Indian J. Plant Physiol., Vol. XXX, No. 1, pp. 96-103 (March, 1987)

REGENERATION OF FRENCH BEAN (*PHASEOLUS VULGARIS* L.) CUTTINGS AS INFLUENCED BY MORPHACTIN PRETREATMENT OF STOCK PLANTS

BINA PUNJABI AND R. N. BASU

University College of Agriculture, Calcutta University,
35 Ballygunge Circular Road, Calcutta 700 019

SUMMARY

French bean (*Phaseolus vulgaris* L. cv. Shravani Ghevada) cuttings, made from stock plants 8-10 days after pretreatment of 10-day-old seedlings with a morphactin formulation, EMD 7301 (10 ppm effective conc., spray application), showed a significant inhibition of adventitious root formation. Rooting was, however, greatly promoted by the pre-planting application of the auxins indoleacetic acid, indolebutyric acid and naphthaleneacetic acid to the base of cuttings resulting in a synergistic effect on regeneration of adventitious roots. When cuttings were made soon after morphactin pretreatment of stock plants auxin-induced rooting of cuttings was inhibited; a time-gap of over a week between spraying and preparation of cuttings was necessary for the synergistic effect.

The beneficial effect of morphactin pretreatment on rooting of cuttings in the presence of auxins cannot be interpreted in terms of differential uptake and distribution of the labelled auxins, IAA-1-^{14}C and IAA-2-^{14}C or the activity of the IAA-oxidizing enzymes. Growth suppression because of morphactin pretreatment resulted in an accumulation of carbohydrates and nitrogenous reserves in shoots. This, coupled with increased synthesis of rooting factors in the pretreated plants, may account for the observed positive interaction between morphactin pretreatment and auxin application.

INTRODUCTION

Suppression of vegetative growth of the stock plants has been found to be conducive to the formation of adventitious roots on cuttings (Sen and Basu, 1960; Sen, *et al.*, 1965; Stoltz and Hess, 1966; Ghosh and Basu, 1973; Basu and Ghosh, 1974; Paria, 1976). Therefore, the possibility of promoting the rooting of daughter cuttings by suppressing the growth of stock plants with growth-inhibiting chemicals was investigated in the present laboratory (Punjabi *et al.*, 1974;

Punjabi and Busu, 1975; Paria, 1976; Punjabi *et al.*, 1977; Bose *et al.*, 1977). It was noted that rooting of cuttings from morphactin (chlorflurenol, 2-chloro-9-hydroxyfluorene-9-carboxylic acid) pretreated french bean and justicia (*Justicia gendarussa* L.) plants in the presence of an exogenous application of IBA (indolebutyric acid) and NAA (naphthaleneacetic acid) exceeded that of the corresponding auxin-treated cuttings from control plants (Punjabi *et al.*, 1974). Rooting of cuttings was, however, greatly inhibited in the absence of an exogenous supply of auxins. The physiological and biochemical bases of the opposing effects of morphactin on adventitious root formation have not been analysed so far. In the present investigation, we have, therefore, studied the involvement of nutritional, enzymatic and hormonal factors in the auxin-induced rooting of morphactin-pretreated French been cuttings.

MATERIALS AND METHODS

Rooting Experiments : French bean seedlings were grown in the nursery beds of the Agri-Horticultural Society of India, Alipore, Calcutta under natural outdoor conditions during the winter months from December to middle of February. Ten-day old seedlings having a shoot length of about 10-12 cm, with the primary leaves fully expanded, were sprayed to the point of run-off with a morphactin (EMD 7301; a chlorohydroxyfluorene carboxylic acid formulation of CELAMERCK GmbH & Co. at 10 ppm effective conc.). Cutting were prepared from the treated stock plants 2 and 10 days after spraying. Each cuttin gwas 10 cm long with a 5 cm long hypocotyl region. The leaf area was kept constant at 10 sq cm per cutting by punching with a metal device and the apical bud was pinched off. Ten cuttings were placed in 40-ml-capacity glass vials containing 30 ml of rooting solution made up of IAA (5×10^{-5}M) or IBA (2×10^{-5}M) or NAA (2×10^{5} M) in phosphate buffer (pH 6.5, 0.01M). Ruquisite controls were provided and there were 3 replications for each treatment. The solution was taken up after 24 hr and the vials were then filled with distilled water which was changed every alternate day till data on number of roots per cutting were recorded. The interaction of auxins, IAA, IBA and NAA with morphactin (1 ppm) supplied to the base of cuttings prepared from 15-day-old control (unsprayed) plants was also studied.

