Effect of growth regulators on rooting cuttings of *Karwinskia* species under *in vivo* conditions

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ABSTRACT

Effect of the growth regulators Atonik, Rastim 30 DKV, Stimulator AS 1, and Stimulax III on rooting of half-woody shoots of the species *Karwinskia humboldtiana* (Roem et Schut) Zucc. and *Karwinskia parvifolia* Rose was studied. Rooting does not occur without stimulation in these species, after stimulation rhizogenesis takes 14 to 16 weeks. Growth regulators, with the exception of the preparation Atonik, showed a significantly stimulating effect on rhizogenesis, and effect of them declined in the order Stimulax III, Stimulator AS, and Rastim 30 DKV. The percentage of rooting in the species *Karwinskia humboldtiana* was higher than that in *Karwinskia parvifolia* and this was dependent on the age of the plants, the type of stimulator, cutting, substrate, and conditions of cultivation.

Keywords: *Karwinskia humboldtiana*; *Karwinskia parvifolia*; plant growth regulators; rooting; cuttings

Of the fifteen *Karwinskia* species described (family *Rhamnaceae*), ten grow in Mexico (Fernandéz 1992). Among them the species *Karwinskia parvifolia* and *Karwinskia humboldtiana* are of special importance for the highest content of toxins (Waksman et al. 1989) based on dimeric anthracenones (Dreyer et al. 1975) with a selective antitumour effect (Piñeyro López et al. 1994). To provide sufficient and continuous production of the most effective toxin T-514 (peroxisomicine A_1) attention was paid to the propagation and cultivation of these species mainly under *in vitro* conditions (Lišková et al. 1994, 1999, Lux et al. 1997/1998).

According to Lišková et al. (1994), rooting under *in vitro* conditions was obtained in stem explants, however, the plants contained lower amount of peroxisomicine A_1 than those cultivated under *in vivo* condition. For this reason cultivation of half-woody summer cuttings of *Karwinskia* species was also started using rhizogenesis stimulators (Lux et al. 2002).

Plant growth regulators are frequently used in vegetative propagation to promote rooting of cuttings (Criley and Parvin 1979, Fibijian et al. 1981, Davis and Hassig 1990, Tantos et al. 2000). Preparations are based mostly on auxinoids (Haissig 1974, Gaspar and Hofinger 1988, Rauscherová et al. 1991, Dolinay and Kohaut 2000, Henselová 2002), retardants (Davis et al. 1985, Upadhyaya et al. 1986, Marino 1988, Šebánek et al. 1991, Tari and Nagy 1996), and eventually their mixtures (Smith and Thorpe 1975, Obdržálek 1987, Pan and Zhao 1994).

The present work summarizes the results of a three-year study of vegetative propagation of two *Karwinskia* species under *in vivo* conditions, with a discussion of the effect of different factors in the rhizogenesis process.

MATERIAL AND METHODS

Plant material. Cuttings from *Karwinskia humboldtiana* (Roem & Schut) Zucc. and *Karwinskia parvifolia* Rose species were taken from one-year old half-woody shoots (these were taken from 5- to 7-year old trees: July 2, 1997, July 15, 1998, July 10, 1999). The *Karwinskia* species grew in the greenhouse at the Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Bratislava and had been grown from seeds originating from the locality Villa de García in the State of Nuevo León (*K. humboldtiana*) and from the state Sinaloa, Mexico (*K. parvifolia*). Specifically, apical and basal cuttings (1997) and their mixture in the ratio 1:1 (1998 and 1999) were cut to the length of 8 to 10 cm, with 2 to 4 leaves left, the leaf area being reduced by one half, and in the case of the apical cuttings, immature terminal parts were also removed.

