EFFECT OF ALUMINIUM ON IN VITRO ROOTING OF BIRCH (*BETULA PENDULA* ROTH.) AND POPLAR (*POPULUS TREMULA* L. × *P. ALBA* L.) MICROCUTTINGS

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ABSTRACT

Poplar (*Populus tremula* L. × *P. alba* L.) and birch (*Betula pendula* Roth.) microcuttings obtained from in vitro cultures on media with aluminium (Al+) or without aluminium (Al-) were rooted in perlite saturated with a liquid 1/4 MS medium. Aluminium was added to the rooting medium in the form of aluminium sulphate or aluminium chloride. In the control, i.e. in the medium without aluminium, Al+ and Al- shoots usually developed similarly. Addition of aluminium to the rooting medium had a negative effect on the development of adventitious roots. Poplar and birch shoots obtained from cultures on media with aluminium (Al+) were distinguished by a greater tolerance of aluminium in the medium than shoots obtained from cultures on media without aluminium (Al-).

KEY WORDS: *Betula pendula* Roth., *Populus tremula* L. × *P. alba* L., in vitro culture, aluminium, rooting of microcuttings.

INTRODUCTION

Environmental pollution is one of the factors inhibiting the growth of trees and shrubs, and may cause decline of whole forest stands in extreme situations. Contamination of the soil has a negative impact on the development of root systems, particularly of long-lived organisms, such as trees. The increasing environmental pollution associated with the development of industry and agronomic practices, leads to a permanent and ever-increasing soil acidification. One of the major causes of soil pollution and lowering of soil pH is the influence of acid rain and salts of toxic metals (Vilkka et al. 1990; Reich et al. 1994).

Aluminium is widespread throughout the earth crust, and its availability to plants increases with decreasing soil pH (Greger et al. 1992; Bojarczuk and Oleksyn 1994). A high concentration of toxic aluminium ions in the substrate leads to a considerable limitation of uptake of some micro- and macronutrients by plants, which results in a severe inhibition of the growth of their above-ground parts (Henriksen et al. 1992; Boudot et al. 1994).

Little is known about the mechanism of tree defence against industrial pollution. Broad-leaved trees are more tolerant to environmental pollution than conifers. Nonetheless, decline of many species of broad-leaved trees and shrubs has been recently observed in industrial regions. Results of research conducted so far, show that individual species and even clones of trees and shrubs differ widely in tolerance to industrial pollution (Oleksyn et al. 1996). Tolerance of plants to toxic metal ions is a genetically controlled trait (Ślaski 1992; Ernst et al. 1998). For a long time, in vitro cultures have been used for investigations of plant responses to toxic metal ions (Meredith 1978). The impact of high concentrations of metal ions upon root and shoot development in plants tolerant and susceptible to industrial pollution has been studied (Arnold et al. 1994).

The objective of the experiments carried out at the Institute of Dendrology of the Polish Academy of Sciences at Kórnik was to investigate the effect of aluminium on root and shoot development in birch and poplar cultured in vitro. This study may increase our knowledge of the mechanisms of plant susceptibility to toxic compounds and allow to obtain plants which are more tolerant to industrial pollution.

MATERIAL AND METHODS

Explants in the form of apical and lateral buds with shoot fragments were obtained from a selected birch clone K-03-144 (*Betula pendula* Roth.) resistant to industrial pollution (Rachwał and Wit-Rzepka 1989), and from three clones of poplar (*Populus tremula* L. × *P. alba* L.) between June and September. The mother plants from which the explants were collected, were grown in a greenhouse. The culture medium was a modified 1/4 strength MS medium (Murashige and Skoog 1962) supplemented with: BAP 0.25 $mg \cdot dm^{-3}$ and

NAA 0.1 mg·dm^{-3}, and solidified with Bactoagar. Microcuttings of about 2 cm in length were obtained from stabilized cultures of adventitious buds, and placed in perlite saturated with the liquid medium (1/4 MS). Two kinds of microcuttings were used in the experiments: Al+, obtained from cultures on media containing aluminium; and Al-, obtained from cultures on media without aluminium (Bojarczuk 1999). Aluminium was added to the liquid rooting medium as aluminium sulphate $Al_2(SO_4)_3 \cdot 18\ H_2O$ (Al concentration of 10-100 mg·dm^{-3}) or aluminium chloride $AlCl_3 \cdot 6\ H_2O$ (Al concentration of 30-60 mg·dm^{-3}). The pH of media used for culture and rooting of microcuttings was 4.5 or 5.5.

