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HORMONAL REGULATION OF ROOT FORMATION IN PACLOBUTRAZOL TREATED BEAN PLANTS

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Abstract

Besides retarding expansion growth paclobutrazol stimulates the root forming process both in intact plants and stem cuttings.

The promoting effect on the rooting process in primary leaves of treated plants was not observable, the formation of root primordia was definitely inhibited in petioles. This inhibition can be overcome by IBA, ABA and ethylene-generator treatment, and all the three cases the initiation of root primordia can be prevented by STS pretreatment.

Results are discussed in connection with the endogenous IAA content and ethylene production of bean plants.

Key words: abscisic acid, bean, IAA content, IBA, ethylene, paclobutrazol, *Phaseolus vulgaris*. rhisogenesis, STS

Introduction

Stimulation of root formation is a known side effect of plant growth retardants.

The triazole derivative paclobutrazol (PP333) is also characterized by such effect both in intact plants and stem cuttings (STEFFENS et al., 1983; LENZ, 1984; STEFFENS and WANG, 1984; DAVIS et al., 1985; SEBANEK et al., 1991).

PP333 originally had been developed as fungicide, but today it is used as a potent growth retardant which is active in a wide range of plant species (LEVER, 1986)

The retarding effect of PP333 on shoot growth is generally explained by the inhibition of gibberellin-biosynthesis (DALZIEL and LAWRENCE, 1984; HEDDEN and GRAEBE, 1985; LEVER, 1986; GRAEBE, 1987). In connection with the rooting process its effect on the indole-3-acetic acid (IAA) metabolism should also be taken into consideration since it is generally well accepted fact that IAA has an important role in rooting process (TORREY , 1976; HARTMAN and KESTER, 1983).

Since the stem-thickening effect of retardants in the basal part of the stem is a characteristic ethylene effect as well, in the induction of rooting the production of ethylene may be also important.

In our present work we investigated the IAA content and its distribution, the ethylene production of the hypocotyls and primary leaves of beans treated with PP333 in connection with their rooting capatity.

Material and Methods

Seeds of *Phaseolus vulgaris L.* c. v. Juliska were soaked in a paclobutrazol (ICI, USA) solution containing 5 $mg \cdot l^{-1}$ of the active component in a thermostat at 25 °C. On the 3rd day they were sowed into garden mould. The plants were grown under controlled conditions (CONVIRON Cabinet model EF7, equipped with 4×50 W Sylvania incandescent lamps, at 25/20 °C day/night temperatures respectively, 16 h illumination with 21 Wm^{-2} and 65% relative humidity). The IAA-content and ethylene production of the hypocotyls was measured in six-day-old plants, while that of the primary leaves in fourteen-day-old ones. The hypocotyls were divided into two parts of the same length, in the case of leaves the laminae and petioles were separate and the amount of IAA was determined separately in both parts.

Measurement of IAA-content: the IAA-contents of hypocotyls and that of primary leaves were determined after extraction with 80% cold methanol.

The extract was evaporated to dryness under reduced pressure, then it was purified and fractionated according to the method of KAMISAKA and LARSEN (1977). The amount of IAA present in the final acidic ether fraction was measured by the indolo-α-pyrone fluorescence method (KNEGT and BRUINSMA, 1973; HEMBERG and TILLBERG, 1980) with a PERKIN-ELMER spectrofluorimeter.

Losses of IAA during extraction and purification were estimated by adding a known amount of [2-^{14}C]-IAA (Amersham, U. K., 120 GBq/mol IAA^{-1}) as an internal standard.

Measurement of ethylene-production: ethylene was measured by a gas chromatograph fitted with a flame ionization detector and an alumina column. Plant parts were enclosed in 20—100 ml gas tight flaske and samples were withdrawn from the flask with a Hamilton gas-tight syringe and analysed isothermally after 6-h-incubation.

All experiments were repeated at least three times and measured 5 parallel samples.

In the experiments on root formation stem and primary leaf cuttings were immersed in fourth strength Hoagland nutrient solution supplemented with 1 ml of complex solution of micronutrients.