Data on number of roots per cutting were taken after 8 days and values for synergism and antagonism were calculated and tested for statistical significance following the method of Roy *et al.* (1972).

Carbohydrate and nitrogen fractions : The different carbohydrate and nitrogen fractions were estimated from the shoot samples (leaf and stem) taken 2 and 10 days after spraying with morphactin. Sugars were estimated following the copper reduction method of Somogyi (1952) and the conventional microkjeldahl method was employed for the estimation of different nitrogen fractions.

The methods of extraction of the IAA-oxidase enzyme from stem and leaf samples were similar to those described by Basu (1970). Extraction, incubation and assay of amylase activity were done following the methods of Punjabi and Basu (1972). Activity of IAA-oxidase was assayed using IAA-1-^{14}C following the procedure described by Basu (1970).

Samples for studying the changes in endogenous hormonal levels were collected 2 and 10 days after spraying with morphactin. Methods employed in extraction, fractionation of the extract for free and bound substances and chromatography were similar to those described by Basu *et al.* (1967). Bioactive regions on the chromatograms were determined by the wheat coleoptile straight growth test of Mer *et al.* (1962). Rooting factor activity was assayed by the kenaf rooting test using 10-day-old kenaf (*Hibiscus cannabinus* L.) plants; the method was, in principle, similar to the mung been rooting of Hess (1964). The original values for each type of bioassay viz. wheat coleoptile straight growth, rice seedling growth and kenaf rooting tests were first transformed to percentages of their respective controls. The net promoting and inhibiting activity was calculated by pooling the different Rf values and then representing them as biohistograms. For the bioassay of gibberellin and gibberellin-like substances on chromatograms the rice seedling test of Murakami (1959) was followed.

Parallel and co-chromatography with authentic sample of morphactin were done to study its zone of resolution in different solvent systems based on bioactivity of eluates of chromatograms in the rice seedling test. Similar chromatographic procedure was followed for the characterization of phenols resolving on chromatograms of stem and leaf but instead of bioassay specific chromogenic reagents for the phenols were employed.

For upake studies, leafless cuttings were prepared from French bean plants 7 days after spraying with morphactin. Radioactive auxins, IAA-1-^{14}C and IAA -2-^{14}C obtained form Radiochemical Centre, Amersham, England, having a specific activity of 52 mCi/mmol each were employed. The methodology was the same as that described by Basu (1971).

RESULTS AND DISCUSSION

The French bean rooting experiments clearly show an opposing effect of morphactin depending upon its mode of application. It promoted auxin-induced root formation on cuttings taken from plants 10 days after pretreatment with the chemical but significantly inhibited rooting when supplied directly to the base of cuttings either alone or in combination with auxins (Table I). In case of cuttings made 2 days after morphactin pretreatment an exogenous application of auxins could not counteract the root-inhibitory effect of morphactin.

Table I : Rooting of French bean cuttings as influenced by (a) basal application of morphactin and auxins to cuttings before pretreatment of stock plants and (b) pretreatment of stock plants with morphactin and basal application of auxins to cuttings made from stock plants 2 and 10 days after morphactin pretreatment

Mean number of roots per cutting (±SE)

(a) Basal application of morphactin

Time of treatment	Treatment	Auxins			
		Control	IAA	IBA	NAA
Before treatment	Control	15.3±1.1	16.6±1.3	89.4±2.8	53.3±0.8
	Morphactin (1 ppm)	0	4.5±1.4	0	3.0±0.6