The rooting of poplar and birch shoots was carried out in jars, incubated at 22-23°C in a culture room illuminated 16 h/day with mercury discharge lamps (7500 mW/m^2). The formation of adventitious roots on the shoots lasted about 6 weeks. Subsequent measurements of development of the root system included the number and length of roots on the microcuttings, and root tip growth assessed on a scale of 0-3 (0, lack of tip growth; 1, poor tip growth, with 1-20% of growing tips; 2, moderate tip growth, with 21-60% of growing tips; 3, substantial tip growth, with 61-100% of growing tips). In addition, the development of the above-ground parts of the microcuttings was evaluated: shoot length, the number of leaves and degree of necrosis on a scale of 0-3 (0, lack of necrosis: 1, poor, 1-20% of necrosis; 2, moderate, with 21-60% of necrosis; 2, great, 61-100% of necrosis).

All the experiments were conducted in randomized designs with four replications and 14 microcuttings in each block. The significance of differences between the individual combinations was determined by the new Duncan's multiple range test for a limit value of 5%. In tables and figures values marked with the same letter do not differ from each other statistically (P=0.05).

RESULTS

Birch shoots, which were rooted in the control (medium without aluminium) produced longer roots and were characterized by faster growth of shoots at pH 4.5 than in the less acidic medium, i.e. at pH 5.5 (Table 1). No significant differences in the rooting of poplar shoots at pH 4.5 and 5.5 were detected (Table 2). In the control (liquid medium without Al) birch and poplar shoots obtained from cultures on the solidified medium with aluminium (Al+) or without aluminium (Al-) rooted similarly as a rule (Tables 1, 2 and 3). In some cases birch microcuttings from cultures on media with aluminium (Al+), rooted on the control medium (without Al), produced a higher number of roots (Table 4). In the control medium microcuttings obtained from cultures on media with aluminium (Al+) were also distinguished by faster growth of shoots and a lower degree of necrosis than microcuttings from cultures on media without aluminium (Tables 1 and 4).

In general, addition of aluminium (both as aluminium sulphate and as aluminium chloride) to the rooting medium had a negative effect on the development of adventitious roots on poplar and birch shoots. There were no differences in the development of roots (root length and root tip growth) when microcuttings were treated these two forms of aluminium at concentrations 25 and 50 mg·dm^{-3} (Table 2). A lower concentration of aluminium in media, particularly in the form of aluminium sulphate, had no or little inhibiting effect on formation of adventitious roots (Table 1). A high concentration of aluminium (Al 100 mg·dm^{-3} as aluminium sulphate and Al 60 mg·dm^{-3} as aluminium chloride) caused a marked inhibition of root development, retardation of shoot growth and severe necrosis of microcuttings (Tables 1 and 3).

Poplar and birch shoots obtained from cultures on media with aluminium (Al+) and rooted in media with aluminium,

TABLE 1. Effect of aluminium on the development of adventitious roots and shoots of birch (*Betula pendula* Roth.) cultured in vitro. The shoots were rooted in perlite saturated with a liquid medium (1/4 MS) containing aluminium sulphate. Al+ shoots, are microcuttings from cultures on media with aluminium, Al- shoots, are microcuttings from cultures on media without aluminium. Values marked with the same letters are not significantly different from one another (P=0.05).

Treatment Al mg·dm^{-3} (medium pH)	No of roots per shoot	Root length (cm)	Root tip growth (scale 0-3)	Shoot length (cm)	No. of leaves per shoot
Al – shoots, Control (5.5)	3.3 bc	9.2 b	1.3 ab	3.4 ab	4.5 b
Al – shoots, Control (4.5)	3.7 cd	13.1 cd	2.2 c	5.1 c	6.2 c
Al + shoots, Control (4.5)	4.4 d	16.0 d	2.4 c	9.0 e	8.9 d
Al – shoots 10 (4.5)	3.1 bc	10.5 bc	1.2 ab	7.1 d	7.9 d
Al – shoots 25 (4.5)	2.6 ab	10.1 bc	1.1 a	5.3 c	6.1 c
Al – shoots 50 (4.5)	2.7 ab	9.5 bc	1.7 b	3.8 bc	3.5 b
Al – shoots 100 (4.5)	1.8 a	3.7 a	0.9 a	2.2 a	1.8 a

TABLE 2. Effect of aluminium on the development of adventitious roots on poplar (*Populus tremula* L. × *P. alba* L.) microcuttings cultured in vitro. The shoots were rooted in perlite saturated with a liquid medium (1/4 MS) containing aluminium sulphate (Al-1) or aluminium chloride (Al-2). Other data as in Table 1.