In order to promote rooting the primary leaves were evenly moistened with 1 μl of 1 mg/l indole-3-butyric acid (IBA, Reanal) and abscisic acid (ABA, Sigma) solution containing 0,05% TWEEN 80 as a detergent.

The ethylene treatment was carried out by „Rol-Fruct" an ethylene-generator, contaning 40% 2-chloroethyl-phosphonic acid (Chinoin Co., Budapest). 0,04 μg of agent was spread over the entire surface of one leaf.

0,1 mM silver thiosulfate (STS, O,1 mM $AgNO_3$ + 0,1 mM sodium thiosulfate) was applied to inhibit the effect of ethylene.

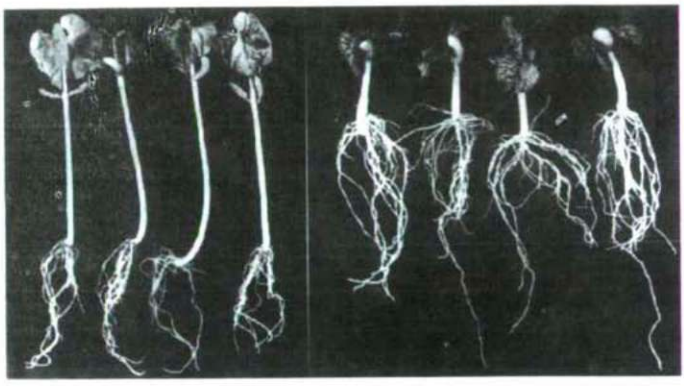

Fig. 1.: Effect of PP333 on the growth of six-day old *Phaseolus vulgaris* cv. Juliska seedlings. (Left: control, right: treated plants)

Results

The effect of PP333 treatment on growth of bean seedlings is presented in Fig. 1. and Table 1.

Besides retarding expansion of shoot growth, stimulation of stem-thickening, richer and longer roots are the main morphological differences between the treated and control plants.

From the point of view of the retarding effect on shoot elongation and the stimulating effect on root growth first of all the knowledge of the effect on IAA content is important.

The results are summarised in Table 2.

In the hypocotyls of PP333 treated plants the total IAA content is higher than that of the control, but the distribution ratio between the apical and basal part was changed comparing to the control. This new proportion is favourable only for rooting process but disadvantageous for elongation growth of the shoot.

Table 1. Effect of paclobutrazol on the growth of bean plants (Data of six-day old plants)

	hypocotyl				root		
	length mm	diameter of basal part mm	fresh weight g	dry weight mg	length of primary root mm	fresh weight g	dry weight mg
Control	108,8±11,2	3,1±0,2	1,052±0,290	55,5±5,1	110,21±11,50	0,464±0,029	41,46±5,05
Treated	37,9±12,1	5,2±0,3	0,534±0,190	33,7±5,9	194,40±14,30	1,027±0,040	56,88±7,16

Table 2. Effect of paclobutrazol on the IAA content and quantitative distribution between apical (A) and basal (B) parts of hypocotyls

	Control		Treated	
	A	B	A	B
ng IAA/hypocotyl part	1,8±0,5	2,4±0,4	2,2±0,6	4,9±0,6
ng IAA/fresh mass	2,7±0,2	3,6±0,5	3,7±0,5	7,2±0,6
distribution in % of the total quantity	42,85	57,15	30,98	69,02

Table 3. Effect of paclobutrazol on the ethylene production of the apical (A) and basal (B) parts of bean hypocotyls

	Control		Treated	
	A	B	A	B
ethylene nl/hyp. part/h	0,093±0,008	0,087±0,006	0,003±0,0001	0,0527±0,004
ethylene/g fresh mass/h	0,130±0,040	0,110±0,030	0,070±0,0100	0,1200±0,030
distribution of ethylene production between A and B parts of hypocotyls as % of total ethylene release	51,7	48,3	5,4	94,6

In the effect of PP333 on the ethylene production of hypocotyls (Table 3.) the effect on the total ethylene production is not so striking than the difference between the levels of ethylene formation in the apical and the basal parts.

