Europ.J.Hort.Sci., **72** (5). S. 198–201, 2007, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Effects of Paclobutrazol and Indole-3-butyric Acid on *in vitro* Rooting and Growth of some Rootstocks of the Genus *Prunus* L.

B. Křižan[1)], E. Ondrušiková[1)], K. Trčková[1)] and D. Benediková[2)]
([1)] Mendel University of Agriculture and Forestry, Brno, Czech Republic and [2)] Research Institute of Plant Production, Pieštany, Slovak Republic)

Summary

In the presented study, the role of paclobutrazol (N-dimethylaminosuccinamic acid) as a growth regulator influencing *in vitro* rooting was not confirmed. A simultaneous application of paclobutrazol and auxin (indole-3-acetic acid potassium salt) did not show any positive effect on rhizogenesis in experiments with four different *Prunus* rootstocks. In variants with paclobutrazol, numbers of roots and their lengths were equal or lower than in controls (i.e. without paclobutrazol and auxin). The application of auxin showed a positive effect on the both number and the length of roots.

Key words. auxin – gibberellin inhibitor – rooting – peach – plant growth retardant – plum

Introduction

When propagating vegetatively *Prunus* L. rootstocks under *in vitro* conditions, the stage of rooting is the most limiting factor. Good rooting is an indispensable precondition of successful growth and acclimatisation of plants under conditions *extra vitrum*. *In vitro* rooting is influenced by many endogenous and exogenous factors that, moreover, mutually influence each other (e.g. genotype, medium composition, growth hormones etc.). It is well known that auxins show a promoting effect on rhizogenesis while cytokinins and gibberellins inhibit it (Haissig 1972; Wiesman et al. 1989). It was found out that rhizogenesis was promoted by growth inhibitors belonging to the group of triazoles (Davis et al. 1985; Fletcher et al. 1988; Geneve 1990). This group involves also the preparation called paclobutrazol (PP 333), which contains N-dimethylaminosuccinamic acid as an efficient agent. Paclobutrazol inhibits the synthesis of gibberellins, increases the stress tolerance and inhibits the senescence (Davis and Sankhla 1988; Lenton et al. 1994). Paclobutrazol is often used as a morphoregulator (Sopher et al. 1999; Bañón et al. 2005). An experimental application of PP 333 on stem cuttings showed a stimulating effect and increased rhizogenesis (Davis and Sankhla 1988; Králík and Šebánek 1989; Šebánek et al. 1991). However, it has not yet been explained in which way paclobutrazol does promote the formation of adventive roots. It is possible that paclobutrazol as an inhibitor of synthesis of gibberellins (that inhibits the rooting of plants) may promote rhizogenesis particularly in plants with a high content of endogenous gibberellins (Králík and Šebánek 1989). A negative effect of gibberellins on rooting was observed also in studies recommending rooting plants after repeated multiplication (sub-cultivation) in cherry and apple trees (Grant and Hammatt 1999). In the course of a long-term cultivation, the content of endogenous gibberellins in plants decreased (Takeno et al. 1983). A positive effect on rhizogenesis in *Vigna radiata* was observed most notably after the application of paclobutrazol in combination with auxin (Wiesman and Riov 1994).

The aim of this study was to evaluate effects of paclobutrazol present in growing medium in combination with auxin on numbers and length of roots on the one hand and on the growth of plant during the rhizogenesis.

Material and Methods

Experiments were carried out with clones MY-KL-A (*P. cerasifera* x *P. cerasifera* var. 'Atropurpurea'), Jaspi® (*P. salicina* var. Methey x *P. spinosa*), VVA-1® (*P. tomentosa* x *P. cerasifera*) and MrS 2/5 (*P. cerasifera* x *P. spinosa*). The rooting took place at the temperature of 24 °C in a medium consisting of basic salts of MS-medium including vitamins and with 20 g l^{-1} of saccharose and 5 g l^{-1} of agar. The addition of IBAK (indole-3-acetic acid potassium salt) at the dose of 0.00 μM (as control), 1.42 μM and 4.14 μM, respectively, was tested either separately or together with PP 333. Concentrations of PP 333 used in this experiment were as follows: 0.00, 0.20 and 0.41 μM. Growing jars were sealed with a plastic foil used in food industry, which prevented the exchange of gases with the laboratory environment.

