MORPHOLOGICAL CHANGES IN YOUNG PLANTS OF *HEVEA BRASILIENSIS* INDUCED BY PACLOBUTRAZOL

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This study was carried out to investigate the effect of paclobutrazol, a gibberellin synthesis inhibitor in young rubber plants and standardise its optimum concentration to develop better root system. Paclobutrazol treatment in young rubber (*Hevea brasiliensis*) plants resulted in short and compact plants having dark green leaves, with more fibrous root development and improved root-to-shoot ratio. Plants of *Hevea* clone RRII 429 were treated with 0, 50, 100, 250, 500 and 750 mg active ingredient of paclobutrazol per plant as soil application. Three months after treatment the leaves showed increased chlorophyll (25.9%) and carotenoid (33.6%) contents over control. The height increment in paclobutrazol (50 mg) treated plants was significantly lower than control plants of same age, whereas, the girth increment was significantly higher (10.9%) in the treated plants. Paclobutrazol treatment remarkably increased root dry weight (maximum of 132 per cent with 50 mg) and root-to-shoot ratio. The results indicated that paclobutrazol at a concentration of 50 mg was optimum for more fibrous root development without any significant changes in stem girth and leaf number. The modified root system may likely be an advantage to young *Hevea* plants during drought.

Keywords: *Growth retardant, Hevea, Paclobutrazol, PBZ, Root morphology*

INTRODUCTION

Young *Hevea* plants are developed through bud grafting and a grafted plant comprises a root system contributed by the stock plant and the shoot system by the scion. Vigorous stocks with better root system can increase the vigour of the scion resulting in reduction of the immaturity period and increased yield of the scion (Dijkman, 1951; Combe and Gener, 1977) and vigorous scions induce more growth in the root system (Dijkman, 1951). In the present scenario of changing climate, drought situations are common occurrence in non-traditional as well as traditional rubber growing regions in India. Soil moisture deficit coupled with high temperature adversely affects the survival and growth of young rubber plants (Jacob *et al.*, 1999). Development of suitable stocks with well-developed root system is helpful to survive the plants under such unfavourable conditions (Combe and Gener, 1977).

The regulation of plant growth with synthetic plant growth regulators has

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become a common agricultural practice. Of the available synthetic plant growth regulators, triazoles are potent at low concentrations to inhibit shoot growth (Davis *et al.*, 1988). Paclobutrazol (PBZ) is a triazole derivative known to interfere with *ent*-kaurene oxidase activity in the *ent*-kaurene oxidase pathway leading to a decrease in endogenous GA levels and ABA catabolism (Rademacher, 1997). PBZ suppressed growth in a wide range of plant species and treated plants exhibited a dark green colour, and are shorter and more compact in appearance (Terri and Millie, 2000; Sebastian *et al.*, 2002). Treating potato plants grown under non-inductive greenhouse conditions with PBZ resulted in compact plants with thicker, dark green leaves, and wider stem and root diameter (Tekalign and Hammes, 2004). It induces leaf morphological and anatomical modifications depending on plant species, growth stage, rate and method of application. In wheat PBZ increased thickness of the leaves by inducing additional layers of palisade mesophyll cell (Gao *et al.*, 1987). The reduction of plant height following PBZ treatment is accompanied by various morphological alterations that depend on species and concentration of PBZ. Berova and Zlatev (2000) observed increased radial extension in tomato stems following PBZ treatment. It increased root diameter in chrysanthemum by increasing the number of rows and diameter of cortical cells (Burrows *et al.*, 1992). PBZ increased root diameter in soybean by increasing the size of cortical parenchyma cells (Barnes *et al.*, 1989).

However, information on plant growth characters, especially shoot and root morphological modifications in *Hevea* in response to PBZ treatment is not available. The study was initiated with the major objectives of investigating the effect of palcobutrazol on growth and morphology of young rubber plants and standardization of an optimum concentration of paclobutrazol required for the development of better root system.

