

Root initiation and development by auxin physiological analogue TA-12

Leonida Novickiene*, Jurate Darginavičiene, Gemir Maksimov

Institute of Botany, Žaliojų Ežerų 49, Vilnius 08406, Lithuania

*Corresponding author, E-mail: gemir@botanika.lt

Abstract

The effect of the compound TA-12 (1-[2-chloroethoxycarbonylmethyl]-4-) naphthalene sulphonic acid calcium salt) on the rooting of difficult-to-root cherry (*Cerasus vulgaris* Mill.) cv. 'Zagarvysne' and various cultivars of hazel (*Coryllus avellana* L.) green cuttings was studied and compared to the activity of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA). Under the effect of TA-12, IAA and IBA, cherry 'Zagarvysne' cuttings undergo complex anatomical and morphological changes in the basal and subbasal parts. Using wheat coleoptile cell plasmalemma as a model test object in IAA investigations, it was shown that TA-12 could be perceived in the plasmalemma under conditions similar to IAA and transduced to the nucleus. It is supposed that TA-12 can affect the plant cell through the IAA receptory system, but TA-12 is distinguished for having high activity in rhizogenesis of difficult-to-root cherry 'Zagarvysne', hazel 'Pervenets' and 'Akademik Jablov' green cuttings, as compared to the activity of optimal concentrations of IAA and IBA.

Key words: cherry *Cerasus vulgaris* Mill., hazel *Coryllus avellana* L, indole-3-acetic acid, indole-3-butyric acid, rooting, TA-12.

Introduction

Organogenesis occurs in various plant tissue cultures and during vegetative propagation, in response to exogenously applied phytohormones, mainly auxin and cytokinin, and also on the ability of the tissue to respond to these phytohormone changes during culture (Sugiyama 1999). Lateral root formation plays a crucial role in plant development by permitting the construction of branched root systems. The process of lateral root formation consists of two major steps: cell cycle reactivation in the xylem pericycle and establishment of a new meristem (Laskowski et al. 1995; Himanen et al. 2002). The first formative divisions in the pericycle depend on the basipetal transport of auxin, whereas shoot-derived auxin regulates the outgrowth of lateral roots (Casimiro et al. 2001). Cuttings of the cherries 'Liubskaja' and 'Zagarvysne', as well as those of numerous varieties of cultivated hazel, are difficult-to-root plants (Novickiene, Darginaviciene 2001). The objectives of the present work were to determine the physiological activity of an auxin analogue, TA-12 (1-[2-chloroethoxycarbonylmethyl]-4-naphthalenesulfonic acid calcium salt); (Merkys et al. 1993)], as well as to compare its effect on the rooting of cherry and hazel sprout cuttings with the effect of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), depending on the parent plant and sprout cutting age.

Materials and methods

Cuttings of cherry (*Cerasus vulgaris* Mill. cv. 'Zagarvysne') and hazel (*Corylus avellana* L.) served as the object of research. Cuttings for rooting were obtained in June from the upper, middle and basal part of annual first and second line sprouts of young five- to six-year-old parent plants. The rooting was carried out according to Turetskaja and Polikarpova (1968) with modifications. The basal ends of cuttings were immersed in compounds at optimal concentrations: TA-12 (1×10^{-3} M), IAA (1×10^{-3} M), IBA (2.5×10^{-4} M) for 15 h. Ninety green cuttings were used for each treatment. The cuttings were planted in a greenhouse and grown at a constant temperature (~ 26 °C) under conditions of artificial mist till October. Then their roots were analysed morphometrically. Samples for anatomical and cytological studies of callus and root formation were taken in the course of five weeks every seven days. Longitudinal and transverse microtomic slices were analyzed by light microscopy.

The plasmalemma vesicle fraction was obtained from wheat (*Triticum aestivum* L. cv. 'Nandu') coleoptiles according to Tichaja et al. (1986). A transmembrane potential was created as described by Maksimov et al. (2004). The physiological activity of IAA and TA-12 complexes with protein in the plasmalemma (pH 7.2) was evaluated from RNA-polymerase II activity in a system of nuclei isolated from wheat coleoptile cells. CTP, GTP, UTP and $8\text{-}^{14}\text{C}$ -ATP (ammonium salt; $2.11 \text{ GBq mmole}^{-1}$ at a final concentration 0.1 mM) were added into the system (Novickienė, Darginavičienė 2001). The label incorporation was sensitive to RNA-polymerase II inhibitor α -amanitine. The label content in the vesicles was determined with the aid of an LS 1801 scintillation counter (Beckman, USA). Data were statistically evaluated. Differences were statistically significant at $p \leq 0.05$.