Shoots, which were about 12 mm long, were cultivated at the photoperiod of 12/12 h (day/night) under light from fluorescent lamps with the intensity 20.25 μmol m^{-2} s^{-1}. During rhizogenesis, the following parameters were recorded and evaluated: number of roots per plant, average length of roots per plant and height of plants. The number and length of roots were evaluated on day 7 and day 14 of cultivation on the rooting medium. The height of plants

Table 1. Root development in media enriched with IBAK (indole-3-acetic acid potassium salt) and PP 333 (paclobutrazol) at different concentrations (left) and growth of plants after 14 days on this media (right). P=0.05.

Prunus rootstock	Media composition (μM)		Number of roots/plant		Average length of roots/plant (mm)		Height of plants (mm)
	IBAK	PP 333	Days on rooting media				
			7 days	14 days	7 days	14 days	14 days
MY-KL-A	0.00	0.00	0.30 ± 0.34	0.30 ± 0.34	0.12 ± 0.34	0.50 ± 0.45	16.35 ± 0.01
	2.49	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15.70 ± 0.02
	4.14	0.00	0.20 ± 0.22	0.25 ± 0.34	0.15 ± 0.11	0.43 ± 0.39	15.80 ± 0.02
	2.49	0.20	0.00 ± 0.00	0.15 ± 0.00	0.00 ± 0.00	0.70 ± 0.00	16.95 ± 0.22
	4.14	0.41	0.00 ± 0.00	0.10 ± 0.00	0.00 ± 0.00	0.40 ± 0.39	18.20 ± 0.22
VV-A-1®	0.00	0.00	0.70 ± 0.56	0.95 ± 0.56	0.62 ± 0.50	2.43 ± 0.89	22.85 ± 0.01
	2.49	0.00	0.28 ± 0.56	3.35 ± 0.22	2.68 ± 0.45	7.05 ± 0.78	22.50 ± 0.03
	4.14	0.00	1.95 ± 0.34	2.25 ± 0.56	1.68 ± 0.56	3.83 ± 1.34	24.15 ± 0.01
	2.49	0.20	0.00 ± 0.00	0.60 ± 0.22	0.00 ± 0.00	1.20 ± 0.78	17.00 ± 0.34
	4.14	0.41	0.00 ± 0.00	0.05 ± 0.00	0.00 ± 0.00	0.25 ± 0.00	21.50 ± 0.11
Jaspi	0.00	0.00	0.30 ± 0.11	0.35 ± 0.34	1.25 ± 0.34	2.95 ± 1.79	17.15 ± 0.02
	2.49	0.00	1.35 ± 0.34	1.55 ± 0.22	1.50 ± 0.34	5.00 ± 0.67	17.90 ± 0.01
	4.14	0.00	0.65 ± 0.34	0.95 ± 0.11	1.62 ± 0.56	5.10 ± 2.24	16.40 ± 0.02
	2.49	0.20	0.00 ± 0.00	0.20 ± 0.00	0.00 ± 0.00	1.45 ± 0.00	14.60 ± 0.34
	4.14	0.41	0.00 ± 0.00	0.10 ± 0.11	0.00 ± 0.00	1.15 ± 0.00	14.20 ± 0.22
MrS 2/5	0.00	0.00	0.20 ± 0.22	0.30 ± 0.22	0.20 ± 0.22	0.27 ± 0.31	22.25 ± 0.04
	2.49	0.00	0.20 ± 0.22	0.20 ± 0.22	0.28 ± 0.25	1.30 ± 1.57	20.45 ± 0.11
	4.14	0.00	1.70 ± 0.45	1.90 ± 0.45	2.64 ± 0.39	7.38 ± 0.89	17.15 ± 0.02
	2.49	0.20	0.00 ± 0.00	0.10 ± 0.22	0.00 ± 0.00	1.00 ± 1.68	18.40 ± 1.12
	4.14	0.41	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	19.20 ± 0.22

was measured on day 14. Altogether 100 plants of each clone were evaluated in each variant media. Results were analysed by of multifactorial variance analysis using the programme Unistat at the probability level of 0.05.