Treatment of cuttings. The bases of the cuttings (50 to 250 specimens per variant in three repetitions) were treated with tested growth regulators (Table 1). The treatment in the case of Stimulator AS 1 was realized by dusting, in that of Stimulax III by dipping the basal part (1 cm) directly in the preparation. In the case of preparation Rastim 30 DKV in 0.03% and that of Atonik, the cuttings were immersed in a 0.3% solution for 12 hours. Control cuttings were immersed for the same time in distilled water or treated with pure talk. Afterwards the cuttings were planted in two substrates: sand + peat in the ratio 2:1 (s_1) and pure sand (s_2). Five days prior to the planting of the cuttings substrate s_1 was chemically sterilized with Previcur 607 SL (containing 607 g/l of the active substance propamocarb – producer AgrEvo Frankfurt am Main, Germany), in a concentration of

Table 1. List of the growth regulators used

Preparate	Active substance	Content of active substance (g or %)	Formulation	Producer
Atonik	sodium 2-nitrophenolate	2 g.l^{-1}	SL	Asahi Chemical Co. Ltd. Tokyo (Japan)
	sodium 4-nitrophenolate	3 g.l^{-1}		
	sodium 2-methoxy-5-nitrophenolate	1 g.l^{-1}		
Rastim 30 DKV	benzolinone	300 g.l^{-1}	SC	Istrochem, Co. Bratislava (Slovakia)
Stimulator AS 1	nicotinic acid	0.06%	DP	Zahrádkářské potřeby Uhřetice (Czech Republic)
	α-naphtylacetic acid	0.072%		
Stimulax III	β-indolylacetic acid	0.06%	G	Hüben Co. Čerčany (Czech Republic)
	α-naphtylacetic acid	0.06%		
	β-naphtyl-*n*-butyric acid	0.05%		

DP = dispersible powder, G = gel, SC = soluble concentrate (flowable), SL = soluble liquid

0.15% solution in a volume of 5 liters per m^2. The cuttings were maintained in a greenhouse, under a plastic foil tunnel, at a day temperature 26 ± 2°C, relative air humidity 80 to 90%, with one-minute fogging during the day, at 2 hour-intervals.

Evaluation of results. The percentage of rooted cuttings and the number of adventitious roots, were evaluated after a period of 14 to 16 weeks. Afterwards the rooted cuttings were transferred into vessels with a mixture of sand and peat in a 3:1 ratio. The plants were cultivated in the greenhouse at the temperature 18 ± 2°C, and subsequently at winter temperature 10 ± 2°C. The results were statistically evaluated using the Student's *t*-test.

RESULTS

The species *K. humboldtiana* and *K. parvifolia* are heavy rooting woody tree species. Their cuttings do not form roots at all unless stimulated. The preparations Stimulax III, Stimulator AS 1, Rastim 30 DKV and Atonik had a significant effect on callogenesis, the callus being predominantly formed on the base, exceptionally also on the lateral parts of cuttings. Stimulated cuttings formed roots in 14 or 16 weeks. Growth regulators, with the exception of Atonik, had significant stimulatory effect on the rooting rate of the species *K. humboldtiana*, the effect declining in the order Stimulax III (15 up to 41%), Stimulator AS 1(10 to 15%) and Rastim 30 DKV (5 to 10%) (Figure 1). Atonik did not significantly influence rhizogenesis and its effect, not exceeding 1%, became apparent only in basal cuttings. In *K. humboldtiana* a better rooting ability was achieved in the basal than in the apical cuttings – 10 to 41% and 3 to 13% respectively (Figure 1). This species also had a superior rooting ability to *K. parvifolia*, but a higher rooting percentage was achieved in both species with the combined substrate sand + peat (Figure 2A, B), than in pure sand (Figure 2C, D). In the years 1998 and 1999, propagation of *Karwinskia* species became restricted to only two of the most active preparations, viz. Stimulax III and Stimulator AS 1. An evident effect of the preparations on the rhizogenesis of cuttings was also achieved in these subsequent years, but it was weaker than that of 1997. In *K. humboldtiana* the efficiency of Stimulax III declined from 28% rooted cuttings (basal + apical) in 1997, to 23.5% in 1998 and to 18.6% in 1999. A similar declining trend in the action was also noted with the preparation Stimulax III where it decreased from 13% in 1997 to 9.5% in 1999 (Figures 1 and 2). In *K. parvifolia* we observed a lower stimulating effect of the prepara-

