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Original Research

Effect of Toxic Metals on the Development of Poplar (*Populus tremula* L. × *P. alba* L.) Cultured *in vitro*.

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Abstract

Adventitious bud cultures were established by using buds of selected poplar clones (*Populus tremula* L. × *P. alba* L.) as initial explants. The Murashige and Skoog medium (½ and ¼ MS) was used for multiplication of shoots. Aluminium and copper were added to the medium in the form of sulphates. Copper, lead and aluminium were also added to the medium in the form of nitrates. Low concentrations of copper had no inhibitory effect on culture quality (i.e. degree of chlorosis and browning) and shoot development. High concentrations, especially of copper and lead, inhibited shoot and root development. Adventitious bud cultures derived from cultures grown on media with aluminium (Al+) were distinguished by a greater tolerance to aluminium and copper in the medium than shoot cultures derived from cultures grown on media without aluminium (Al−).

Keywords: *Populus tremula* L. x *P. alba* L. copper, lead, aluminium, development, *in vitro* cultures.

Introduction

The increasing environmental pollution associated with industrial development and intensive farming, leads to continuous and aggravating acidification of soils [8, 20]. In degraded soils, toxic metal ions are accumulated, including copper, lead, zinc, cadmium and aluminium. As soil pH decreases, metals are transformed into easily soluble, ionic forms, so their availability to plants increases [19]. High concentrations of metal ions in the soil limit the assimilation of important micro- and macronutrients by plants [9, 23]. Research on birch seedlings (*Betula pendula* Roth.) has shown that high concentrations of aluminium in the substrate diminish assimilation of calcium, potassium, phosphorus, iron and zinc by plants, causing strong inhibition of root and shoot development [7, 13]. Toxic metal ions not only limit the uptake of elements from the soil, but also block their transport and utilization in important metabolic processes, such as photosynthesis and respiration [15, 26].

The mechanisms of plant defence against industrial pollution are relatively poorly studied [8]. For a long time already, *in vitro* culture has been used to investigate responses of plants to toxic metal ions [2, 21, 35]. With the use of plant culture *in vitro*, researchers have selected plants tolerant to some metal ions, including aluminium, copper, manganese and nickel [12, 30, 33]. Studies *in vitro* have shown that microcuttings of poplar and birch, produced in cultures on media with aluminium, proved to be more tolerant to aluminium than microcuttings obtained from cultures without aluminium [5, 6]. Further experiments aimed to analyse the effect of other metals, such as copper and lead, on the growth of those plants *in vitro*. This may enable selection of trees with a higher tolerance to industrial pollution. Such plants would also be valuable material for research on mechanisms of plant sensitivity to toxic metal ions.

Material and Methods

Apical and lateral buds, as explants from poplar clones (*Populus tremula* L. × *P. alba* L.), were collected from June till September. The MS medium developed

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Table 1. Effect of toxic metal ions on the development of poplar (*Populus tremula* L. × *P. alba* L.) cultured *in vitro*. Cu, Pb, Al added as nitrates, concentration in mM. Data are the means of four analyses ± standard deviation. Mean values marked with the same letters are not significantly different at P< 0.05.

Treatment (mM)	Shoot number	Shoot length (cm)	Degree of chlorosis (scale 0-5)	Degree of browning (scale 0-5)	Degree of root development (scale 0-5)
Control	13.9 ± 0.4 c	4.2 ± 0.5 d	1.2 ± 0.3 a	1.3 ± 0.4 a	2.3 ± 0.4 bc
Cu 0.1	11.2 ± 1.6 bc	3.3 ± 1.0 d	0.9 ± 0.1 a	2.1 ± 0.2 ab	3.3 ± 0.3 c
Cu 0.25	7.3 ± 2.9 b	1.8 ± 0.2 bc	2.8 ± 0.3 b	2.9 ± 0.1 bc	1.7 ± 0.2 bc
Cu 1.0	1.3 ± 0.6 a	0.5 ± 0.1 a	5.0 ± 0.1 c	5.0 ± 0.1 d	0 a
Pb 1.0	12.6 ± 2.6 c	1.9 ± 0.1 bc	1.0 ± 0.3 a	2.0 ± 0.1 ab	1.7 ± 0.2 b
Pb 2.0	6.3 ± 1.9 b	0.7 ± 0.3 ab	3.7 ± 1.5 bc	3.9 ± 1.3 cd	0.2 ± 0.1 a
Al 1.0	9.8 ± 0.4 bc	2.0 ± 0.3 c	1.2 ± 0.5 a	1.7 ± 0.4 a	1.5 ± 0.2 b
Al 2.0	7.6 ± 1.4 b	1.4 ± 0.4 abc	1.0 ± 0.1 a	1.4 ± 0.2 a	1.2 ± 0.2 b

