Bot. Bull. Academia Sinica (1991) **32:** 9-14

# Metabolic changes associated with paclobutrazol induced rootings in hypocotyl cuttings of mung bean

K. K. Bora[1,3], Jaishree Munot[2] and Sudha Mathur[1]

*Department of Plant Physiology[1] and Biochemistry[2], Rajasthan College of Agriculture, RAJAU, Udaipur-313001, India*

(*Received May 21, 1990; Accepted August 24, 1990*)

**Abstract.** In comparison to control, paclobutrazol significantly increased number of adventitious roots on the cuttings of mung bean, however, the chemical reduced length of root and hypocotyl of seedling. The treated cuttings exhibited increased activities of peroxidase, polyphenol oxidase, acid phosphatase and protease in the base of cuttings. The highest paclobutrazol-induced promotion of enzyme activity occurred with peroxidase compared with controls. In contrast, paclobutrazol reduced amylase activity, soluble sugars, in comparison to control, suggesting that this enzyme in particular may not be associated with root formation. The decrease in phenolic compound in treated cuttings were also evident. The results indicate that enhancement of adventitious root formation by paclobutrazol is accompanied by changes in enzyme activities and level of chemical constituents which have previously been postulated to be involved in rooting.

**Key words:** Enzyme activity; Growth regulator; Metabolic constituents; Paclobutrazol; Rooting; Triazole; *Vigna radiata* L.

## Introduction

Triazoles has recently emerged as a new group of synthetic plant growth regulators. Paclobutrazol [(2 RS, 3 RS)-1-(4 chlorophenyl)-4-4-dimethyl-2-1, 4 triazol-yl-pentan-3-ol)] is an experimental antigibberellin plant growth regulator which reduces plant growth by suppressing the synthesis of gibberellins and its effects are reversed by gibberellin application (Davis *et al.*, 1986). This compound affects several physiological and biochemical processes in plants including reduction of internodal length and leaf expansion (Davis *et al.*, 1986) chlorophyll content (Wood, 1984) and enzyme activities (Sankhla *et al.*, 1985). Recently, paclobutrazol has been reported to induce adventitious root formations in hypocotyl cuttings of some species (Davis *et al.*, 1985; Bora and Mathur, 1989). The mode by which paclobutrazol increased root formation is not clear and needs investigation.

The changes in enzyme activities and metabolic constituents are known to occur during the rooting process and it has been suggested that some of these enzymes and metabolic constituents may be involved in root formation (Bhattacharya *et al.*, 1978; Bassuk *et al.*, 1981; Swarnkar *et al.*, 1987; Bhattacharya, 1988; Kumar and Kakkar, 1989). The objectives of the current investigation was to study the paclobutrazol induced changes in some enzyme activities and metabolic constituents which may be involved in adventitious root formation on mung bean hypocotyl cuttings.

## Materials and Methods

Mung bean (*Vigna radiata* L.) seeds were surface sterillized with 0.1% mercuric chloride solution for five minutes and after thorough rinsing in distilled water, were transferred to sterilized Petri dishes lined with filter paper and moistened with 5 ml distilled water.

[3]To whom correspondence should be addressed.

**Abbreviations:** IAA = Indole acetic acid.

Seven seeds were germinated in each Petri dish under continuous light from fluorescent tubes in a growth chamber maintained at 28±2°C. The hypocotyl cuttings were obtained after excising roots of 48 h old seedling. Each seedling consists of hypocotyl, cotyledons and epicotyl with small primary leaves. These cuttings were then transferred to Petri dishes containing distilled water (control) and paclobutrazol (5 ppm) respectively, and placed under similar experimental conditions for rooting. Enzyme activities and metabolic constituents were measured 48, 72 and 96 h after seedling transfer. For each experiment three samples were taken for biochemical assaying. Rooting and shoot elongation were evaluated after 120 h. Each set consisted of three replications and seven seedlings in each Petri dish served as one replication. The experiments were then repeated twice.