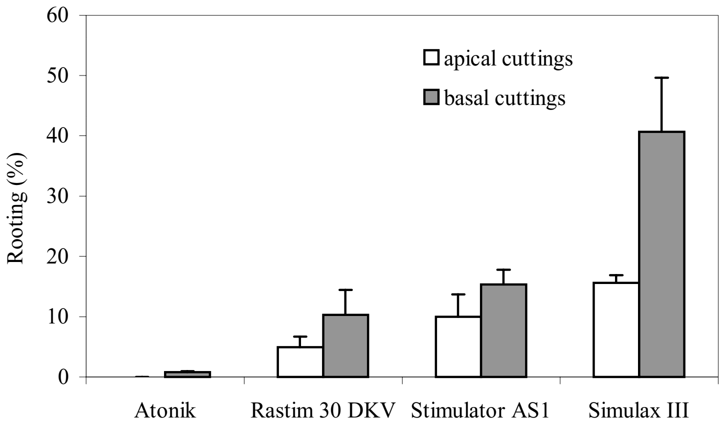

Figure 1. Effect of plant growth regulators on adventitious roots formation of *Karwinskia humboldtiana* in the year 1997; values presented are means ± *SE*, $n = 3$

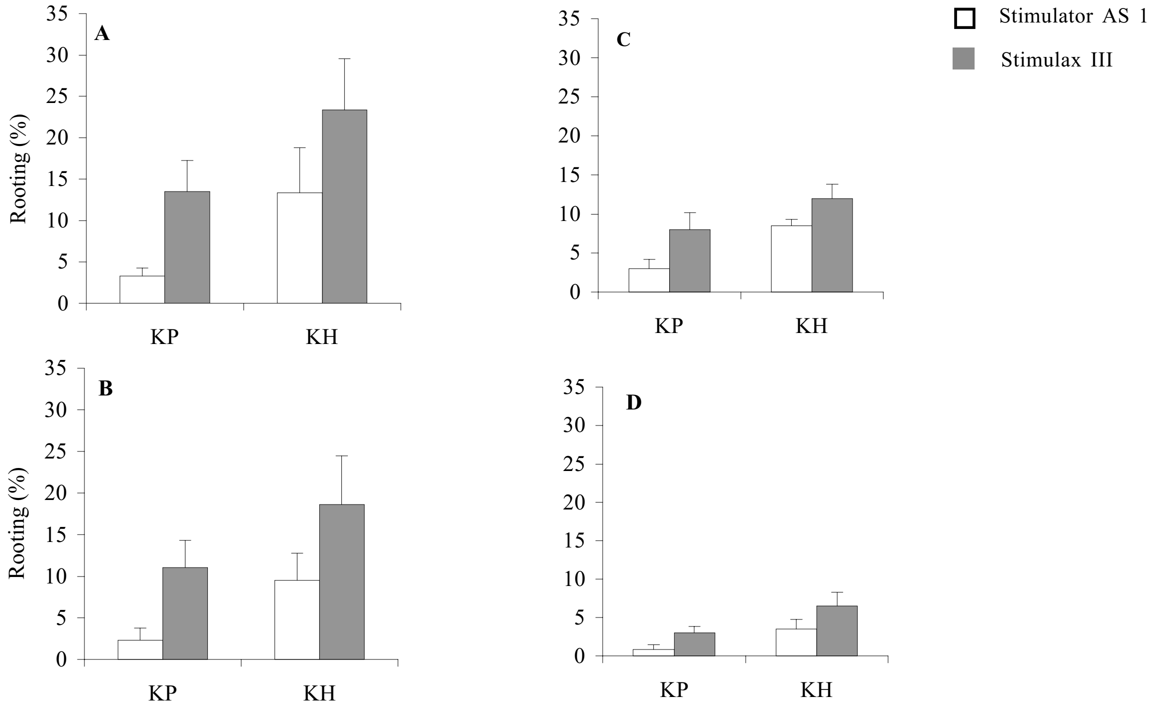

Figure 2. Effect of plant growth regulators on rooting of *Karwinskia parvifolia* (KP) and *K. humboldtiana* (KH) cuttings in substrates sand + peat in 1998 (A) and 1999 (B) and sand in 1998 (C) and 1999 (D); values presented are means ± *SE* (n = 3)

tions Stimulax III and Stimulator AS 1 on rooting of cuttings, with different effect in individual years.