Results

The effect of TA-12 and IAA on rooting of cherry 'Zagarvysne' cuttings depends on the sprout age and its position in the tree crown

In our studies cuttings were prepared from the upper, middle and basal sprout parts of five-year-old plants and exposed to TA-12 (1×10^{-3} M) and IAA (1×10^{-3} M). Cuttings taken from the middle part were found to root best, while the rooting of cuttings from the upper and basal parts was the weakest. The experiments showed the advantage of the compound TA-12 over IAA. Under the effect of TA-12 and IAA the number of main and lateral roots increased in all parts of sprouts, especially in the middle part (Table 1). Under the effect of TA-12, the length of lateral roots in the upper, middle and basal part cuttings increased 5.2-, 11.9- and 10.0-fold as compared to the control, while under the effect of IAA the increase was 2.0-, 3.75- and 3.2-fold, respectively.

It seemed worthwhile to study the dependence of rooting on the sprout position in the tree crown. Cuttings were taken from the middle part of the sprout. In the control variant, no significant difference in the rooting of the first and second row sprouts was detected. However, under the effect of TA-12 and IAA the rooting of cuttings of the first row branching sprouts was higher by 14 % and 11 %, respectively, while the formation of main roots per cutting was higher by 66 % and 91 %, respectively, as compared to the cuttings of the second branching row sprouts (Table 1).

Table 1. Effect of TA-12 and indole acetic acid (IAA) on rooting of cherry 'Zagarvysne' green cuttings taken from different sprout parent plant parts (data are means \pm SE from 90 cuttings)

Parent plant parts	Variant	Rooted cuttings (%)	Main roots		Lateral roots	
			Number	Length (mm)	Number	Length (mm)
Upper sprout part	Control	15.5	1.5 \pm 0.2	19 \pm 2	1.2 \pm 0.1	2.1 \pm 0.6
	TA-12 10 ⁻³ M	44.4	6.0 \pm 0.5	68 \pm 2	21.5 \pm 1.2	11.0 \pm 0.1
	IAA 10 ⁻³ M	34.4	4.6 \pm 0.4	53 \pm 2	17.0 \pm 1.3	4.0 \pm 0.2
Middle sprout part	Control	17.8	1.8 \pm 0.1	25 \pm 2	1.3 \pm 0.1	2.6 \pm 0.4
	TA-12 10 ⁻³ M	63.3	7.6 \pm 0.4	79 \pm 1	26.1 \pm 2.1	19.0 \pm 0.3
	IAA 10 ⁻³ M	53.3	6.3 \pm 0.3	71 \pm 2	32.0 \pm 2.1	6.0 \pm 0.1
Basal sprout part	Control	11.1	1.3 \pm 0.1	16 \pm 1	1.0 \pm 0.1	1.9 \pm 0.3
	TA-12 10 ⁻³ M	31.1	5.1 \pm 0.5	61 \pm 3	20.0 \pm 2.2	19.0 \pm 0.3
	IAA 10 ⁻³ M	26.7	3.9 \pm 0.1	47 \pm 3	21.0 \pm 1.2	6.1 \pm 0.3
Branching of the first row	Control	12.6	1.4 \pm 0.2	39 \pm 1	1.3 \pm 0.1	2.7 \pm 0.3
	TA-12 10 ⁻³ M	68.4	7.5 \pm 0.3	131 \pm 2	37.0 \pm 2.1	17.4 \pm 0.2
	IAA 10 ⁻³ M	61.6	6.9 \pm 0.4	77 \pm 2	32.0 \pm 1.3	12.2 \pm 0.2
Branching of the second row	Control	10.6	1.6 \pm 0.3	41 \pm 2	1.1 \pm 0.1	2.7 \pm 0.7
	TA-12 10 ⁻³ M	54.2	6.2 \pm 0.3	116 \pm 2	33.0 \pm 3.4	14.9 \pm 0.1
	IAA 10 ⁻³ M	50.8	5.3 \pm 0.4	73 \pm 2	29.0 \pm 2.5	8.0 \pm 0.2

The effect of TA-12 and IBA on rooting of cuttings of various hazel cultivars

The effect of TA-12 and IBA, the classic compound for stimulation of rooting, was studied on various cultivars of hazel green cuttings: 'Pervenec', 'Akademik Jablokov', 'Tambovskij rannij' and 'Moskovskij rubin'. The effect of the tested compounds on rooting was especially high in the cvs. 'Pervenec' and 'Akademik Jablokov'. No significant differences in rooting under the effect of TA-12 and IBA were detected, except for cuttings of hazel 'Akademik Jablokov' in which the effect of TA-12 on rooting was higher than that of IBA (Table 2).