by Murashige and Skoog [22] was modified by dilution to ½ or ¼ concentrations of micro- and macronutrients. The media were supplemented with plant hormones: BAP 0.25-0.5 mg·dm^{-3} and NAA 0.1 mg·dm^{-3}, and solidified with Bactoagar (8 g·dm^{-3}). Also, ions of copper, lead and aluminium were added to the medium as nitrates (0.1-2.0 mM), copper ions as copper sulphate (0.2 -1.0 mM), and aluminium ions as aluminium sulphate (0.5-1.0 mM). The pH of media used in the experiment was 4.5.

Poplar culture was conducted in 300-ml jars (4 callus culture of about 1 cm in diameter in each jar), kept in a growth chamber at 22-23°C, with fluorescent mercury illumination (7500 mW/m^2) for 16 h/day.

After 12 months of growth on media with aluminium (1.0 mM aluminium sulphate), the cultures were transferred to a medium with aluminium sulphate (0.5 and 1.0 mM) or copper sulphate (0.2 and 0.5 mM).

After about 5 weeks, an assessment of culture development was started. The assessment involved measurements of length and number of shoots, and evaluation of culture quality, i.e. the level of chlorosis and browning on a scale of 0-5: 0 - no chlorosis and browning; 1 - very low level (1-20%); 2 - low level (21-40%); 3 - moderate level (41-60%); 4 - high level (61-80%); 5 - very high level (81-100%). In some media, poplars formed root systems, whose development was assessed on a scale of 0-5: 0 - no roots; 1 - very poor development (1-20% roots in medium); 2 - poor development (21-40%); 3 - moderate development (41-60%); 4 - strong development (61-80%); 5 - very strong development (81-100%).

All experiments were conducted in a randomized block design with 4 replications of 6 jars each. Tukey's test was used to determine the significance of differences between combinations at significance levels 0.01 and 0.05. In tables and figures, values marked with the same letters are not significantly different at P<0.05.

Results

Research on the effects of toxic ions in media on development of poplar culture revealed the following ranking of toxicity of the studied ions: Cu>Pb>Al (Table 1). The most toxic were copper ions (irrespective of the applied sulphate or nitrate form). At concentrations of 0.25-1.0 mM, copper strongly inhibited shoot development (number and length of shoots) and deteriorated culture quality, reflected in an increase in chlorosis and browning (Tables 1 and 2). Copper nitrate added to media at a low level of 0.1 mM, did not inhibit culture development (number and length of shoots and quality of cultures) and even slightly stimulated root development (Table 1). As the concentration of the studied ions in media increased, their toxic influence was enhanced (Tables 1, 2, Figs. 1, 2). For example, 1 mM copper nitrate and 2 mM lead nitrate caused a strong inhibition of shoot development, deterioration of culture quality (increase level of chlorosis and browning), and limited root development (Table 1). Copper sulphate, at concentrations of 0.5-1.0 mM, completely blocked root development (Table 2). Aluminium ions proved to be less toxic. If added as 2 mM aluminium nitrate, they reduced shoot number and length, but no significant effects on culture quality and root growth were observed (Table 1).

Poplars cultured for 12 months on a medium with aluminium (Al+), after transfer to a control medium were characterized with better development than those cultured without aluminium (Al–) (Figs. 1, 2). Plants obtained from (Al+) cultures, after transfer to a medium containing copper sulphate or aluminium sulphate, were distinguished by more intensive shoot development (greater number and length of shoots) and higher culture quality (lower level of chlorosis and browning) than plants from (Al–) cultures (Figs. 1, 2). A particularly strong tolerance to toxic ions was observed in plants from (Al+) cultures, if higher concentrations of aluminium ions (1 mM aluminium sulphate) and copper ions (0.5 mM copper sulphate) were applied (Figs. 1, 2).