Treatment Al mg·dm^{-3} (medium pH)	No of roots per shoot	Root length (cm)	Root tip growth (scale 0-3)	Degree of shoot necrosis (scale 0-3)
Al – shoots, Control (5.5)	4.0 bc	13.8 ab	2.5 ab	0.6 a
Al – shoots, Control (4.5)	4.6 c	13.9 ab	2.5 ab	1.2 ab
Al + shoots, Control (4.5)	4.5 c	15.3 b	2.4 ab	1.6 bc
Al – shoots (Al-1) 25 (4.5)	3.6 ab	14.1 ab	2.5 ab	0.5 a
Al – shoots (Al-1) 50 (4.5)	3.1 a	9.9 a	2.0 a	2.1 c
Al – shoots (Al-2) 25 (4.5)	3.8 abc	14.6 ab	2.8 b	0.7 a
Al – shoots (Al-2) 50 (4.5)	3.3 ab	11.7 ab	2.1 a	1.6 bc

TABLE 3. Effect of aluminium on the development of adventitious roots on poplar (*Populus tremula* L. × *P. alba* L.) microcuttings cultured in vitro. The shoots were rooted in perlite saturated with a liquid medium (1/4 MS) containing aluminium chloride. Other data as in Table 1.

Treatment Al mg·dm^{-3} (medium pH 4.5)	No of roots per shoot	Root length (cm)	Root tip growth (scale 0-3)	Degree of shoot necrosis (scale 0-3)
Al – shoots, Control	5.5 b	15.0 b	4.0 b	2.1 b
Al + shoots, Control	5.9 b	15.5 b	4.1 b	2.2 b
Al – shoots 30	5.4 b	14.2 ab	3.4 ab	1.4 ab
Al + shoots 30	8.2 c	22.7 c	3.9 ab	0.8 a
Al – shoots 60	3.0 a	7.9 a	2.8 a	4.7 d
Al + shoots 60	5.2 b	14.2 ab	2.9 a	3.2 c

TABLE 4. Effect of aluminium on the development of adventitious roots of birch (*Betula pendula* Roth.) microcuttings cultured in vitro. The shoots were rooted in perlite saturated with a liquid medium (1/4 MS) containing aluminium chloride. Other data as in Table 1.

Treatment Al mg·dm^{-3} (medium pH 4.5)	No of roots per shoot	Root length (cm)	Root tip growth (scale 0-3)	Degree of shoot necrosis (scale 0-3)
Al – shoots, Control	2.6 a	12.7 bc	2.5 b	1.4 b
Al + shoots, Control	3.8 c	16.7 c	2.2 b	0.8 a
Al – shoots 30	2.8 ab	9.1 ab	1.2 a	2.5 c
Al + shoots 30	3.7 bc	16.8 c	2.7 b	0.6 a
Al – shoots 60	2.4 a	6.7 a	1.3 a	2.6 c
Al + shoots 60	2.9 abc	12.2 bc	2.7 b	1.4 b

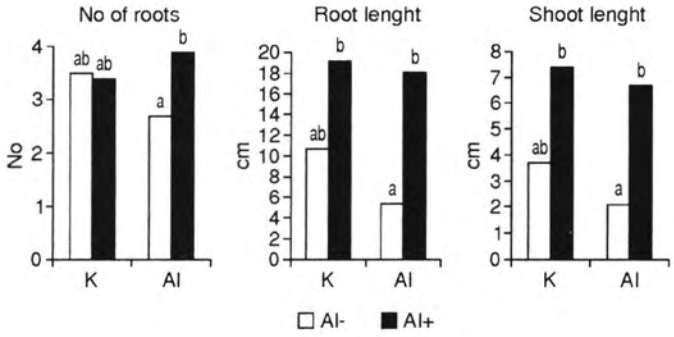

Fig. 1. Development of poplar (*Populus tremula* L. × *P. alba* L.) microcuttings obtained from cultures on media with aluminium (Al+) and without aluminium (Al-). The microcuttings were rooted in perlite saturated with a liquid medium (1/4 MS, pH 4.5) without aluminium (K) or containing aluminium sulphate at a concentration of Al 50 mg·dm^{-3} (Al).

produced more numerous and longer roots than shoots cultured on media without aluminium (Al-) (Fig. 1, Tables 3 and 4). A higher capacity of those shoots for root regeneration was particularly conspicuous in the case of media containing a high concentration of aluminium (Fig. 1, Tables 3 and 4). Shoots obtained from cultures on media with aluminium (Al+) were characterized not only by a stronger root system but also by a faster growth of the above-ground parts and a lower degree of necrosis than shoots cultured on media without aluminium (Fig. 1, Table 4).