(b) Pretreatment of stock plants with morphactin

Days after pre-treatment	Pretreatment	Auxins			
		Control	IAA	IBA	NAA
2 days	Control	27.2±0.74	42.1±1.99	120.8±3.70	61.9±0.22
	Morphactin (10 ppm)	19.2±0.54	36.8±1.06 (+2.7)	110.8±2.02 (—2.0)	50.9±1.38 (—3.0)
10 days	Control	17.9+0.34	27.9±0.19	94.9±2.10	50.9±0.66
	Morphactin (10 ppm)	16.1±1.64	44.6±3.18 (+18.5**)	177.1±1.60 (+84.0**)	82.1±1.38 (+33.0**)

Values in parentheses under morphactin pretreatment denote synergism (+) or antagonism (—)

Concentration of basally applied auxins : IAA 5×10^{-5}M, IBA and NAA 2×10^{-5}M

** Significant at 0.01P.

As regards the direct root-inhibiting effect of morphactin, it has been suggested that morphactins exert differential effects on the different phases of adventitious root formation on woody and non-woody cuttings (Schneider, 1970). Thus induction of cell division is stimulated and the number of primordia may be increased, but the organization of those primordia seems to be often histologically disturbed so that extension growth of the root may not take place normally or is retarded. (Kochar *et al.*, 1972).

The mechanism of the promoting effect of morphactin pretreatment of the stock plants on the auxin-induced rooting of cuttings has been analyzed in terms of the effect of the chemical on carbohydrate and nitrogen metabolism, transport and distribution of exogenously applied auxins, auxin economy of cells and synthesis of rooting factors in tissues.

Pretreatment of the stock plants with morphactin inhibited the elongation growth of the second internode. The concentration of sugars and reserve polysaccharides, proteins and soluble nitrogen fractions were much higher in the shoots of morphactin-pretreated plants (Fig. 1). Amylase activity was also higher in the pretreated shoots than in the controls. There was very little difference in the activity of the IAA-oxidizing system between shoots of control plants and morphactin-pretreated ones (Fig. 1). The uptake and distribution of radioactive auxins (IAA-^{14}C and IAA 2-^{14}C) were also similar in cuttings from morphactin-pretreated and control plants.

An enhanced biogenesis of rooting factors (auxin-synergists) were noted in shoots 10 days after spraying with the chemical. Two peaks of rooting factor activity were present on the chromatograms of extracts of pretreated shoots. A smaller zone of activity was noted around Rf 0.2 and was due to the phenolic inhibitors characterized as p-hydroxybenzoic p-coumaric and ferulic acids. The bigger peak between Rf 0.7-1.0 was due to certain unidentified factors which caused significant inhibition of wheat coleoptile straight growth.

The data on net promotion and inhibition in Fig. 2 would show that total rice root growth inhibiting activity was more at S_1 in the free fraction of leaf extracts of morphactin plants. Rooting of kenaf cuttings was also inhibited to a greater extent by inhibitors at S_1 due to unmetabolized morphactin itself. At S_2, however, this inhibition was not seen and greater root promoting activity could be noted in free fractions of both stem and leaf samples from morphactin pretreated plants. Total wheat coleoptile growth inhibitor activity was more in the control than morphactin treated plants, at both S_1 and S_2 stages.

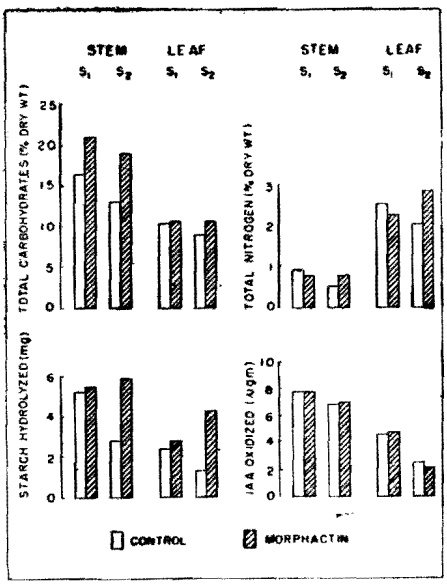

Fig. 1: Total carbohydrate and nitrogen contents and activity of amylase and IAA-oxidizing enzymes in stem and leaf samples of control (unsprayed) and morphactin pretreated French bean plants 2 days (S_1) and 10 days after spraying (S_2).