Results and Discussion

As compared with controls, rootstocks Jaspi and VVA-1®, which were cultivated on media containing 2.49 and 4.14 μM IBAK, produced more roots (Table 1). After the supplementation of PP 333 into the medium, the number of roots decreased and was equal to controls. On the control medium without growth regulators rhizogenesis was observed only in case of the rootstock VVA-1®. Blazkova et al. (1997), using clones of *Sequoia sempervirens*, mentioned that not all plants species require exogenous auxin to produce roots.

The rootstock VVA-1® produced the highest number of roots while the lowest one was produced by the rootstock MY-KL-A (Fig. 1). Rootstocks Jaspi® and MrS 2/5 created a homogenous group. When cultivating *Prunus* rootstocks, also Franc (1998) observed different requirements of individual rootstocks as far as the content of auxin was concerned. The rootstock MrS 2/5 rooted at best on the medium with 4.14 μM IBAK. On the other side, Morini et al. (1990) mentioned that this rootstock required only a half concentration when IBA was used. However, the difference could result from the physiological status of the plants investigated.

The rootstock MY-KL-A had the shortest roots and the difference from other clones was significant. In the presence of auxin, the length of roots was higher than in controls while in the presence of auxin + paclobutrazol, the length of roots was lower (Fig. 2). Marino (1986) mentioned that on a medium containing paclobutrazol rootstocks of genus *Prunus* L. produced shorter and thicker roots than on media containing only auxin. This was corroborated also in our study but on media containing PP 333 only a minimum rhizogenesis was observed.

The height of individual rootstock clones was rather different and there was an interaction between the growing medium variant and the genotype used. The lowest intensity of growth was recorded in Jaspi®, followed by MY-KL-A, MrS 2/5 and VVA-1®. As compared with control, shorter plants were obtained after cultivation on a medium containing 0.20 μM PP 333 + 2.49 μM IBAK. The presence of paclobutrazol inhibited the growth of plants (Davis et al. 1986, Šebánek et al. 1991). In our study, however, a higher concentration of PP 333 (0.41 μM) did not influence the height of plants and there were no differences between the height of controls and plants cultivated on this medium in case of MY-KL-A, MrS 2/5 and VVA-1® (Fig. 3). This observation dffers from data published by Marino (1988), who reported a more pronounced inhibition in plants cultivated on media containing higher levels of PP 333.

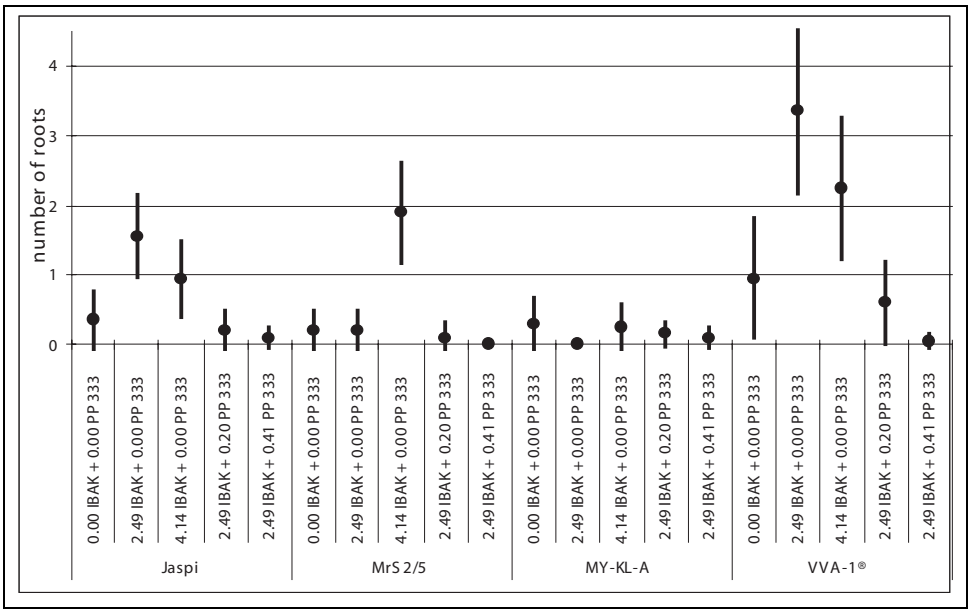


Fig. 1. The influence of different concentrations of IBAK (indole-3-acetic acid potassium salt) and PP 333 (paclobutrazol) on number of roots after 14 days on rooting media. P=0.05.