The effect of Stimulax III decreased from 13.5% in 1998 to 11% in 1999 and that of Stimulator AS 1 from 3.3% to 2.3% (Figures 2A, B). A similar declining tendency in the effect of the preparations was observed in the sand substrate (Figures 2C, D) during the periods followed. An inter-specific difference also manifested not only in the percentage yield of rooted cuttings, but also in the quality of the root system (Figure 3). A higher abundance of roots was produced by cuttings in the combined substrate sand + peat, as well as in pure sand (Table 2). In *K. humboldtiana,* the number of roots per cutting ranged from 4.4 ± 0.34 to 5.1 ± 0.48 in (S_2) and from 3.3 ± 0.26 to 3.5 ± 0.22 in (S_1), and in *K. parvifolia*, from 1.8 ± 0.25 to 2.9 ± 0.31 in (S_2) and from 1.4 ± 0.16 to 1.9 ± 0.26 in (S_1) (Table 2).

DISCUSSION

The production of adventitious roots in plants is controlled by growth substances (Davis and Hassig 1990), a key role in this process being played by auxins. This is especially true of nonrooting, or heavy-rooting cuttings, such as were those in the present study. The preparations Stimulax III, Stimulator AS 1, and Rastim 30 DKV based on active substances of the auxinoid type exerted an evident effect on rhizogenesis of both *Karwinskia* species. A higher percentage of rooted stem explants in *K. humboldtiana* than in *K. parvifolia* under *in vitro* conditions was also obtained by Lišková et al. (1994) and Lux et al. (1997/1998). There may be several reasons for the different rooting ability in the *Karwinskia* species under *in vivo* conditions. A low rooting capacity of the

Table 2. The number of roots of *Karwinskia humboldtiana* (KH) and *K. parvifolia* (KP) after 16 weeks of growth of cuttings in two substrates (sand and sand + peat); values presented are means ± *SE*, n = 20

Treatment	Root number per cuttings			
	sand		sand + peat (2:1)	
	KH	KP	KH	KP
Stimulax III	3.5 ± 0.22	1.9 ± 0.26	5.1 ± 0.48	2.9 ± 0.31
Stimulator AS 1	3.3 ± 0.26	1.4 ± 0.16	4.4 ± 0.34	1.8 ± 0.25

Values between *K. humboldtia* and *K. parvifolia* are significantly different at P = 0.01
Values between Stimulax III and Stimulator AS 1 are significantly different at P = 0.05 in the substrate sand + peat only