In the primary leaves of PP333 treated plants an increase in rooting capacity was not observable, on the contrary the formation of root primordia were definitely inhibited in petioles (Fig. 2.). After PP333 treatment the distribution of IAA between petiole and blade was changed (Table 4.).

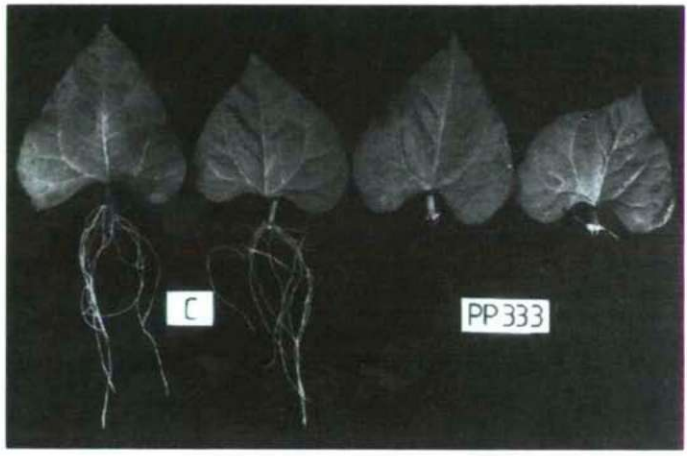

Fig. 2.: Adventitious root formation of detached primary leaves of PP333 treated bean plant

Table 4. Effect of paclobutrazol on IAA content and its distribution in blade and petioles of bean leaves

	Control		Treated	
	blade	petiole	blade	petiole
ng IAA/leaf part	3,5±0,6	3,2±0,5	7,8±0,6	3,1±0,6
ng IAA/g fresh mass	4,4±0,1	8,6±0,4	9,7±0,5	6,2±0,8
distribution in % of the total IAA quantity between blade and petiole	52,23	47,77	71,55	28,45

Table 5. Effect of paclobutrazol on the ethylene production of the blade and petiole of bean leaves

	Control		Treated	
	blade	petiole	blade	petiole
ethylene nl/plant part/h	0,790±0,07	0,0520±0,01	2,690±0,62	0,0310±0,01
ethylene nl/g fresh mass/h	1,780±0,67	0,5400±0,02	7,150±0,43	0,6900±0,02
distribution of ethylene production between blade and petiole as % of total ethylene release	93,8	6,2	98,9	1,1

The quantity of ethylene production is also changed in the blade and petiole; in the petioles of primary leaves of treated plants only a lower ethylene production can be measured (Table 5.).

The inhibiting effect of PP333 on rooting of primary leaves can be overcome by IBA (Fig. 3.), ABA (Fig. 4.) and ethylenegenerator (Fig. 5.) treatment, and in all three cases the inhibition can be restored by STS.

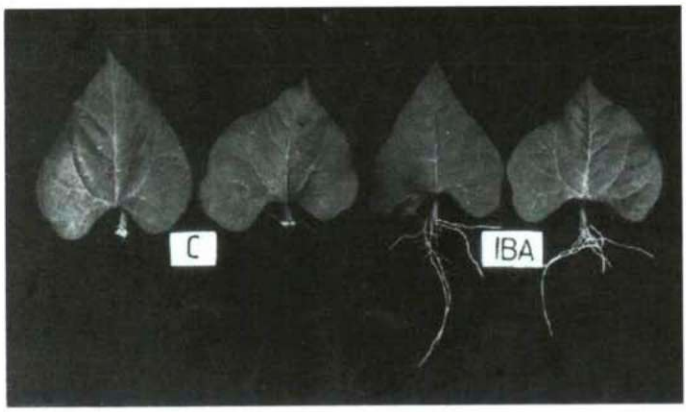

Fig. 3.: Effect of IBA on the adventitious root formation of detached primary leaves of bean plant pretreated with PP333.