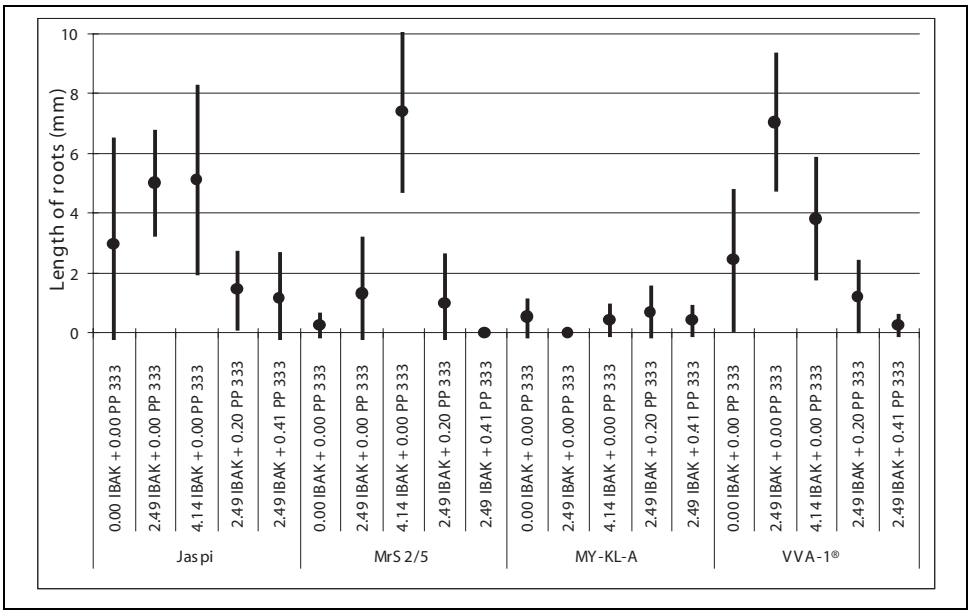


Fig. 2. The influence of different concentrations of IBAK (indole-3-acetic acid potassium salt) and PP 333 (paclobutrazol) on average length of roots per plant after 14 days on rooting media. P=0.05.

In contrast to results published by Rauscherová and Tesfa (1993) and Šebánek et al. (1991), we were not able to demonstrate a positive effect of PP 333 on the number of roots. The expectation that paclobutrazol, when applied in combination with auxin, could promote the formation of roots was not corroborated in experiments with rootstocks under study. In our experimental material, the capacity of rootstocks to produce roots may not be influenced by a high level of endogenous gibberellins, the inhibiting effect of which can be suppressed by paclobutrazol. So, for example, Marino (1988) observed a positive effect of paclobutrazol on rhizogenesis in the hybrid rootstock S479 x S1419 (*P. persica* x *P. kansuensis*). In the same study, however, PP 333 did not show any effect on rhizogenesis of CAB 11E (*P. cerasus*) rootstocks. This can be explained through different requirements of these plant species. In a hybrid rootstock GF 677 (*P. persica* x *P. amygdalus*), paclobutrazol in concentration of 0.43 μM slightly increased the number of produced roots and improved also the subsequent acclimatisation of plants (Křižan et al. 2006).

Conclusions

In the genus *Prunus* L., rhizogenesis is influenced by a number of factors that influence each other. The obtained results corroborated that auxin (a potassium salt of indolyl-3-butyric acid) is the phytohormone, which promoted rhizogenesis.