DISCUSSION

Studies of aluminium phytotoxicity in vitro have been conducted with the use of media with many components. It was found that some components of the media, e.g. chelate compounds of phosphorus, calcium and iron, may protect plants by lowering aluminium toxicity. Therefore, media with a diminished concentration of some nutrients or diluted media have usually been applied in research on this subject (Borkowska 1988, Wilkins and Hodson 1989). In this study of poplar and birch regeneration in vitro, media with lower nutrient concentrations than standard media (1/4 MS) were used, so that the effect of aluminium on plants should not be alleviated. The shoots were cultured and rooted at pH 4.5 or, in some cases, at pH 5.5. In alkaline conditions aluminium is present exclusively in the form of anions, in neutral substrates the dominant form is aluminium hydroxide, whereas in an acidic environment the cationic form (Al^{3+}) prevails. The cationic form is the only mobile form regarded as toxic to plants. Dependence of aluminium toxicity on substrate acidity has been confirmed in experiments on in vitro regeneration of birch and poplar (Bojarczuk 1997, 1998, 1999). Birch microcuttings in the control (medium without aluminium) rooted best at 4.5, while poplar shoots rooted well both at pH 4.5 and 5.5 (Tables 1 and 2). Birch shoots rooted in the more acidic media were also distinguished by faster development of shoots (Table 1). Good rooting of shoots in a poor medium (1/4 MS) at pH 4.5 could result from more efficient assimilation of nutrients than at pH 5.5. In the control (medium without Al), birch and poplar shoots obtained from cultures on media with aluminium (Al+) and without aluminium (Al-) usually rooted similarly (Tables 1 and 2).

For rooting of poplar shoots obtained from cultures on media with aluminium (Al+) or without aluminium (Al-), media with a pH of 4.5 were used, so that the added aluminium could be readily assimilable by the plants. Plant susceptibility to toxic metal ions in the substrate depends on the type and concentration of the applied compound (Keltiens and Loenen 1989; Arnold et al. 1994). In this study, low concentrations of aluminium in media, particularly in the form of aluminium sulphate, had no or little inhibiting effect on formation of adventitious roots, whereas high doses of aluminium clearly inhibited root development and caused necrosis of microcuttings (Table 1). The negative effect of aluminium on plant development was also reflected in a marked inhibi-

tion of root tip growth (Tables 1 and 4). Roots are most exposed to the toxic impact of aluminium. Tips of the main and lateral roots turn brown and stop growing (Gabara 1992; Lima and Copeland 1994). Such injuries may result from a deficiency of calcium, phosphorus or magnesium (Rengel 1992). Aluminium both limits the uptake of those nutrients by plants (Göransson and Eldhuset 1991; Kochian 1995) and blocks their transportation and utilization in metabolic processes (Borkowska 1988; Farid 1991). This is followed by an adverse change of the ratio Ca/Al (in favour of Al) and a reduction in the magnesium content of plants (Henriksen et al. 1992). This, in turn, results in retardation of plant growth and development, and inhibition of important physiological processes like photosynthesis and respiration (Rengel 1992; Lima and Copeland 1994). The results of this study show that in birch and poplar aluminium not only affects the root system development, but also inhibits shoot growth (Table 1, Fig. 1).

There are substantial differences in tolerance for aluminium in the substrate between species and between varieties or clones of the same plant species (Foy et al. 1987; Kinrade 1991). A lower susceptibility of some plants to toxic compounds may be linked with limited uptake of toxic metals from the substrate and their detoxication inside cells (Foy et al. 1987; Farid 1991). Using in vitro cultures, Conner and Meredith (1985) have selected tobacco mutants resistant to aluminium toxicity. In this study poplar and birch shoots cultured in vitro on media with aluminium (Al+) were distinguished by a greater tolerance for aluminium during rooting (Fig. 1, Tables 3 and 4).