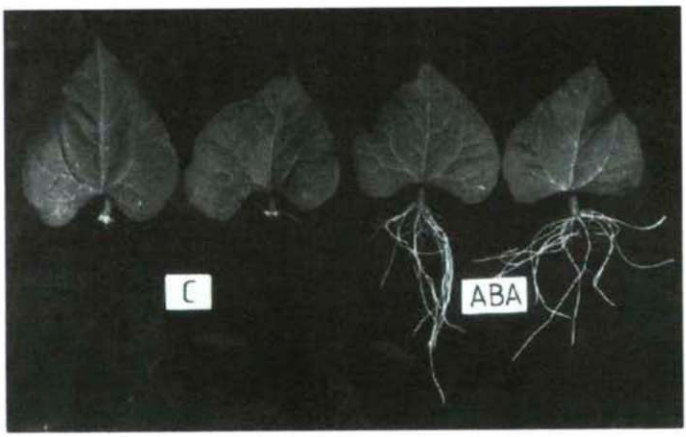

Fig. 4.: Effect of ABA treatment on the adventitious root formation of detached primary leaves of bean plant pretreated with PP333.

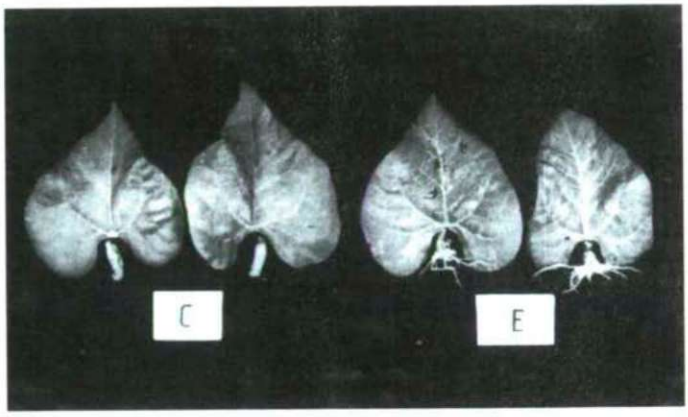

Fig. 5.: Effect of ethylene-generator (E) treatment on the adventitious root formation of detached primary leaves of bean plant pretreated with PP333.

Discussion

Hormonal regulation of root formation was studied in bean hypocotyls and primary leaves treated with PP333.

In the hypocotyls of treated plants positive correlation can be observed between the effect of rooting capacity and the change of IAA distribution.

The higher IAA content measurable in the basal part of hypocotyls is advantageous for the induction of rooting process. The change in the IAA content involves the change in the ethylene production as well because of the known effect of auxin on the ethylene production (SHINGO and IMASEKI, 1971; BURG, 1973; LAU and YUNG, 1974; MALLOCH and OSBORNE, 1976).

The ethylene production of hypocotyl parts correlates well with their IAA content. The higher ethylene production in basal hypocotyl parts is advantageous for the root initiation and this is the cause of the stem-thickening too, observable at the basal part of hypocotyls which comes into beeing by stimulation of lateral expansion of the cells (CAMP and WICKLIFF, 1981; PINFIELD et al., 1984).

The PP333 treated primary leaves contain higher IAA amount than that of the control. Inspite of this root initiation is inhibited. The reason of this phenomenon may be partly the changed IAA-distribution in the primary leaf between the blade and petiole, on the other hand the high cytokinin content because of the treatment with a triazole derivative (FLETCHER and ARNOLD, 1986; GROSSMANN et al., 1987; IZUMI et al. 1988) don't make possible the advantageous IAA/cytokinin ratio for rooting. In the originating of favourable ratio ethylene has an important role by decreasing the cytokinin level (VAN STADEN et al., 1987; BOLLMARK and ELIASSON, 1990), but the ethylene amounts produced by PP333 treated leaves are not sufficient to this.

According to our results ethylene is primely important in this system, because the hormones which are capable of increasing the ethylene production of leaf blade and petiole are effective in promoting the formation of adventitious roots.

Besides the ethylene-generator „Rol-Fruct", abscisic acid (ABA) is also suitable to increase the ethylene production because the higher ABA level brings about higher ethylene production (MAYAK and DILLEY, 1976; GOREN, 1979; LIEBERMANN et al., 1977; SAGEE et al., 1980; WRIGHT, 1980; RIOV et al., 1990). Otherwise PP333 treatment decreases the endogenous ABA level in the leaves (WANG et al., 1987; BUTA and SPAULDING, 1991).