The application of paclobutrazol was not efficient when rooting the following rootstocks: MY-KL-A (*P. cerasifera* x *P. cerasifera* var. Atropurpurea), Jaspi® (*P. salicina* var. Methey x *P. spinosa*), VVA-1® (*P. tomentosa* x *P. cerasifera*) and MrS 2/5 (*P. cerasifera* x *P. spinosa*). In variants with paclobutrazol, the parameters 'number of roots' and 'length of roots' were equal or not significantly lower than

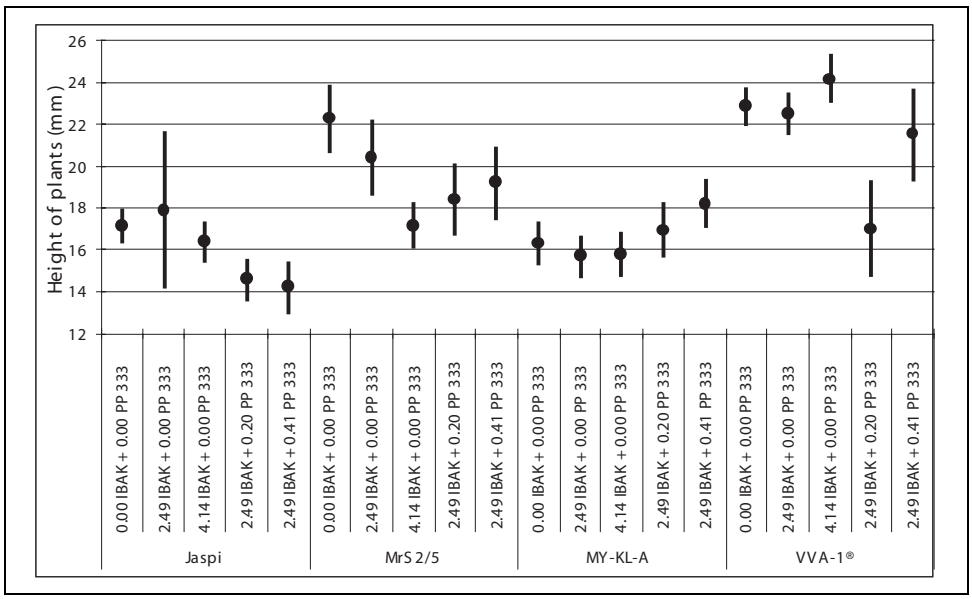


Fig. 3. The influence of different concentrations of IBAK (indole-3-acetic acid potassium salt) and PP 333 (paclobutrazol) on height of plants after 14 days on rooting media. P=0.05.

in plants cultivated on a control medium without any growth regulators.

We cannot recommend the using of paclobutrazol for propagation of the plants investigated. With regard to obtained results, it will be necessary to study underlying mechanisms responsible for changes in gibberellin biosynthesis, induced by paclobutrazol. For better understanding of these mechanisms, the endogennous level of gibberellins in plants during rhizogenesis will be determined in a further study.

References

Baňón, S., J.A. Fernandez, J. Ochoa and M.J. Sánchez-Blanco 2005: Paclobutrazol as an aid to reduce some effects of salt stress in oleander seedlings. *Europ. J. Hort. Sci.* **70**, 43–49.

Blazkova, A., B. Sotta, H. Travan, R. Maldiney, M. Bonnet, J. Einhorn, L. Kerhoas and E. Miginiac 1997: Auxin metabolism and rooting in young and mature clones of *Sequoia sempervirens*. Physiol. Plant. **99**, 73–80.

Davis, T.D., N. Sankhla and R.H. Walser 1985: Promotion of adventitious root formation in cuttings by paclobutrazol. HortScience **20**, 883–884.

Davis, T.D., R.H. Walser, K. Soerenson and N. Sankhla 1986: Rooting and subsequent growth of cuttings treated by paclobutrazol. Plant. Prop. **32**, 7–9.

Davis, T.D. and N. Sankhla 1988: Effect of shoot growth retardants and inhibitors on adventitious rooting. In: Dudley, T. R. (ed.): Adventitious root formation in cuttings. Dioscorides Press, Portland, 174–185.