The fact that microcuttings with a lower susceptibility to toxic aluminium ions were obtained by means of tissue culture, suggests that it is possible to obtain plants more tolerant to pollution with other toxic metals, too (Conner and Meredith 1985; Samantaray et al. 1994). Vegetative propagation of those plants and their introduction to degraded habitats may improve the results of management of contaminated grounds (e.g. industrial areas and vicinities of highways).

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LITERATURE CITED

ARNOLD M.A., LINEBERGER R.D., STRUVE D.K. 1994. Copper compounds influence in vitro rooting of birch microcuttings. J. Amer. Soc. Hort. Sci. 119 (1): 74-79.

BOJARCZUK K. 1997. Wpływ toksycznych jonów glinu na rozwój mikrosadzonek brzozy (*Betula pendula* Roth.) w kulturach in vitro. Arobor. Kórnickie 42: 217-228.

BOJARCZUK K. 1998. Wpływ jonów glinu na rozwój pędów i korzeni topoli (*Populus tremula* L. × *P. alba* L.) w kulturach in vitro. "Zastosowanie kultur in vitro w fizjologii roślin". Zakład Fizjologii Roślin PAN Kraków: 29-34.

BOJARCZUK K. 1999. Effect of aluminium toxicity on the development of poplar (*Populu tremula* L. × *P. alba* L.) cultured in vitro. Acta Soc. Bot. Pol. Vol. 68(4): 245-250.

BOJARCZUK K., OLEKSYN J. 1994. Rozwój jednorocznych sadzonek sosny (*Pinus sylvestris* L.) i brzozy (*Betula pendula* Roth.) w zanieczyszczonych podłożach inokulowanych grzybem *Trichoderma harzianum* Rifai. Arbor. Kórnickie 39: 163-177.

BORKOWSKA B. 1988. Toksyczność glinu (Al). Wiadomości Botaniczne 32 (3): 167-168.

BOUDOT J.P., BECQUER T., MERLET D., ROUILLER J. 1994. Aluminium toxicity in declining forest: A general overview with a seasonal assessment in silver fir forest in the Vosges mountains (France) Ann. Sci. For. 51: 27-51.

CONNER A.J., MEREDITH C.P. 1985. Stimulating the mineral environment of aluminium toxic soils in plant cell culture. J. Exp. Botany 36, 167: 870-880.

ERNST W.H.O., VERKLEJ J.A.C., SCHAT H. 1998. Metal tolerant plants. Acta. Bot. Neerl. 41 (3): 229-248.

FARID J. 1991. Aluminium effects on growth, nutrient net uptake and transport in 3 rice (*Oryza sativa*) cultivars with different sensitivity to aluminium. Physiol. Plantarum 83: 441-448.

FOY C.D., LEE E.H., WILDING S.B. 1987. Differential aluminium tolerances of two barley cultivars related to organic acid in their roots. J. Plant Nut. 10: 1089-1101.

GABARA B. 1992. Reakcje roślin na metale ciężkie na poziomie organizmu i komórki. Materiały z V Ogól. Konf. "Mechanizm regulacji morfogenezy roślin oraz funkcjonowanie w warunkach stresowych". Rogów 15-16 VI 1992: 37-47.

GÖRANSSON A., ELDHUSET T.D. 1991. Effects of aluminium on growth and nutrient uptake of small *Picea abies* and *Pinus sylvestris* plants. Trees 5: 136-142.

GREGER M., TILLBERG J.E., JOHANSSON M. 1992. Aluminium effects on *Scenedesmus obtusiusculatus* with different phosphorus status. I. Mineral uptake. Physiol. Plantarum 84: 193-201.

HENRIKSEN T.M., ELDHUSET T.D., STUANSE A.D., LANGERUD B.R. 1992. Effects of aluminium and calcium on *Picea abies* seedlings. Scand. J. For. Res. 7: 63-70.

KELTIENS W.G., VAN LOENEN E. 1989. Effects of aluminium and mineral nutrition on growth and chemical composition of hydroponically grown seedlings of five different forest tree species. Plant and Soil 119, 1: 39-50.

KINRADE T.B. 1991. Identity of the rhizotoxic aluminium species. Plant and Soil 134: 167-178.

KOCHIAN L.V. 1995. Cellural mechanisms of aluminium toxicity and resistant in plants. Ann. Rev. Plant Physiol. Mol. Biol. 46: 237-260.