Table 2. Effect of auxin physiological analogues on rooting of hazel green cuttings (average % from 100 cuttings)

Variant	Cultivar			
	'Pervenec'	'Akademik Jablokov'	'Tambovskij rannij'	'Moskovskij rubin'
Control	9	1	1	0
TA-12 1 \times 10 ⁻³ M	71	9	6	2
TA-12 5 \times 10 ⁻⁴ M	83	20	11	2
TA-12 5 \times 10 ⁻⁵ M	58	7	1	0
IBA 1 \times 10 ⁻³ M	88	10	8	4
IBA 2.5 \times 10 ⁻⁴ M	77	8	9	2
IBA 1 \times 10 ⁻⁴ M	43	8	1	2

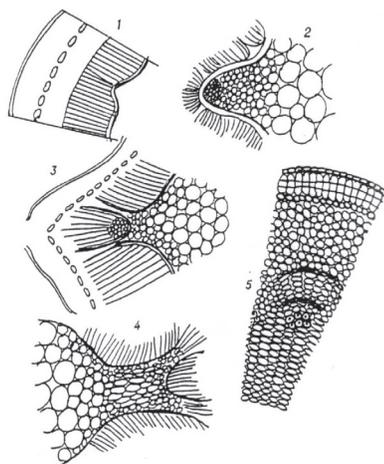


Fig. 1. Scheme of root formation of cherry 'Zagarvysne' green cuttings under the effect of IAA (1×10^{-3} M) and IBA (2.5×10^{-4} M). 1, secondary xylem layer (curvature); 2, 3, formation of initial cells in the area of the peripith; 4, stretching of initial cells in transverse direction; 5, formation of secondary phloem.

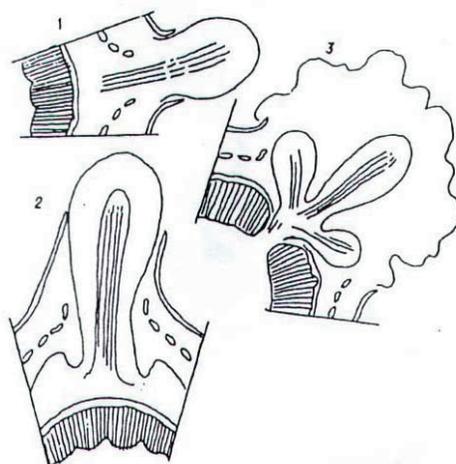


Fig. 2. Scheme of root formation in cherry 'Zagarvysne' green cuttings under the effect of IAA (1×10^{-3} M), IBA (2.5×10^{-4} M) and TA-12 (1×10^{-3} M). 1, 2, on day 21 and 28 roots are formed from secondary phloem under the effect of IAA and IBA; 3, on day 35 roots (by 3) are formed from the peripith area under the effect of TA-12.

Anatomical features of root primordium formation and root development

Root primordium formation as well as their further growth was strongly modified by the test compounds IAA, IBA and TA-12. On the 7th day from the beginning of rooting, parenchyma became narrow on the layer of cortex in transverse section of the cutting and a bend of certain tissues was notable. This was followed by formation of initial cells in the peripith area and stretching of initial cells in transverse direction. These initial cells in cutting segments were vascularized and intended specifically for root formation. In cuttings treated with IAA, also the semicircular secondary phloem with a thin secondary cambium layer was formed (Fig. 1). The structure of the control cutting sections remained the same and primordium initiation did not occur. Quantitative and qualitative differences between the test variants become significant only on day 21. On day 28, under the effect of TA-12, IAA and IBA, on average 3.7, 3.6 and 4.1 roots had formed on each sprout, respectively. After 35 days, under the effect of IAA and ABA the anatomical structure of roots did not change, while in the variant with TA-12, two to three roots from one group of initial cells were formed (Fig. 2). No such cases were found in other variants of the experiments.

Is the similarity in the action of TA-12 and IAA in the cell?

To answer this question, as a model object we used wheat coleoptile segments whose growth is controlled by IAA. TA-12 (1×10^{-4} M), like IAA, also stimulates their growth. We previously showed that the functional activity of IAA-protein complexes formed in the system *in vitro* is potential-dependent (Maksimov et al. 2004). What events are

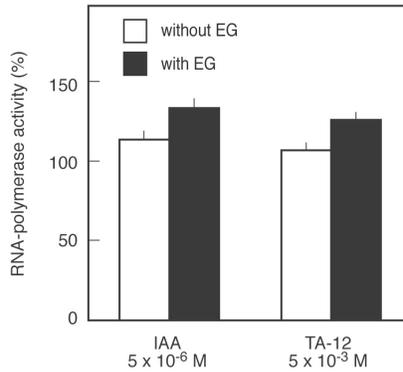


Fig. 3. Activity of RNA polymerase II (cpm $100 \mu\text{g}^{-1}$ plasmalemmal protein, %) in the system of isolated nuclei under the effect of IAA (5×10^{-6} M) or TA-12 (5×10^{-5} M), both 30 min *in vitro* treated plasmalemma. Control (non treated plasmalemma addition) = 100 %. White columns, electrochemical gradient (EG) not created on membrane of plasmalemma vesicles. Black columns, the same in the conditions of a created electrochemical gradient.