Table 2. Effect of copper sulphate (concentration in mM) on the development of poplar (*Populus tremula* L. × *P. alba* L.) cultured *in vitro*. Other data as in Table 1.

Treatment (mM)	Shoot number	Shoot length (cm)	Degree of chlorosis (scale 0-5)	Degree of browning (scale 0-5)	Degree of root development (scale 0-5)
Control	16.6 ± 2.1 c	3.4 ± 0.2 c	0.8 ± 0.2 a	0.8 ± 0.2 a	2.3 ± 0.6 b
Cu 0.25	11.0 ± 1.3 b	2.1 ± 0.4 b	1.7 ± 0.1 b	1.5 ± 0.3 b	2.7 ± 0.3 b
Cu 0.5	2.4 ± 0.4 a	0.8 ± 0.1 a	3.2 ± 0.2 c	3.5 ± 0.4 c	0 a
Cu 1.0	1.0 ± 0.3 a	0.6 ± 0.1 a	4.7 ± 0.2 d	4.9 ± 0.2 d	0 a

Discussion

Numerous *in vitro* studies have been concerned with plant sensitivity to toxic metal ions [4, 10, 28, 29]. A lower sensitivity of plants to toxic ions in media can be reflected in their limited uptake, or synthesis of compounds (enzymes, lipids or chelates) that cause their detoxification inside cells [17, 18, 31]. Plant sensitivity to toxic metal ions depends on the kinds of compounds present in the substrate and on their concentrations [2]. In the above experiments, both copper sulphate and nitrate showed a reducing effect on poplar development (Tables 1, 2, Figs. 2). Copper nitrate at a low concentration (0.1 mM) did not limit the development of cultures, and even slightly stimulated poplar root development (Table 1). A similar effect has been observed in the case of low concentrations of aluminium ions, which stimulate rooting of poplar and birch microcuttings [6], while some heavy metals have a positive effect on callus tissue morphogenesis [1, 24]. Such a stimulating effect of some metal ions on plant development may be due to their impact on solubility and hence availability of some nutrients to plants [14, 16]. In cultures *in vitro*, heavy metals at low concentrations inhibit production of ethylene and stimulate somatic embryogenesis [27].