MATERIALS AND METHODS

Planting material

A nursery level experiment was carried out at the Rubber Research Institute of India, Kottayam, Kerala during 2013. Budded stumps of *Hevea* clone RRII 429 were raised in polybags containing ten kg of soil mixed with organic manure. The plants were grown under normal nursery conditions in open sunlight with monthly application of recommended dose of fertilizers (10:10:4:1.5 NPKMg mixture @ 10g per plant) and irrigated regularly to avoid water stress.

Treatments

Six months after planting, during the initiation of second leaf whorl, the plants were treated with PBZ with six concentrations of 0, 50, 100, 250, 500 and 750 mg active ingredient per plant with six replications as soil drench mixed with one litre distilled water and the control plants were treated only with distilled water of equal volume.

Chlorophyll and carotenoids content

Pigments were extracted from 100 mg leaf discs in 10 mL of acetone:dimethyl sulphoxide (1:1 v/v) solution and filtered supernatant was read at 470, 645 and 663 nm. Estimation of chlorophyll *a*, *b* and total chlorophyll was carried out by the method of Arnon (1949) and the quantity of carotenoids was estimated by the method of Lichtenthaler (1987).

Plant height and girth

Plant height was measured from the bud union to the apex of the stem. Basal girth was calculated from stem diameter measured at five centimeter away from the bud union.

Dry weights

The intact root system was collected by tearing the polybag and removing the entire soil by washing under running water. Roots and shoots were separated carefully and dried inside a hot air oven at a constant temperature (60 °C) until the dry weights were stable.

All the data were analyzed following completely randomized design (CRD) at $P \leq 0.05$.

Fig. 1. Control (left) and paclobutrazol-50 mg (right) treated polybag plants of *Hevea*

RESULTS AND DISCUSSION

Number of leaf and leaf whorl

PBZ treated *Hevea* plants were compact with less number of leaf and leaf whorl than the control (Fig. 1). The average number of leaf whorl was 4.3 in control plants followed by plants treated with 50 and 100 mg PBZ (Table 1). Number of leaf whorl was lesser in plants treated with higher concentrations of PBZ. There was no significant difference in leaf number per plant among the control and treated plants with 50 and 100 mg of PBZ. The leaf number were recorded less with higher concentrations of PBZ. In young scions of *Hevea*, growth in the length of stem is discontinuous, with rapid elongation of an internode towards the end of which a cluster of leaves is produced. This will be followed by a rest period of 2-3 weeks for the scale leaves to develop around the terminal bud. This sequence is repeated and leaves are produced in whorls separated by bare stem. Although the elongation of stems is intermittent, their girth increases continuously (Mercykutty *et al.*, 2002). Paclobutrazol inhibits cell elongation and internodal growth resulting in retarded plant growth by inhibition of gibberellin

Table 1. **Number of leaf and leaf whorl before and after application of paclobutrazol (PBZ) in young polybag plants of *Hevea***

Treatment	Before application		Six months after application	
	No. of leaf whorl	No. of leaves	No. of leaf whorl	No. of leaves
Control	2.0^a	$17.0^a \pm 1.89$	$4.3^a \pm 0.33$	$34.3^a \pm 3.24$
PBZ-50 mg	2.0^a	$19.0^a \pm 2.12$	$3.0^b \pm 0.31$	$33.0^a \pm 2.98$
PBZ-100 mg	2.0^a	$19.0^a \pm 2.06$	$3.0^b \pm 0.29$	$30.7^a \pm 2.80$
PBZ-250 mg	2.0^a	$19.7^a \pm 1.78$	$2.7^b \pm 0.19$	$19.0^b \pm 3.14$
PBZ-500 mg	2.0^a	$15.0^a \pm 2.26$	$2.3^b \pm 0.27$	$18.7^b \pm 2.25$
PBZ-750 mg	2.0^a	$20.0^a \pm 2.03$	$2.7^b \pm 0.24$	$18.3^b \pm 3.12$

The values are mean ± SE (n=6)

biosynthesis. When gibberellin production is inhibited, cell division still occurs, but the new cells do not elongate; which develop shoots with the equal numbers of leaves and internodes compressed into a shorter length (Chaney, 2003). With higher concentrations of PBZ (250-750 mg) there were negligible stem elongation as compared to control, might be due to higher levels of inhibition of gibberellin biosynthesis without further stem elongation and development of new leaves and leaf whorl.