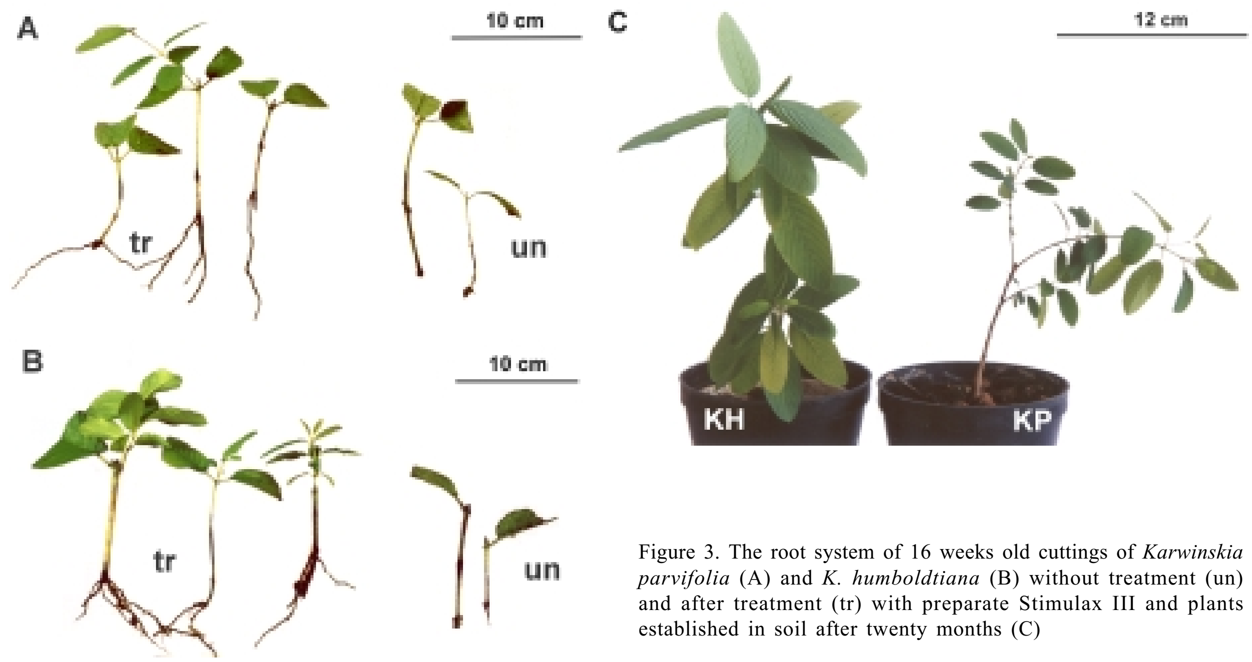

Figure 3. The root system of 16 weeks old cuttings of *Karwinskia parvifolia* (A) and *K. humboldtiana* (B) without treatment (un) and after treatment (tr) with preparate Stimulax III and plants established in soil after twenty months (C)

species may be related to a low content of endogenous auxins. According to Šebánek and Králik (1983), the correlation had been found in plants between the content of their endogenous auxins and rooting ability. Hence, an exogenous application of synthetic auxinoids in the form of the preparations studied here may have reinforced the effect of endogenous auxins or directly induced rhizogenesis. The inter-specific difference has also been found in the propagation of poplars (Králik and Šebánek 1980), and also of cherry-trees, or cherry rootstocks (Marino 1988, Kosina and Kracíková 1999). Differences in the rooting of *Karwinskia* species may likewise be genetically determined and come from their disparate natural occurrence. The species *K. humboldtiana* is characterized by a greater adaptability. It occurs in broader range of altitudes and grows over a wider area than the species *K. parvifolia*. The degree of maturity is manifested in a better rooting of the basal rather than the apical cuttings, even though Rauscherová et al. (1991), on the contrary, reported a better rooting ability of the apical cuttings in *Ligustrum vulgare* L.

According to our opinion, the higher rooting of basal cuttings may be determined by higher resistance of these cuttings to higher temperature during the propagation. In addition, basal cuttings proved to be more resistant to botrytide, which often attacked the less mature apical cuttings under the plastic cover. Rooting ability of *Karwinskia* species also decreased with the age of the plants. Rooting was highest in 5-year old and lowest in 7-year old trees, which may be related to their different regeneration capacity. This is also supported by the results of Králik et al. (1989) who obtained 95% rooting ability in two-year old plants of *Ligustrum vulgare*, while in plants over 10 years of age the rooting ability decreased to 45–66%. A lack of plant material prevented us to compare the rooting ability in younger plants. Cuttings from *Karwinskia* species were taken in July. According to Tureckaya and Polikarpova (1968), the best month for majority of woody species is June, but of course, this depends on the start of growing season. An earlier date for cutting *Karwinskia* species is not recommended, because these plants tiller intensively during May and June and simultaneous flowering also culminates (Lux et al. 2002). Obdržálek (1983) obtained the best rooting in Japanese maple trees likewise when the cuttings were taken prior to, or just after the growth had been completed and rooting on the contrary was bad if that was done during intensive tillering. Propagation with hard cuttings in February was unsuccessful with *Karwinskia* species (Henselová, unpublished data). This may be related to the ongoing endogenous dormancy of the species, even though Carey (1974) and Carville (1975) achieved satisfactory rooting of Japanese maples and corresponding to the period before the start of growing season. According to Králik et al. (1989) endogenous dormancy of woody plants strongly inhibits the formation of adventitious roots. Inter-specific differences also appeared in the quality of the root system of the cuttings. A higher number of roots were formed by cuttings of *K. humboldtiana*, in contrast to those of *K. parvifolia*. Similarly Lišková et al. (1994) and Lux et al. (1997/1998) observed a better root formation under *in vitro* conditions in *K. humboldtiana*, than in *K. parvifolia*. Rhizogenesis in *Karwinskia* species lasts from July until October, a period unsuitable for transplanting cuttings, because of high loses (70–80%) during wintering. It was better to leave the cuttings in the substrate and transplant them the next spring, however, the greenhouse temperature should not fall below 15°C, eventually the transplanted rooted cuttings should be transferred into conditions with a temperature gradually rising up to 25°C. According to Lišková et al. (1994), rooted stem segments of *K. humboldtiana* were capable of adaptation from *in vitro* conditions into those of the greenhouse if the temperature gradually increased with a decreasing humidity for at least one month. In the view of the above authors, such a progressive adaptability simulates semi-arid conditions of their natural habitat. *In vivo* propagation of *Karwinskia* species depends on controllable conditions, chiefly those of the temperature, both of the air (which should not exceed 28°C), and of the substrate, which on the contrary should be somewhat higher, in the first weeks of rhizogenesis. It was noted that a temperature of the air higher than that of the substrate causes new shoots to sprout, however, the cuttings rooted badly.