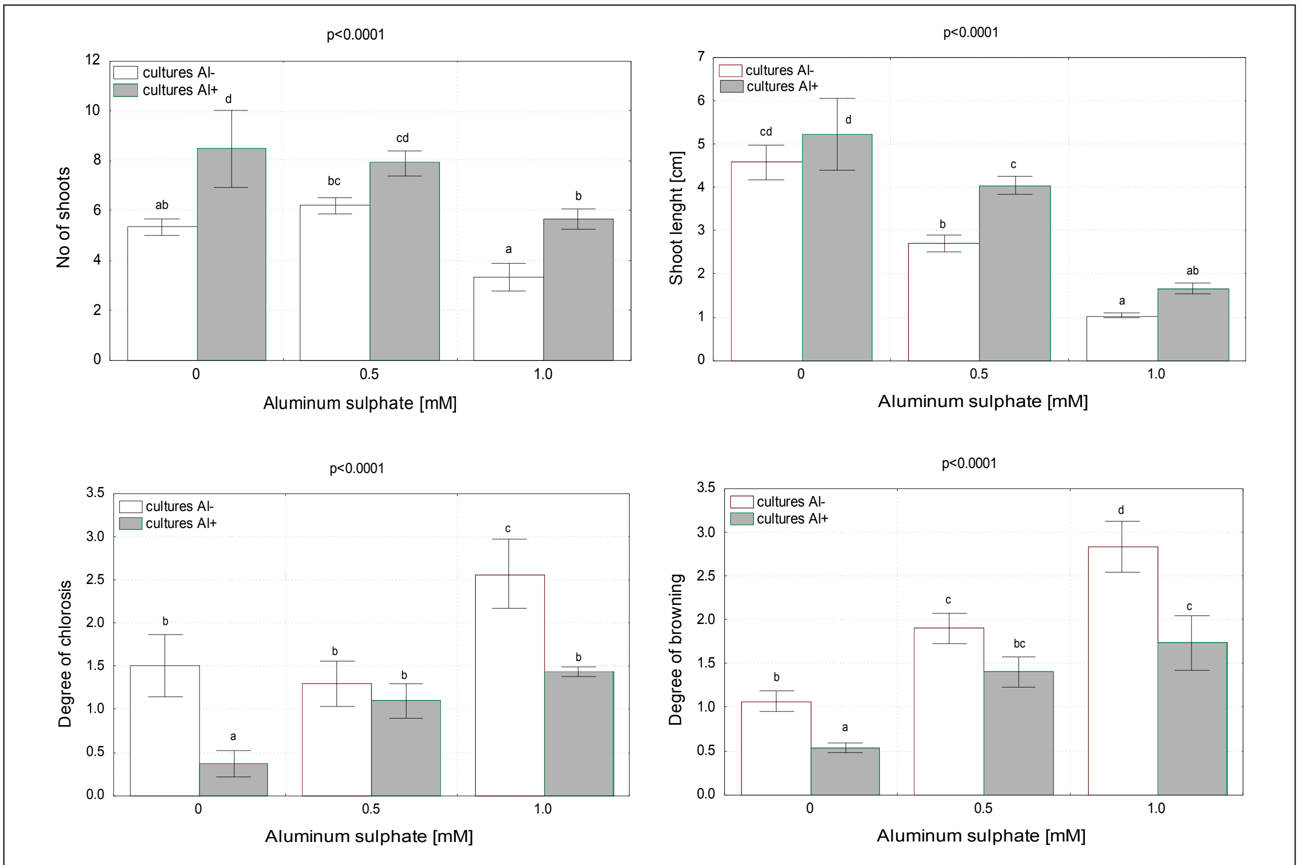

Fig 1. Effect of aluminium on the development of poplar cultures derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al−). Concentration of aluminium sulphate: 0 - control, 0.5 and 1.0 [mM].