Chlorophyll and carotenoids content

PBZ treated leaves were visibly dark green due to high chlorophyll *a* (3.38 mg g^{-1} fresh weight) and chlorophyll *b* (0.87 mg g^{-1} fresh weight) contents (Table 2). Leaves of the control plants had 2.69 and 0.63 mg g^{-1} fresh weight chlorophyll *a* and *b*, respectively. The total chlorophyll and carotenoids content were 25.9 and 33.6 per cent higher in PBZ treated plants than the control plants. The increase in chlorophyll content may be attributed to an enhanced chlorophyll biosynthesis and/or more densely packed chloroplasts per unit leaf area. Sebastian *et al.* (2002) reported enhanced rate of chlorophyll synthesis in *Dianthus caryophyllus* and Khalil (1995) observed more densely packed chloroplast per unit leaf area in response to PBZ treatment. Increased chlorophyll content in potato due to PBZ treatment was observed by Balamani and Pooviah (1985) and Bandara and Tanimo (1995). The higher chlorophyll content of PBZ treated leaves had been attributed to the influence of PBZ on endogenous cytokinin levels in mango (Adil *et al.*, 2011). It has been demonstrated that PBZ induced stimulation resulted in cytokinin synthesis that enhances chloroplast differentiation, chlorophyll biosynthesis, and prevents chloroplast degradation (Fletcher *et al.*, 1982).

Stem morphology

PBZ treatment resulted in shorter stems compared to the control plants (Table 3). The mean plant height was reduced by 53.3 to 62.5 per cent over control in response to PBZ treatment while stem girth was reduced by 16.4 to 31.6 per cent over the control except in PBZ-50 mg treatment where stem girth increased by 10.9 per cent over control. The highest increment (76.2%) in stem girth after PBZ application was noticed in PBZ-50 mg better than control (64.9%) and least was noticed with PBZ-750 mg after six months of application (Fig. 2). PBZ-treated potato plants were shorter and had thicker stems than the control. Reduced internode length caused height reduction. Davis and Curry (1991) reported that shoot growth reduction in

Table 2: **Effect of paclobutrazol (PBZ) on leaf pigment three months after application**

Treatment	Chlorophyll content (mg g^{-1} FW)			Carotenoids
	Chl a	*Chl b*	Total	(mg g^{-1} FW)
Control	$2.69^b \pm 0.032$	$0.63^c \pm 0.027$	$3.32^b \pm 0.044$	$1.43^c \pm 0.028$
PBZ-50 mg	$3.34^a \pm 0.029$	$0.72^b \pm 0.016$	$4.06^a \pm 0.019$ (22.3)*	$1.75^b \pm 0.036$ (18.3)
PBZ-100 mg	$3.28^a \pm 0.033$	$0.87^a \pm 0.027$	$4.15^a \pm 0.042$ (25.9)	$1.81^{ab} \pm 0.036$ (26.6)
PBZ-250 mg	$3.30^a \pm 0.041$	$0.82^a \pm 0.025$	$4.12^a \pm 0.033$ (24.1)	$1.72^b \pm 0.030$ (20.3)
PBZ-500 mg	$3.38^a \pm 0.026$	$0.79^{ab} \pm 0.028$	$4.17^a \pm 0.031$ (25.6)	$1.77^b \pm 0.031$ (23.8)
PBZ-750 mg	$3.28^a \pm 0.027$	$0.86^a \pm 0.023$	$4.14^a \pm 0.039$ (24.7)	$1.91^a \pm 0.022$ (33.6)

The values are mean ± SE (n=6). *Values in parenthesis are per cent increase over control

Table 3: **Effect of paclobutrazol (PBZ) on plant height and stem girth six months after application**