LIMA M.L., COPELAND L. 1994. The effect of aluminium on respiration of wheat roots. Physiol. Plantarum 90: 51-58.

MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bio-assays with Tobacco tissue cultures. Physiol. Plantarum 15: 473-497.

MEREDITH C.P. 1978. Selection and characterization of aluminium-resistant variants from tomato cell cultures. Plant Science Letters 12: 25-84.

OLEKSYN J., KAROLEWSKI P., GIERTYCH M.J., WERNER A., TJOELKER M.G., REICH P.B. 1996. Altered root growth and plant chemistry of *Pinus sylvestris* seedlings subjected to aluminium in nutrient solution. Trees 10: 135-144.

RACHWAŁ L., WIT-RZEPKA M. 1989. Reakcje brzóz na zanieczyszczenia z hut miedzi. Część II. Wyniki badań terenowych. Arbor. Kórnickie 34: 185-205.

REICH P.B., OLEKSYN J., TJOELKER M.G. 1994. Relationship of aluminium and calcium to net CO_2 exchange among diverse Scots pine provenances under pollution stress in Poland. Oecologia 97: 82-92.

RENGEL Z. 1992. Role of calcium in aluminium toxicity New Phytologist 121: 499- 513.

SAMANTARAY S., ROUT G.R., DAS P. 1994. An in vitro study on organogenesis in *Trema orientalis* (Blume) Linn. Plant Science 105: 87-94.

ŚLASKI J. 1992. Mechanizm tolerancyjności na toksyczne działanie jonów glinu u roślin wyższych. Wiadomości Botaniczne 36 (1/2): 31-45.

VILKKA L., AULA L., NUORTEVA P. 1990. Comparison of the levels of some metals in roots and needles *Pinus sylvestris* in urban and rural environment at two times in growing season. Ann. Bot. Fennici 27: 53-57.

WILKINS D.A., HODSON M.J. 1989. The effects of aluminium and *Paxillus involutus* Fr. on the growth of Norway spruce (L. Karst). New Phytol. 113: 225-232.

WPŁYW GLINU NA UKORZENIANIE MIKROSADZONEK BRZOZY (*BETULA PENDULA* ROTH.) I TOPOLI (*POPULUS TREMULA* L. × *P. ALBA* L.) W KULTURACH IN VITRO

STRESZCZENIE

Mikrosadzonki topoli (*Populus tremula* L. × *P. alba* L.) i brzozy (*B. pendula* Roth.) pozyskane z kultur in vitro, hodowanych na pożywkach z glinem (Al+) i bez glinu (Al-) ukorzeniano w perlicie nasyconym płynną pożywką (1/4 MS). Do pożywek, w których ukorzeniano pędy dodawano glin w postaci siarczanu i chlorku glinu. Wartość pH pożywek ustalano w granicach 4,5 i 5,5. Mikrosadzonki brzozy w kontroli (pożywka bez glinu) wytworzyły dłuższe korzenie oraz odznaczały się lepszym rozwojem pędów w pożywce o pH 4,5, niż w pożywce o wyższym odczynie tj. pH 5,5. W kontroli tj. w pożywce bez glinu pędy Al+ i Al- na ogół ukorzeniały się podobnie. Dodanie glinu do pożywek, w których ukorzeniane były mikrosadzonki, wpływało negatywnie na rozwój korzeni przybyszowych. Duże stężenie glinu (100 $mg \cdot dm^{-3}$ Al, w postaci siarczanu glinu i 60 $mg \cdot dm^{-3}$, w postaci chlorku glinu) wpłynęło na zahamowanie rozwoju korzeni, ograniczenie wzrostu pędów oraz silną nekrozę mikrosadzonek. Pędy topoli i brzozy otrzymane z kultur hodowanych z glinem (Al+) i ukorzeniane w pożywce z dodatkiem glinu tworzyły większą liczbę korzeni o silniejszym wzroście niż pędy z kultur hodowanych bez glinu (Al-). Uzyskanie poprzez kultury tkankowe mikrosadzonek o mniejszej wrażliwości na toksyczne jony glinu pozwala przypuszczać, że istnieje możliwość otrzymania tą drogą roślin o zwiększonej tolerancji na skażone podłoże.

SŁOWA KLUCZOWE: *Betula pendula* Roth., *Populus tremula* L. × *P. alba* L., in vitro, glin, mikrosadzonki, ukorzenianie.