Enzyme activities were measured in the basal 2 cm hypocotyl section of the cuttings, which is where adventitious roots emerged about 58 h after transfer in both the treatments. Basal hypocotyl sections from the two cuttings (100 mg f.wt) were used for each individual analysis.

For enzyme assays, the basal sections were homogenised in 0.05 M Tris-HCl buffer (pH 7) containing 0.001 M EDTA and 0.003 M $MgCl_2$ and the extract prepared as described earlier (Bora *et al.*, 1980).

Peroxidase activity was measured as a change in absorbance by incubating enzyme with O-dianisidine and hydrogen peroxide (Shannon *et al.*, 1971). Polyphenol oxidase activity was determined using catechol as substrate (Farkas and Kiraly, 1962) and expressed in terms of a change in absorbance $h^{-1}$ g.f.wt.$^{-1}$. The assay method of Bernfeld (1955) was used for estimating $\alpha$-amylase activity and activity was expressed in terms of $\mu$g maltose liberated $min^{-1}$ g.f.wt.$^{-1}$. P-Nitrophenyl phosphate was used as substrate for assaying the acid phosphatase activity (Zink and Veliky, 1979) and the activity was expressed as $\mu$g nitrophenol released $h^{-1}$ g.f.wt.$^{-1}$. Protease activity was determined as $\mu$g tyrosine released $h^{-1}$ g.f.wt.$^{-1}$ (Bergmeyer, 1974). The metabolic constituents like soluble sugar was estimated by the method of Roe (1955) using anthrone reagent. And phenols was assayed by the method of Farkas and Kiraly (1962). Results are averages of two experiments each with three replicates.

**Table 1.** *Effect of paclobutrazol on number of roots and seedling growth of mungbean cuttings after 120 hours of treatment*

| Parameters | Treatment | |
|---|---|---|
| | Control | Paclobutrazol (5 ppm) |
| Mean number of roots per cutting | 6.6 | 11.6 |
| Mean length of longest root (mm) | 3.3 | 3.4 |
| Mean hypocotyl length (mm) | 30.5 | 8.2 |
| Mean epicotyl length (mm) | 21.0 | 2.5 |

Average of 42 seedlings

## Results and Discussion

Paclobutrazol treated cuttings exhibited 80% more number of roots compared with controls (Table 1). A slow growth of shoot and root was evident in treated plants (Table 1). These responses to paclobutrazol are consistent with our previous work (Bora and Mathur, 1989) and with that of others (Krishnamoorthy, 1972; Davis and Sankhla, 1988) who have reported that some other antigibberellin compounds promote root formations on cuttings. The application of gibberellins to cuttings generally inhibit rooting (Krishnamoorthy, 1972; Hansen, 1988) and promoting effect of paclobutrazol on rooting may be related to its antigibberellin activity. It should be noted, however, that not all antigibberellins promote rooting (Read and Hoysler, 1969).

Treated cuttings exhibited higher peroxidase activity at all the stages of rooting (Fig. 1). The activity of peroxidase may be related to rooting (Nanda *et al.*, 1973; Bhattacharya *et al.*, 1978) and our results are similar to those previously reported (Molnar and Lacroix, 1972; Bhattacharya and Nanda, 1979). Furthermore, the enhancement of peroxidase activity by paclobutrazol is similar to that of IAA, which also promotes rooting (Bhattacharya, 1988). Hence, it is possible that paclobutrazol stimulates rooting in part by enhancing peroxidase activity in the base of the cuttings.