Treatment	Initial		After six months	
	Stem girth (cm)	*Height (cm)*	*Stem girth (cm)*	*Height (cm)*
Control	$2.67^{a} \pm 0.13$	$46.33^{a} \pm 3.58$	$4.39^{ab} \pm 0.30$	$120.00^{a} \pm 7.21$
PBZ-50 mg	$2.78^{a} \pm 0.11$	$38.00^{a} \pm 4.21$	$4.87^{a} \pm 0.27$ (+10.9)*	$56.00^{b} \pm 5.52$ (-53.3)
PBZ-100 mg	$2.72^{a} \pm 0.14$	$46.33^{a} \pm 2.98$	$3.67^{b} \pm 0.34$ (-16.4)	$53.83^{b} \pm 4.61$ (-55.1)
PBZ-250 mg	$2.62^{a} \pm 0.12$	$41.33^{a} \pm 3.21$	$3.30^{b} \pm 0.24$ (-24.8)	$52.17^{b} \pm 5.91$ (-56.5)
PBZ-500 mg	$2.57^{a} \pm 0.11$	$36.50^{a} \pm 3.67$	$3.00^{b} \pm 0.23$ (-31.6)	$45.00^{b} \pm 4.78$ (-62.5)
PBZ-750 mg	$2.62^{a} \pm 0.10$	$42.83^{a} \pm 3.85$	$3.00^{b} \pm 0.25$ (-31.6)	$48.00^{b} \pm 4.92$ (-60.0)

The values are mean ± SE (n=6). *Values in parenthesis are per cent increase over control

response to PBZ treatment occurs primarily due to a decrease in internode length, and the effective dose varies with species and cultivar. This response may probably be explained by the reduction in the endogenous GA level. GA enhances internode elongation of intact stems (Salisbury and Ross, 1992). Similar reduction in shoot growth in response to PBZ was reported in *Scaevola* (Terri and Millie, 2000) and *Dianthus caryophyllus* (Sebastian *et al.*, 2002). More recently, Suzuki *et al.* (2004) reported that the presence of PBZ in the medium strongly inhibited etiolated and non-etiolated longitudinal shoot growth of *Catasetum fimbriatum*. PBZ treatment increased cortex thickness, size of the vascular bundles, and pith diameter and resulted thicker stems. This modification may be attributed to radial expansion of cells due to reduced endogenous GA activities in response to the treatment. Wenzel *et al.* (2000) reported that GA limits the extent of radial expansion of plant organs. In dicot stems, cell shape alterations are apparently caused by a more longitudinal orientation of cellulose microfibrils being deposited in the cell walls, preventing expansion parallel to these microfibrils but allowing expansion perpendicular to them (Eisinger, 1983). It has been also reported that PBZ promoted radial expansion of the pea cells (Wang and Lin, 1992).

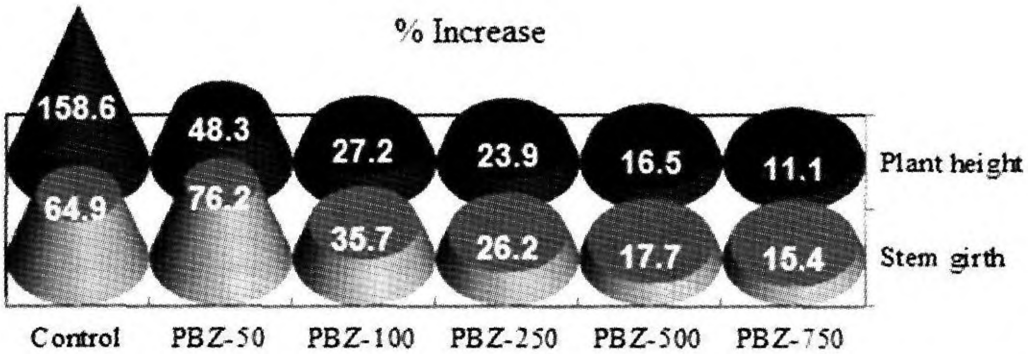

Fig. 2. Per cent increase in plant height and stem girth six months after paclobutrazol application over initial growth. CD (P=0.05) for plant height and stem girth is 11.74 and 15.19, respectively