The type of growth regulator, as also the mode of stimulation decisively influenced the percentage of rooted cuttings. The rooting potential of the species differs and some species are weakly rooting such as *Fagus* and *Betula* (Spethman 1982), other are not rooting at all, such as *Magnolia stellata* (Henselová 2002), and in *Karwinskia* species rooting depends on stimulation. The preparations Stimulax III and Stimulator AS 1, on the basis of several effective auxinoids and a short-term stimulation of 3 to 5 seconds, are more advantageous to *Karwinskia* species. On the other hand a 12-hour stimulation of the cuttings was less efficient with the preparation Rastim 30 DKV and little efficient with that of Atonik. This is also supported by the results of Lux et al. (2002) where a short-term stimulation of a few seconds with a concentrated ethanol solution of a mixture on the basis of IAA + IBA + NAA, had a stimulating effect on rooting of *Karwinskia* species comparable to that exerted by the preparation Stimulax III. The most evident stimulating effect on the rhizogenesis of *Karwinskia* species by the preparations Stimulax III and Stimulator AS 1 is probably achieved by a mutual synergism of the active substances. A similar effective synergism was also achieved by Tantos et al. (2000) between IBA and the preparation Triacontanol in propagating *Malus domestica*, and *Cerasus vulgaris*, Pan and Zhao (1994) between IBA and growth retardants with *Phaseolus aureus* L., and also Henselová (2002) in the mixture benzolinon + IBA + fungicides (captan or *Trichoderma harzianum*), when propagating deciduous woody plants.

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Abbreviations

IAA – indole-3-acetic acid, IBA – indole-3-butyric acid, NAA – 1-naphtyl-acetic acid, NA – nicotinic acid, KH – *Karwinskia humboldtiana*, KP – *Karwinskia parvifolia*

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ABSTRAKT

Vliv růstových regulátorů na zakořeňování řízků druhů *Karwinskia* v podmínkách *in vivo*

Byl sledován vliv růstových regulátorů Atonik, Rastim 30 DKV, Stimulator AS 1 a Stimulax III na zakořeňování polozdřevnatělých řízků druhů *Karwinskia humboldtiana* (Roem et Schut) Zucc. a *Karwinskia parvifolia* Rose. Řízky druhů *Karwinskia* bez stimulace nezakořeňují, se stimulací rhizogeneze trvá 14 až 16 týdnů. Regulátory růstu s výjimkou přípravku Atonik průkazně stimulovaly rhizogenezi, vliv se snižoval v pořadí Stimulax III, Stimulátor AS 1 a Rastim 30 DKV. Vyšší procento zakořeněných řízků bylo zjištěno u druhu *Karwinskia humboldtiana* než u druhu *Karwinskia parvifolia*, přičemž bylo závislé na stáří rostliny, typu stimulátoru, řízku, substrátu a podmínkách množení.

Klíčová slova: *Karwinskia humboldtiana*; *Karwinskia parvifolia*; regulátory růstu rostlin; zakořeňování; řízky

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