The activity of polyphenol oxidase was also promoted by paclobutrazol (Fig. 2). The endogenous levels of phenolic compounds, however, remained low

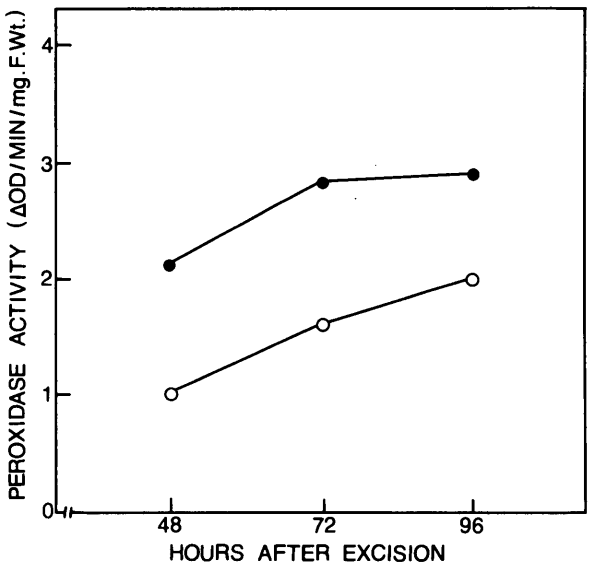


Fig. 1. Effect of paclobutrazol (paclo) on the activity of peroxidase in the base of hypocotyl cuttings. ○—○ Control; ●—● Paclobutrazol.

in treated plants. Similar inverse relationship between polyphenol oxidase and endogenous phenolic contents during rooting has been reported by Bhattacharya (1988). Polyphenol oxidase may oxidize endogenous phenolics which then conjugate with oxidation products of auxin to form phenolic cofactors necessary for rooting (Bhattacharya, 1988). Hence it can not be ruled out that paclobutrazol promotes rooting in part by enhancing the synthesis of phenolic cofactors through the increased enzyme activity.

Previous workers had shown high sugar levels, enhanced amylase activity and disappearance of starch during rooting (Swarnkar *et al.*, 1987). On the other hand, in the present study, paclobutrazol induced rooting was associated with a significant reduced amylase activity and low soluble sugars (Fig. 3). The results of the study suggest that amylase activity is not directly

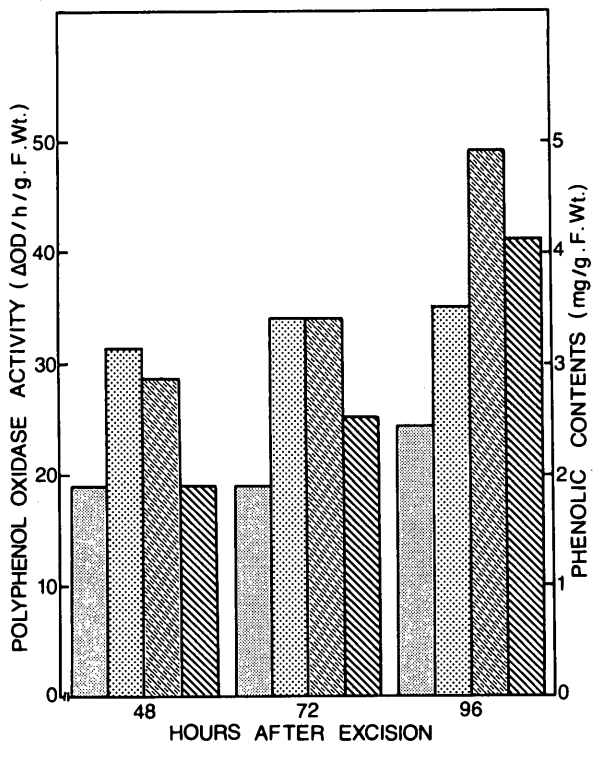


Fig. 2. Polyphenol oxidase activity and phenolic contents in the base of hypocotyl cuttings as affected by paclobutrazol (Paclo) control (Cont). ▨ Cont - Polyphenol oxidase; ⁙ Paclo - Polyphenol oxidase; ▧ Cont - Phenolic contents; ▩ Paclo - Phenolic contents.