related with TA-12 action on plasmalemma level? To generate an electrochemical gradient on the membrane, plasmalemma vesicles were filled (by means of osmotic shock) with 150 mM K_2SO_4 , transferred into equimolar solution of Na_2SO_4 , and to induce membrane potential generation valinomycin (8.3×10^{-9} M) was added. The resulting complexes of TA-12-plasmalemmal protein, upon addition into the system of isolated nuclei, activated RNA-polymerase II by 26 % above the control (without TA-12 treatment), whereas in the absence of the potential, the activity of TA-12 was weak (Fig. 3). The activity of the IAA-protein complexes exceeded the control by 14 % and 34 %, respectively.

Discussion

This study focuses on root primordium formation and further root development under the effect of a classical natural auxin IAA, its physiological analogue, IBA, and the newly synthesized compound TA-12 of the same class, in difficult-to-root cherry and hazel green cuttings. Under the effect of TA-12, root primordia are mostly formed from the peripith area, while under the effect of IBA and IAA they are formed also from secondary phloem. Under the effect of compound TA-12, root primordia are formed in a different way: two-three roots are formed from a group of initial cells. Despite the anatomical differences in the effects of the physiologically active compounds studied, under the effect of TA-12, IAA and IBA, the root regeneration stages occur three weeks earlier and are five to seven times more intensive than in the control (Novickiene, Darginaviciene 2001). The action of TA-12 on the cell level, as shown using wheat coleoptile cells as a model object, is like IAA. TA-12, as IAA, activates growth of coleoptile segments and simultaneously is able to compete for IAA binding sites in the cell plasmalemma during formation of complexes with proteins that realize physiological function in the nucleus. The events that occur in cell plasmalemma under the influence of TA-12 depended on the transmembrane potential, as in the case of IAA.

TA-12 and IBA molecules have two methylene groups, which most likely determine

the activity of these compounds. However, the molecules differ: the TA-12 molecule contains a naphthalene ring and IAA and IBA contain an aromatic indole ring.

In summary, TA-12 can affect the plant cell through the IAA receptor system, but TA-12 is distinguished for high activity in rhizogenesis of difficult-to-root cherry 'Zagarvysne', hazel 'Pervenets' and 'Akademik Jablokov' green cuttings, as compared to the activity of optimal concentrations of IAA and IBA.

References

- Casimiro I., Marchant A., Bhalerao R.P., Beeckman T., Dhooge S., Swarup R., Graham N., Inze D., Sandberg G., Casero P.J., Bennett M. 2001. Auxin transport promotes *Arabidopsis* lateral root initiation. *Plant Cell* 13: 843–852.
- Himanen K., Boucheron E., Vanneste S., Engler J.A., Inze D., Beeckman T. 2002. Auxin-mediated cell cycle activation during early lateral root initiation. *Plant Cell* 14: 2339–2351.
- Laskowski M.J., Williams M.E., Nusbaum H.C., Sussex I.M. 1995. Formation of lateral root meristems is a two-stage process. *Development* 121: 3303–3310.
- Maksimov G., Darginavičienė J., Baniene J., Siemaite J., Sveikauskas V. 2004. The role of transmembrane potential of plant cell plasmalemma *in vitro* in the functional activity of IAA-receptor complexes. *Biologija* 1: 42–44.
- Merkys A., Novickiene L., Miliuviene L., Saltyte Z. 1993. New growth regulators and evaluation of their physiological activity. 2. Compounds of auxin. *Biologija* 4: 54–56.
- Novickiene L., Darginavičienė J. 2001. The course of morphogenetic processes in the rooting of green cherry cuttings. *Horticulture and Vegetable Growing* 20: 160–175.
- Sugiyama M. 1999. Organogenesis *in vitro*. *Curr. Opin. Plant Biol.* 2: 61–64.
- Tichaja N.I., Maksimov G.B. 1986. Isolation of plasmalemma from plant cells. In: Polevoj V.V., Maksimov G.B., Siniutina N.F. (eds.) *Methods of Plant Membrane Investigation*. Publishing house LSU, Leningrad. pp. 20–29.
- Turetskaja R.C., Polikarpova F.J. 1968. *Vegetative Propagation of Plants with Application of Growth Stimulators*. Nauka, Moscow. 91 p.