The effectiveness of IBA in initiation of root primordia may be due to the induction of high ethylene production in the region of adventitious root formation (RIOV and YANG, 1989). Effect of IBA on decreasing of cytokinin level in petioles (BRIDGALL and VAN STADEN, 1985; BOLLMARK and ELIASSON, 1990) may be connected with the great ethylene production too.

On the bases of our results ethylene can play a key role in this system in the origination of IAA/cytokinin ratio advantageous for the root forming process. Our suggestions are supported by the effect of Ag^+, an inhibitor of ethylene action which can prevent the stimulation of root formation in petioles of all three applied hormones.

References

BOLLMARK, M. and ELIASSON, L. (1990): Ethylene accelerates the breakdown of cytokinins and thereby stimulates rooting in Norway spruce hypocotyl cuttings. — Physiol. Plant. *80*, 534—540.

BRIDGALL, S. S. and VAN STADEN, J. (1985): Effect of auxin on rooting and endogenous cytokinin levels in leaf cuttings of *Phaseolus vulgaris* L. — J. Plant Physiol. *117*, 287—292.

BURG S. P. (1973): Ethylene in plant growth. — Proc. Natl. Acad. Sci. USA. *79*, 591—597.

BUTA, J. G. and SPAULDING, D. W. (1991): Effect of paclobutrazol on abscisic acid levels in wheat seedlings. — J. Plant Growth Reg. *10*, 59—61.

CAMP, P. J. and WICKLIFF, J. L. (1981): Light or ethylene treatments induce transverse cell enlargement etiolated maize mesocotyls. — Plant Physiol. *67*, 125—129.

DALZIEL, J. and LAWRENCE, D. K. (1984): Biochemical and biological effects of kaurene oxidase inhibitors such as paclobutrazol — British Plant Growth Reg, Group. *11*, 43—57.

FLETCHER, R. A. and ARNOLD, V. (1986): Stimulation of cytokinins and chlorophyll synthesis in cucumber cotyledons by triadimefon. — Physiol. Plant. *66*, 197—201.

GOREN, R., AETMAN, A. and GILADI, J. (1979): Role of ethylene in abscisic acid-induced callus formation in citrus bud cultures. — Plant Physiol. *63*, 280—282.

GRAEBE, J. E. (1987): Gibberellin biosynthesis and control. -Annu. Rev. Plant Physiol. *38*, 419—465.

GROSSMANN, K., KWIATKOWSKI, J., SIEBECKER, H. and JUNG, J. (1987): Regulation of plant morphology by growth retardants. Effects on phytohormone levels in soybean seedlings determined by immunoassay. — Plant Physiol. *84*, 1018—1021.

HARTMANN, H. T. and KESTER, D. E. (1983): Plant propagation. Principles and practices. — Prentice Hall Englewood.

HEDDEN, P. and GRAEBE, J. E. (1985): Inhibition of gibberellin biosynthesis by paclobutrazol in cell free hormogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. — J. Plant Growth Reg. *4*, 111—122.

HEMBERG, T. and TILLBERG, E. (1980): The influence of the extraction procedure on yield of indole-3-acetic acid in plant extracts. — Physiol. Plant. *50*, 178—182.

IZUMI, L., NAKAGAWA, S., KOBAYASHI, M., OSHIO, H., SAKURAI, A. and TAKAHASHI, N. (1988): Levels of IAA, cytokinins, ABA and ethylene in rice plants as affected by a gibberellin biosynthesis inhibitor, uniconazole-P. — Plant Cell Physiol. *29*, 97—104.

LAU, O. L. and YUNG, K. H. (1974): Synergistic effect of kinetin on IAA-induced ethylene production. — Plant Cell Physiol. *15*, 29—35.

LENZ, F. (1984): PP333 — ein interessanter Wachstumshemmer. — Erwerbsobstbau. *26*, 244.

LEVER, B. G. (1986): 'Cultar — a technical overview. — Acta Hort. *179*, 459—466.