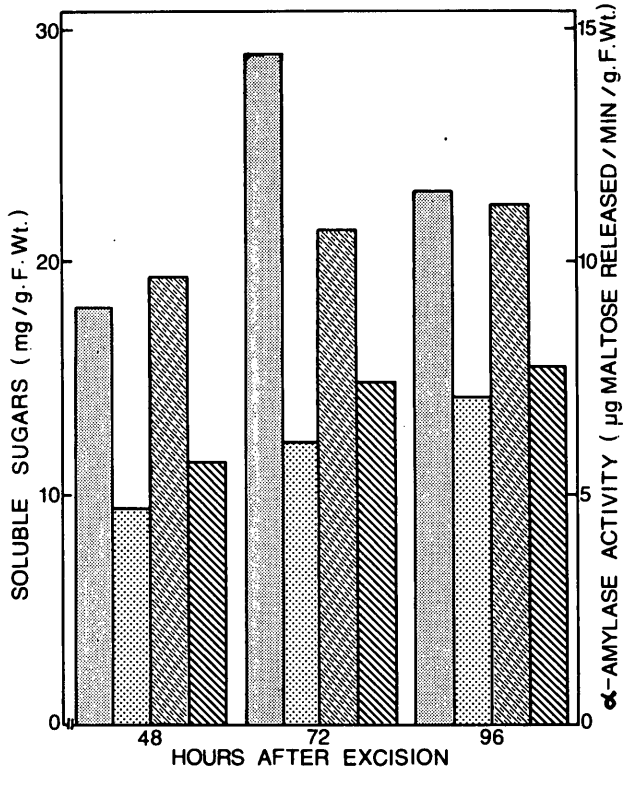


Fig. 3. Activity of α-amylase and soluble sugar content in the base of hypocotyl cuttings as affected by paclobutrazol (Paclo) control (Cont). ▨ Cont - Soluble sugar; ⁙ Paclo - Soluble sugar; ▧ Cont - α- amylase; ▩ Paclo - α-amylase.

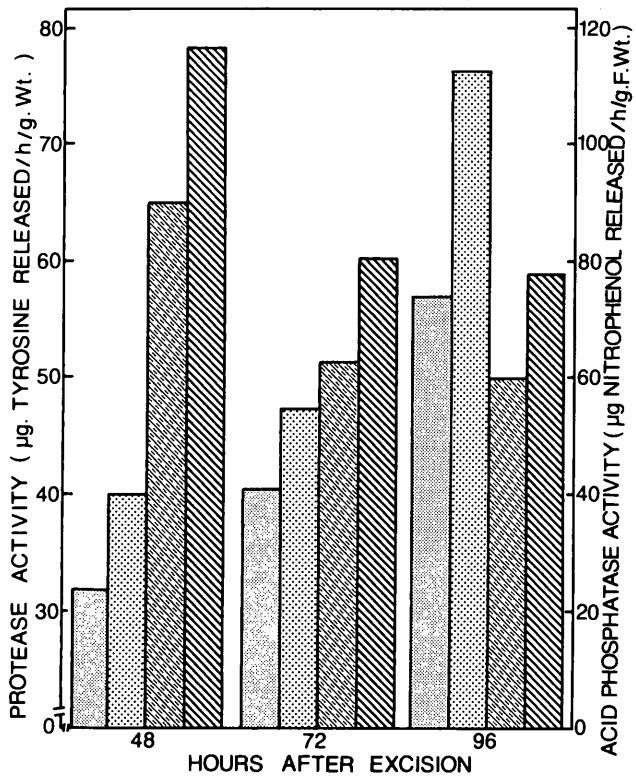


Fig. 4. Protease and acid phosphatase activities in the base of hypocotyl cuttings as influenced by paclobutrazol (Paclo) control (Cont). ▨ Cont - Protease; ▩ Paclo - Protease; ▧ Cont - Acid phosphatase; ▨ Paclo - Acid phosphatase.

related to rooting and that paclobutrazol does not promote rooting by increasing starch hydrolysis in the base of the cuttings.