Root morphology

Three months after PBZ treatment average root diameter of newly developed roots was 4.4 mm and 120 per cent thicker than the roots of the control plants (Fig. 3). These thicker roots were gradually found to develop fibrous roots when examined after six months of application in 50 and 100 mg treatments (Fig. 4). Whereas plants treated with PBZ-250 to 750 mg, continued to produce thicker roots with less fibrous roots as found with PBZ-250 mg treatment. PBZ-50, PBZ-100 and PBZ-250 mg recorded significantly higher root dry weights after three months of application over control. Enormous effect on root growth was noticed after six months of application. There was 132 per cent increase in root dry weight with PBZ-50 mg over control followed by PBZ-100 mg, whereas, it was 11 per cent lower in PBZ-750 mg treated plants than control (Fig. 5). Root-to-shoot ratio was found to be improved by application of PBZ. It was higher in PBZ treated plants (1.32 to 1.53) than control (0.85).

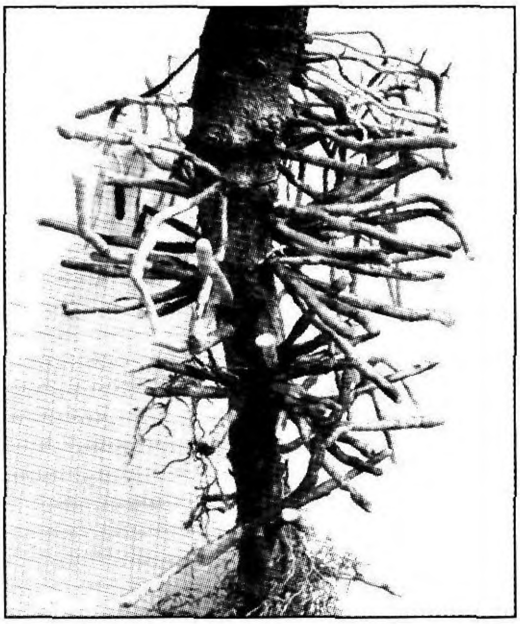

Fig. 3. Development of new and thicker roots in *Hevea* induced by PBZ three months after application

Gibberellins are produced in actively and newly growing tips which include shoots and roots. Following PBZ soil application, uptake occurs through established roots, translocate only in xylem tissues. Since the active ingredient can only

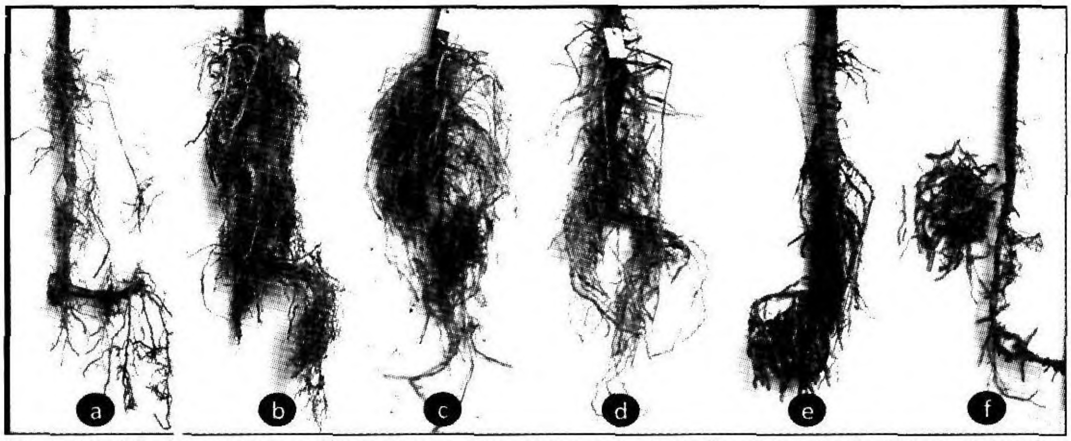

Fig. 4. Modified root system of *Hevea*, six months after application of paclobutrazol. a) Control, b) PBZ-50 mg, c) PBZ-100 mg, d) PBZ-250 mg, e) PBZ-500 mg, f) PBZ-750 mg