LIEBERMAN, M., BAKER, J. E. and SLOGER, M. (1977): Influence of plant hormones on ethylene production in apple, tomato and avocado slices during maturation and senescence. — Plant. Physiol. *60*, 214—217.

KAMISAKA, S. and LARSEN, P. (1977): Improvement of the indole-α-pyrone fluorescence method for quantitative determination of endogenous indole-3-acetic in lettuce seedlings. — Plant Cell Physiol. *18*, 595—602.

KNEGT, E. and BRUINSMA, J. (1973): A rapid, sensitive and accurate determination of indolyl-3-acetic acid. — Phytochem. *12*, 753—756.

MALLOCH, K. R. and OSBORNE, D. J. (1976): Auxin and ethylene control of growth in seedlings of *Zea mays* L. and *Avena sativa* L. — J. Exp. Bot. *27*, 992—1003.

MAYAK, S. and DILLEY, D. R. (1976): Regulation of senescence in carnation (*Dianthus caryophyllus*). Effect of abscisic acid and carbon dioxide on ethylene production. — Plant Physiol. *58*, 663—665.

NAGY, M. and TARI, I. (1986): Ethylene production and IAA distribution in bean hypocotyls treated with CCC. — Biochem. Physiol. Pflanzen. *181*, 611—614.

PINFIELD, N. J., SANCHEZ-TORREZ, J. O. and MCDERMOTT, C. N. (1984): The modifying effect of gibberellic acid and kinetin on the growth abnormalities induced by 2,4-D in marrow seedling and the possible involvement of endogenous ethylene. — Plant Growth Reg. *2*, 99—109.

RIOV, J., DAGEN, E., GOREN, R. and YANG, S. F. (1990): Characterization of abscisic acid-induced ethylene production in citrus leaf and tomato fruit tissues. — Plant. Physiol. *92*, 48—53.

RIOV, J. and YANG, S. F. (1989): Ethylene and auxin — ethylene interaction in adventitious root formation in mung (*Vigna radiata*) cuttings. — Plant Growth Reg. *8*, 131—141.

IAGEE, O., GOREN, R. and RIOV, J. (1980): Abscission of citrus leaf explants: Interrelationships of abscisic acid, ethylene and hydrolytic enzymes. — Plant. Physiol. *66*, 550—753.

STEFFENS, G. L., WANG, S. Y., STEFFENS, C. I. and BRENNAN, T. (1983): Influence of paclobutrazol (PP333) on apple seedling growth and physiology. — Proc. 10th Ann. Meet. PGR Soc. Amer. *43*, 195—206.

STEFFENS, G. L., WANG, S. Y. (1984): Physiological changes induced by paclobutrazol (PP333) in apple. — Acta Horticult. *146*, 135—142.

SEBANEK, J., KRÁLIK, J., PSOTA, V., VITKOVÁ, H., KUDOVÁ, D. and REINOHL, V. (1991): The effect of paclobutrazol on the level of endogenous IAA in relation to the rooting of cuttings and abscission of petioles. — Biochem, Physiol. Pflanzen. *187*, 89—94.

SHINGO, S. and IMASEKI, H. (1971): Auxin-induced ethylene production by mungbean hypocotyl segments. — Plant Cell Physiol. *12*, 349—359.

TORREY, J. G. (1976): Root hormones and plant growth. — Annu. Rev. Plant. Physiol. *27*, 435—459.

VAN STADEN, J., FEATONBY-SMITH, B. C., MAYAK, S., SPIEGELSTEIN, H. and HALEVY, A. H. (1987): Cytokinins in cut carnation flowers. II. Relationship between endogenous ethylene and cytokinin levels in petals. — Plant Growth Reg. *5*, 75—86.

WANG, S. Y., SUN, T., JI, Z. L. and FAUST, M. (1987): Effect of paclobutrazol on water stress-induced abscisic acid in apple seedling leaves. — Plant Physiol. *84*, 1051—1054.

WRIGHT, S. T. C. (1980): The effect of plant growth regulator treatments on the levels of ethylene emanating from excised turgid and wilted wheat leaves. — Planta. *148*, 381—388.