The activities of acid phosphatase and protease in the base of the paclobutrazol treated hypocotyl cuttings were higher than untreated control (Fig. 4). Bhattacharya (1988) reported high activity of the enzymes during rootings. Hydrolytic enzymes (e.g., protease etc.) facilitate root primordia emergence from cuttings following digestion of middle lamella of cortical tissues (Bell and McCulley, 1970). Similarly, phosphatases have also been reported to be involved in the process of root emergence from the cuttings of *Phaseolus mungo* (Bhattacharya and Nanda, 1979). The increased activities of these enzymes by paclobutrazol may facilitate emergence of greater number of root primordia through biochemical degradation of cortical tissues and, assist in the absorption of material derived from these tissues (Malik and Kumari, 1977; Kumar and Kakkar, 1989). Furthermore, the increase of acid phosphatase activity by paclobutrazol is similar to that of IAA, which also promotes rooting (Kumar and Kakkar, 1989). Hence, paclobutrazol may stimulates rooting by enhancing acid phosphatase activity in the cuttings.

The current investigation indicates that paclobutrazol induced adventitious root formations in mung bean is accompanied by changes in the activity of a number of enzymes and metabolic constituents which have been postulated to be involved in rooting. The enhanced rooting seems to have been achieved through cumulative metabolic changes by paclobutrazol. Further, work will be required to elucidate fully the mode by which the chemical stimulates rooting.

**Acknowledgements.** Constant encouragement and help received from Prof. R.C. Mehta, Dean, Rajasthan College of Agriculture, Udaipur is acknowledged. The authors thank Dr. Tim D. Davis, BYU, Provo USA for supplying paclobutrazol.

## Literature Cited

Bassuk, N. L., L. D. Hunter, and B. H. Howard. 1981. The apparant involvement of polyphenol oxidase and phloridzin in production of apple rooting cofactors. J. Hort. Sci. **56**: 313-322.

Bell, J. K. and M. E. McCully. 1970. A histological study of lateral root initiation and development of *Zea mays*. Protoplasma **70**: 179-205.

Bernfeld, P. 1955. Amylases, $\alpha$ and $\beta$. Methods in Enzymology. **1**: 149-158.

Bergmeyer, H. U. (ed.) 1974. Methods in Enzymatic Analysis. Verlag Chemie, New York.

Bhattacharya, N. C. 1988. Enzyme activities during adventitious rooting. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds.), Adventitious Root Formations in Cuttings, Volume 2, Dioscorides Press, Portland, Oregon, pp. 88-101.

Bhattacharya, N. C., S. Bhattacharya, C. P. Malik, and K. K. Nanda. 1978. Isoenzyme polymorphism of peroxidase, IAA-oxidase, catalase and amylase in rooting of etiolated stem segments of *Salix tetrasperma*. Biochem. Physiol. Pflazen. **172**: 439-452.

Bhattacharya, S. and K. K. Nanda. 1979. Promotive effect of purine and pyrimidine bases and their role in mediation of auxin action through regulation of oxidases and phosphatases in rooting of cuttings of *Phaseolus mungo*. Indian J. Exp. Biol. **17**: 40-45.

Bora, K. K. and S. Mathur. 1989. Paclobutrazol-a promising new PGR for promotion of rooting of cuttings. J. Curr. Bio. Sci. **6(3)**: 99-102.

Bora, K. K., A. Upadhyaya, K. Vaid, D. Sankhla, and N. Sankhla. 1980. Pollutants and growth of Indian desert plants II. Role of Lead in early seedlings growth and enzyme activity. Trans. Isdt. & Ucds. **5(2)**: 39-44.

Davis, T. D. and N. Sänkhla. 1988. Effect of shoot growth retardants and inhibitors on adventitious rooting. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds.), Adventitious Root Formation in Cuttings, Volume 2. Dioscorides Press, Portland, Oregon, pp. 174-183.

Davis, T. D., N. Sankhla, and A. Upadhyaya. 1986. Paclobutrazol -a promising plant growth regulator. *In* S. S. Purohit (ed.), Hormonal Regulation of Plant Growth and Development, Volume 3. Agro Botanical Publishers (India) Bikaner, pp. 311-322.

Davis, T. D., N. Sankhla, R. H. Walser, and A. Upadhyaya. 1985. Promotion of adventitious root formation on cuttings by paclobutrazol. Hort. Sci. **20**: 883-884.