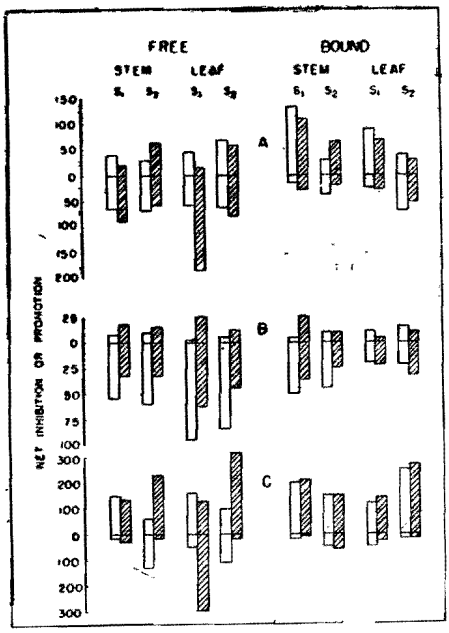

Fig. 2: Net promoting and inhibiting activity in French bean stem and leaf extracts as reflected by promotion or inhibition of rice root growth (A), wheat coleoptile straight growth (B) and rooting of kenaf cuttings (C). Shading of histograms as in Fig. 1.

The beneficial effect of morphactin pretreatment cannot be interpreted in terms of a differential uptake and transport of exogenously supplied auxins or the activity of the IAA-oxidizing system. Greater rooting factor activity in the pretreated shoots corresponded with a high degree of adventitious root formation on cuttings supplied with auxins. In the absence of the auxins the root-forming potentiality of cuttings could not be fully expressed as the rooting factors accumulated are ineffective alone and promote rooting by interacting with exogenously supplied auxins (Gorter, 1969).

The observations that the growth-inhibiting zones corresponded with high rooting factor activity of the same zones, confirm the root promoting activity of growth inhibitors (Basu *et al.*, 1968; Gorter, 1969: Roy *et al.*, 1972; Bose *et al.*, 1973; Ghosh and Basu, 1973; Basu and Ghosh, 1974).

The accumulation of carbohydrates due to growth suppression in morphactin-treated French-bean plants may also be relevant in understanding the

mechanism of the synergistic effect. Under conditions which favour accumulation of carbohydrates, phenolic and other growth-inhibiting compounds have been shown to accumulate (Basu *et al.*, 1967; Choudhuri and Rudra, 1971; Kefeli and Kadyrov, 1973 b; Busu and Ghosh, 1974).

The inhibition of rooting which was observed in cuttings made 2 days after morphactin pretreatment, in presence or absence of auxins, was possibly due to unmetabolised morphactin itself. When the time-gap was more morphactin was detoxified and it is also possible that such detoxification products reacted synergistically with the exogenously supplied auxins.

Further studies are considered necessary to elucidate the mode of action of morphactin pretreatment in the rooting of cuttings. Apart from the fundamental considerations the results presented in this paper open up possibilities of utilising the growth-inhibiting chemicals in commercial plant propagation.

REFERENCES

Basu, R. N. (1970). Indoleacetic acid oxidizing system in relation to synergism and antagonism between auxins and non-auxinic chemicals in rooting of cuttings. *Indian J. Plant Physiol.*, **13**: 251-264.

Basu, R. N. (1971a). Transport of indoleacetic acid in bean cuttings in relation to root ormation. *Cur. Sci.*, **40**: 427-429.

Basu, R. N. and Ghosh, S.K. (1974). Effect of nitrogen nutrition of stock plants of *Justicia gendarussa* L. on the rooting of cuttings. *J. Hort. Sci.*, **49** : 245-252.

Basu, R. N., Roychoudhury, N. and Sen, P. K. (1967b). Analysis of the effect of ringing and auxin treatment on rooting of mango (*Mangifera indica* L.) seedling cuttings. *Proc. Int. Symp. Plant Growth Substances*, Ed. S.M. Sircar, Cal, pp. 271-281.