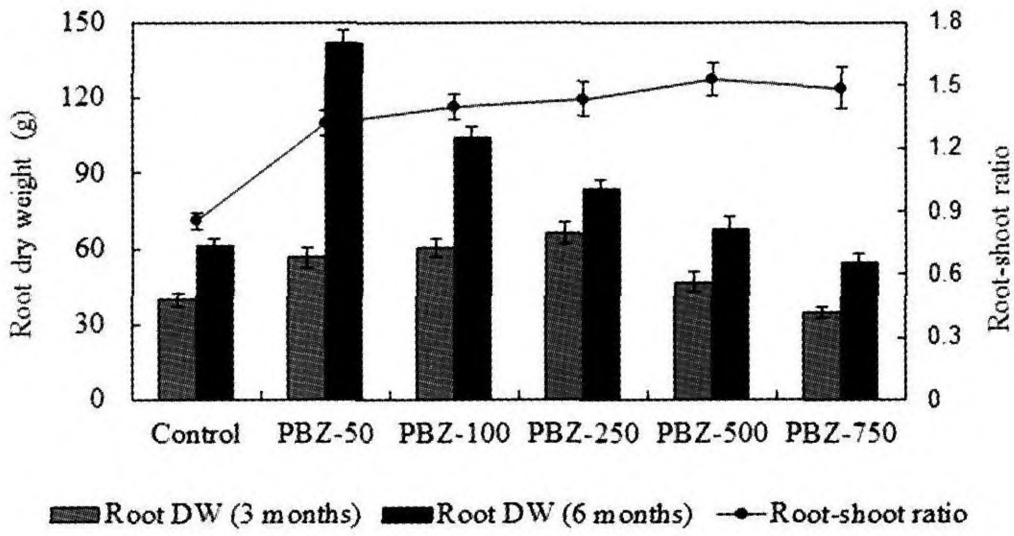

Fig. 5. Effects of paclobutrazol on root dry weights and root-to-shoot ratio. CD (P=0.05) for Root DW-3 months, 6 months and root-to-shoot ratio is 10.69, 12.79 and 0.21 respectively. Bars indicate standard errors (n=6)

move upwards, it can only affect above ground GA production with no effect on root tips and maximum effect on shoot tips (Rademacher, 2000). When PBZ reduces the growth rate of the tree and the energy, carbon assimilates and other substrates produced by photosynthesis in the leaves cannot be utilized fully by the shoots and these get shunted back into the tree and are available for use for other functions including development of new and fibrous roots, enhancement of existing roots and reallocation of carbohydrates towards radial growth of roots and shoots. The enhanced root development may dramatically result in increased root dry weight and root-to-shoot ratio. This resulted in increased water and nutrient uptake and eventually drought tolerance, which produce healthier trees by enhancing the root dry weights and root to shoot ratio (Chaney, 2003). Baluska *et al.* (1993) reported thickening of maize roots and increased starch content when treated with PBZ. A stimulatory effect of PBZ on root growth has also reported in English ivy (Geneve, 1990) and mung bean (Porlingis and Koukourikou-Petridou, 1996).

CONCLUSION

Paclobutrazol modified the morphology of the young *Hevea* plant in such a way that treated plants appeared to be dark green with more chlorophyll and carotenoids contents in leaves; short and compact plants. It induced morphological alterations such as increasing the diameter of the stems and roots at 50 mg concentration. Further, it induced vigorous fibrous root system and increased root-to-shoot ratio. The well-developed fibrous root system may help *Hevea* plants to mine more water and nutrient uptake from the deeper soil layers during moisture stress.

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