Farkas, G. L. and Z. Kiraly. 1962. Role of phenolic compounds in physiology of plant disease and disease resistance. Phytopathol. Z. **44**: 105-150.

Hansen, J. 1988. Influence of gibberellins on adventitious root formation. *In* T. D. Davis, B. E. Haissig and N. Sankhla (eds.), Adventitious Root Formation in Cuttings, Volume 2. Dioscorides Press, Portland, Oregon, pp. 162-173.

Krishnamoorthy, H. N. 1972. Effect of growth retardants and abscisic acid on the rooting of hypocotyl cuttings of muskmelon (*Cucumis melo* cv. Kutana). Biochem. Physiol. Pflazen. **163**: 513-517.

Kumar, K. and R. K. Kakkar. 1989. Changes in activities of some hydrolyses and oxidoreductases in IAA-treated hypocotyl cuttings of *Phaseolus vulgaris* L. during adventitious root initiation and development. Curr. Sci. **58(12)**: 694-696.

Malik, C. P. and U. Kumari. 1977. Histochemical studies of metabolic reserves and enzymes during initiation and formation of adventitious roots in *Impatiens balsamina* L. New Bot. **4**: 113.

Molnar, J. M. and L. J. Lacroix. 1972. Studies on the rooting of cuttings of *Hydrangea macrophylla* enzyme changes. Can. J. Bot. **50**: 315-322.

Nanda, K. K., N. C. Bhattacharya, and N. P. Kaur. 1973. Effect of morphactin on peroxidases and its relationship with rooting of hypocotyl cuttings on *Impatiens balsamina*. Plant Cell Physiol. **14**: 297-311.

Read, P. E. and V. C. Hoysler. 1969. Stimulation and retardation of adventitious root formation by application of B-nine and cycocel. J. Ame. Soc. Hort. Sci. **94**: 314-316.

Roe, J. H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. J. Bio. Chem. **212**: 335-343.

Sankhla, N., T. D. Davis, A. Upadhayaya, D. Sankhla, R. H. Walser, and B. N. Smith. 1985. Growth and metabolism of soybean as affected by paclobutrazol. Plant Cell Physiol. **26**: 913-921.

Shannon, L. H., L. Uritaric, and A. Imaski. 1971. De novo synthesis of peroxidase isoenzymes in sweet potato slices. Plant Physiol. **47**: 493-498.

Swarnkar, P. L., S. P. Bohra, and N. Chandra. 1987. Biochemical changes during rooting in cultural stem segments of *Petunia hybrida*. Ann. Biol. **3(1)**: 32-35.

Wood, B. M. 1984. Influence of paclobutrazol on selected growth and chemical characteristics of young pecan seedlings. Hort. Sci. **19**: 837-839.

Zink, M. W. and I. A. Veliky. 1979. Acid phosphatases of *Ipomoea* sp. cultured *in vitro* I. Influence of pH and inorganic phosphate on the formation of phosphatases. Can. J. Bot. **57**: 739 -753.

# 以 paclobutrazol 誘導綠豆胚軸插枝生根時之相關代謝變化

K. K. Bora, Jaishree Munot and Sudha Mathur

Department of Plant Physiology and Biochemistry
Rajasthan College of Agriculture
RAJAU, Udaipur-313001, India

以 paclobutrazol 處理，會增加綠豆插枝所產生的不定根數目，然而，這種化學物質也降低了幼苗胚軸和根的長度。處理過的插枝，其基部的過氧化酶，多酚氧化酶、磷酸酶和蛋白酶均會增加。經 paclobutrazol 誘導，酵素的活性被提昇最高的爲過氧化酶。相反的，paclobutrazol 可使澱粉酶活性和可溶性糖類減少，因此，推測此澱粉酶可能和根的形成無關。處理後之插枝，其酚類化合物亦減少。這些結果顯示 paclobutrazol 在增加不定根形成時，就如先前對發根研究之推測，也同時造成有關酵素活性及化學成份濃度的改變。