Basu, R. N., Ghosh, B. and Sen, P. K. (1961). Naturally-occurring rooting factors in mango (*Mangifera indica* L.). *Indian Agric.*, **12**: 194-196.

Bose, T. K., Mukhopadhyay, T. P. and Basu, R. N. (1976). Effect of ethylene and acetylene on the rooting of cuttings. *Indian J. Plant Physiol.*, **20**: 134-139.

Choudhuri, J. M. and Rudra, P. (1971). Physiological studies on chemical control of growth and flowering in mango (*Mangifera Indica* L.). *Indian Agric.*, **15**: 127-135.

Ghosh, S. K. and Basu, R. N. (1973b). Effect of nutrition of stock plant on rooting of cuttings. *Indian Agric.*, **17**: 7-16.

Gorter, C. J. (1969). Auxin-synergists in the rooting of cuttings. *Physiol. Plant*, **22**: 493-502.

Hess, C. E. (1964). Naturally-occuring substances which stimulate root initiation. *In* : *Regulateurs Naturels dc la Croissance Vegetale, Ed.* J. P. Nitsch, C. N. R. S., Paris, pp. 517-527.

Kefeli, V. I. and Kadyrov, C. S. (1971). Natural growth inhibitors, their chemical and physiological properties. *Ann. Rev. Plant Physiol.*, **22**: 185-196.

Mer, C. L., Choudhury, S. H., Dattaray, P. and Hafeez, U. (1962). The influence of light and temperature on the estimation of auxin by a straight growth technique. *Indian J. Plant Physiol.*, 5: 97-116.

Murakami, Y. (1959). A paper chromatographic survey of gibberellins and auxins in immature seeds of leguminous plants. *Bot Mag.* Tokyo, 72: 36-43.

Paria, N. C. (1976). Studies on the effect of nutrition of stock plant on the rooting of cutting. *Ph. D. (Agri.) thesis, Cal. Univ.*

Punjabi, B. and Basu, R. N. (1975). Effect of treatment of stock plants with Ethrel and Morphactin on the rooting of French bean (*Phaseolus vulgoris* L.) cuttings. *Proc. Symp.* Recent Adv. Plant Sci., B.C.K.V.V., Kalyani, W.B.

Punjabi, B. and Basu, R. N. (1978). Amylolytic activity in relation to adventitious root formation on stem cuttings. *Indian Biol.*, 10: 65-71.

Punjabi, B., Dewan, A. and Basu, R. N. (1977). Effect of pretreatment of stock plants with growth suppressing chemicals on the rooting of *Hibiscus cannabinus* L. cuttings. *Plant Sci.*, 9: 21-23.

Punjabi, B., Paria, N. C. and Basu, R. N. (1974). Opposing effects of morphactin on the rooting of cuttings in the presence or absence of auxin. *J. Hort. Sci.*, 49: 253-256.

Roy, B. N., Basu, R.N. and Bose, T. K. (1972). Interaction of auxins with growth-retarding, -inhibiting and ethylene-producing chemicals in rooting of cuttings. *Plant Cell Physiol.*, 13: 1123-1127.

Sen, P. K. and Basu, R. N. (1960). Effect of growth substances on root formation cuttings of *Justicia gendarussa* L. as influenced by varying levels of nitrogen nutrition of stock plants. *Indian J. Plant Physiol.*, 3: 72-83.

Sen, P. K., Sen, S. and Basu, R. N. (1965). Studies on rooting of cuttings : Effects of auxins and organic nutrients on rooting of cuttings of *Justicia gendarussa* Linn. taken from stock plants grown under varying levels of nitrogen. *Indian J. Plant Physiol.*, 8: 36-49.

Somogyi, M. (1952) Notes on Sugar determination. *J. Biol. Chem.*, 200: 145-154.

Stoltz, L. P. and Hess, C. E. (1966). Effect of girdling upon root initiation, carbohydrate and amino acids. *Proc. Am. Soc. Hort. Sci.*, 89: 734-743.