZ treatment has also been reported earlier (Davies *et al.* 1988, Sankhla *et al.* 1996).

Endogenous PGSs

PBZ treated mango seedlings showed marked changes in the level of endogenous PGSs (Table 5). The contents of ABA and cytokinins, *t*-ZR and DHZR, in treated mango seedlings were found to increase over control, both after 30 and 60 days. However, there was a marginal decline in IAA concentration (Table 5). These results indicate a role for these PGSs in PBZ induced vigour regulation in mango. Jindal *et al.* (1974) showed that the enhanced levels of ABA are associated with shoot growth retardation in apple. Kojima *et al.* (1996) reported a 4-fold increase in ABA levels in mandarin fruitlets treated with uniconazole (a triazole compound having growth inhibitory activity similar to PBZ). In earlier studies, we reported higher ABA coupled with lower IAA levels during active shoot growth responsible for vigour restriction in mango (Murti and Upreti, 1999). Murti and Upreti (2003) also reported that ABA and seedling vigour of polyembryonic mango cultivars are inversely related. Although there is decrease in most of the morphological characters, we witnessed an increase in growth promoting PGSs, cytokinins in the PBZ treated mango seedling. The role of increased levels of cytokinins in PBZ induced vigour restriction could not be explained from present study. It is possible that the enhanced ABA and phenolic levels observed as a consequence of PBZ application could counteract the growth promoting effects of cytokinins. Also higher chlorophyll levels observed in the leaves of PBZ treated seedlings could be the result of

Table 4. Effect of paclobutrazol on chlorophyll content (mg/g fw) in mango seedlings cv. Bappakai.

Treatments	Chlorophyll a		Chlorophyll b		Total chlorophyll content		a/b ratio	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
Control	2.49	1.95	1.14	1.18	3.63	3.13	2.18	1.65
Paclobutrazol								
0.34 mM	2.44	1.89	1.12	1.25	3.56	3.14	2.18	1.51
0.85 mM	2.72	2.09	1.28	1.37	4.00	3.46	2.13	1.52
1.71 mM	3.45	3.05	1.71	1.75	5.16	4.80	2.02	1.74
CD at 5%, p<0.05	0.43	0.26	0.11	0.24	0.95	0.73	NS	NS

Table 5. Effect of paclobutrazol on endogenous levels of PGSs in mango seedlings cv. Bappakai.

Treatments	ABA (ng/g fw)		*t*-ZR (pg/g fw)		DHZR (pg/g fw)		IAA (ng/g fw)	
	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
Control	34.8	47.2	79.9	85.1	83.2	79.8	13.5	11.7
Paclobutrazol								
0.34 mM	37.5	41.2	83.9	84.5	89.6	81.7	13.4	12.4
0.85 mM	39.5	47.1	100.2	101.5	79.6	95.0	12.8	10.8
1.71 mM	54.2	66.3	126.5	142.5	114.7	124.1	9.0	8.5
CD at 5%, p<0.05	1.62	2.95	4.80	6.14	3.43	5.36	0.82	1.12

increased cytokinins as reported by Fletcher *et al.* (1982). The observed decline in IAA levels is in line with Law and Hamilton (1989) who reported decline in IAA responsible for uniconazole induced reduction in internodal length in peas. Buta *et al.* (1989) also reported that the IAA levels in the leaves of vigorous apple trees were higher in early and late growing season as compared with those in dwarf ones.

Thus the seedling growth inhibition in mango by PBZ is clearly evident after 60 days of application, and this response to PBZ is characterized by increased hormonal levels of ABA and cytokinins together with phenols and chlorophyll, and less negative Ψ_w values.

ACKNOWLEDGEMENTS

The authors are thankful to the Director of the institute for providing facilities and to Shri H.L. Jayaram and Shri K.R. Earanna for technical help.

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