Fletcher, R.A., N.K. Asare-Boamah and L.C. Krieg 1988: Triadimefon stimulates rooting in bean hypocotyl. Physiol. Plant. **73**, 401–405.

Franc, P. 1998: The effect of different carbon sources and cultivars on *in vitro* micropropagation of some rootstocks of the genus *Prunus* L. Hort. Sci. (Prague) **25**, 41–46.

Geneve, R.L. 1990: Root formation in cuttings of English ivy treated with paclobutrazole or uniconazole. HortScience **25**, 709.

Grant, N.J. and N. Hammatt 1999: Increased root and shoot production during micropropagation of cherry and apple rootstocks: effect of subculture frequency. Tree Physiol. **19**, 899–903.

Haissig, B.E. 1972: Meristematic activity during adventitious root primordium development. Influence of endogenous auxin and applied gibberellic acid. Plant Physiol. **49**, 886–892.

Králík J. and J. Šebánek 1989: On the stimulation effects of paclobutrazol on the rhizogenesis of *Ligustrum vulgare* L. cuttings. Acta Univ. Agric. Brno A. **37**, 51–54, ISSN: 1211–8516.

Křižan, B., E. Ondrušiková, G. Dradi and R. Roncasaglia 2006: The effect of paclobutrazol on *in vitro* rooting and growth of GF-677 hybrid peach rootstock. Acta Physiol. Plant. **28**, 21–26.

Lenton, J.R., N.E.J. Appleford and K.E. Temple-Smith 1994: Growth retardant activity of paclobutrazol enantiomers in wheat seedlings. Plant Growth Regul. **15**, 281–291.

Marino, G. 1986: Effetto del patrobutrazolo sula radicazione *in vitro* di un portainnesto ibrido di *Prunus* spp. Riv. Ortoflorofrutt. **70**, 267–275.

Marino, G. 1988: The effect of paclobutrazol on *in vitro* rooting, transplant establishment and growth of fruit plants. Plant Growth Regul. **7**, 237–248.

Morini, S., F. Loreti and R. Sciutti 1990: Response of some Mr.S plum clones to *in vitro* propagation. Acta Hort. (ISHS) **283**, 207–212.

Rauscherová, L. and L. Tesfa 1993: The stimulating effect of paclobutrazol on the rhizogenesis of *Ligustrum vulgare* L. cuttings. Sci. Hort. **53**, 167–173.

Sopher, C.R., M. Król, N.P.A. Huner, A.E. Moore and R.A. Fletcher 1999: Chloroplastic changes associated with paclobutrazol-induced stress protection in maize seedlings. Can. J. Bot. **77**, 279–290.

Šebánek, J., Š. Klíčová, J. Králík, V. Psota, H. Vítková and D. Kudová 1991: The effect of paclobutrazol on the level of endogenous IAA in relation to the rooting of cuttings. Biochem. Physiol. Pflanzen **187**, 89–94.

Takeno, K., J.S. Taylor, S. Riskandarajah, R.P. Pharis and M.G. Mullins 1983: Endogennous gibberellin- and cytokinin-like substances in cultured shoot tissues of apple, *Malus pumila* cv. 'Jonathan', in relation to adventitious root formation. Plant Growth Regul. **1**, 261–268.

Wiesman, Z., J. Riov and E. Epstein 1989: Paclobutrazol and ureaphosphate increase rooting and survival of peach 'Maravilha' softwood cuttings. HortScience **24**, 908–909.

Wiesman, Z. and J. Riov 1994: Interaction of paclobutrazol and indole-3-butyric acid in relation to rooting of mung bean (*Vigna radiata*) cuttings. Physiol. Plant. **92**, 608–612.

Received March 21, 2006 / Accepted December 21, 2006

Addresses of authors: Bř etislav Křižan (corresponding author), Kamila Trčková and Eva Ondrušiková, Mendel University of Agriculture and Forestry, Faculty of Horticulture, Valtická 337, 691 44 Lednice, Czech Republic and Daniela Benediková, Gene Bank, Research Institute of Plant production, Bratislavská 122, 921 68 Pieštany, Slovak Republic, e-mail: bretislav.krizan@seznam.cz.