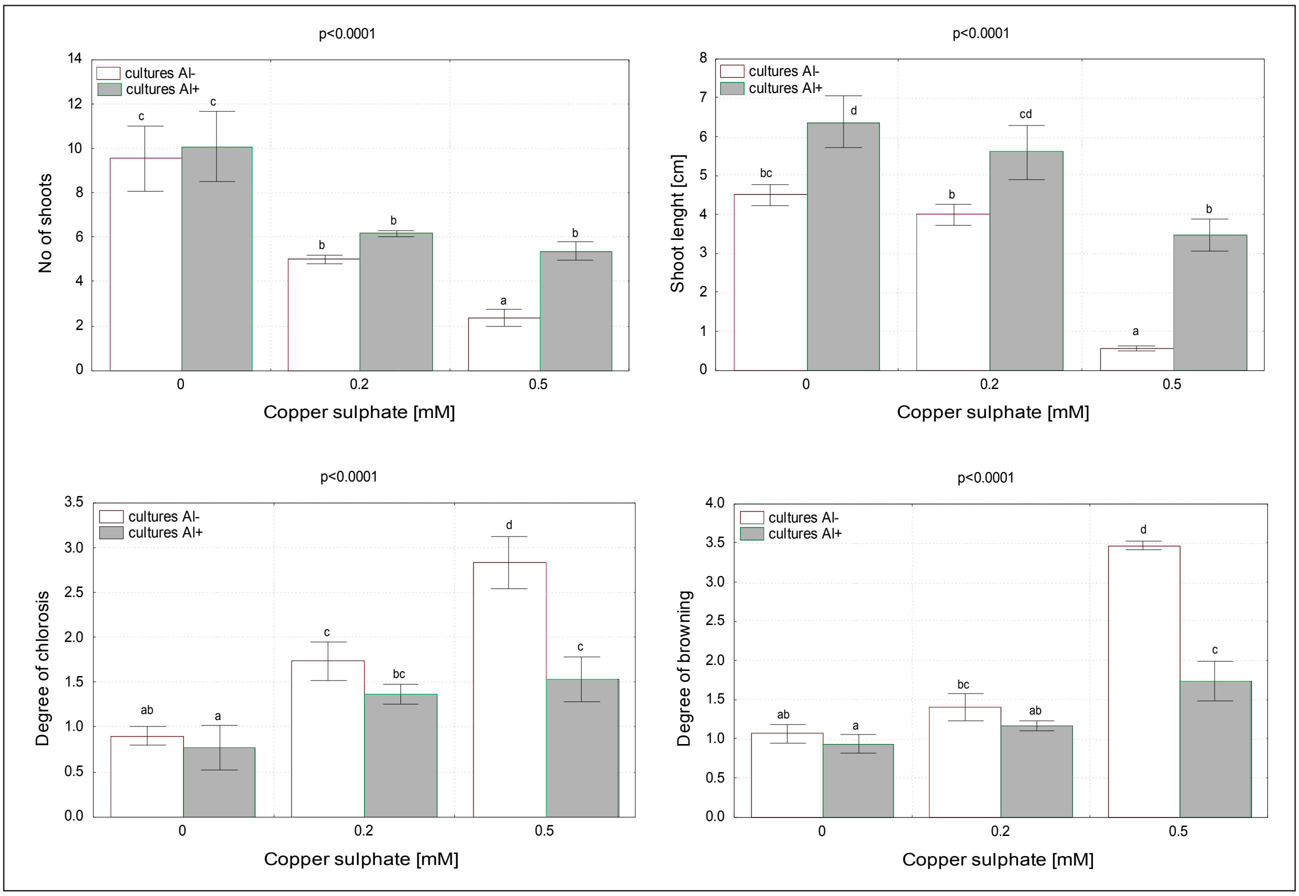

Fig.2. Effect of copper on the development of poplar cultures derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al−). Concentration of copper sulphate: 0 - control, 0.2 and 0.5 [mM].

The study on development of poplar cultures in media containing toxic metal ions in the form of nitrates attests to the following ranking of their toxicity: Cu>Pb>Al (Table 1). The most toxic were copper ions, added as 0.25 or 1.0 mM copper nitrate, which strongly inhibited shoot and root development and deteriorated culture quality (higher level of chlorosis and browning), whereas lead and aluminium nitrate, at a concentration of 1 mM, did not cause such negative effects, especially on root system development. Higher concentrations of toxic ions in media, strongly inhibited culture development and lowered culture quality, i.e. increased the level of chlorosis and browning. Also 1 mM copper nitrate and 2 mM lead nitrate caused a nearly complete inhibition of root development. Toxic ions, e.g. aluminium, contained in the media, effect on development and morphology of poplar and birch shoots and roots [5, 6]. Copper and lead ions damage root apices, thus causing the formation of short and thin roots with necrotic lesions [4, 32].

Numerous *in vitro* experiments have focused on the effects of high concentrations of heavy metals on the regeneration of plants tolerant or sensitive to industrial pollution [11, 25, 35]. Selection of plants under natural conditions of environmental pollution or *in vitro* may result in the selection of clones tolerant to toxic metal ions [3, 12, 28, 30, 34].

Repeated passaging of poplar cultures to media containing aluminium (about 12 months of culture), resulted in the production of a line of cultures, which under stress conditions (in media with aluminium and copper ions) regenerated better than cultures grown earlier on control media (without aluminium). Plants derived from those cultures (grown with aluminium), were characterized with more intensive development, particularly in media containing higher concentrations of toxic ions, than control plants (grown earlier without aluminium). Poplar cultures tolerant to aluminium were also more tolerant to copper ions. Chakravarty and Srivastava [10] found that plants tolerant to zinc ions grew well in media containing zinc and cadmium ions, separately and jointly. Thus, induction of tolerance to one toxic metal can result in reduced sensitivity to other heavy metals. Stress-tolerant plants produced *in vitro* preserve a greater tolerance to toxic metal ions in the next few generations, also after transfer to natural conditions [1, 33].

In vitro selection of plants tolerant to toxic ions contained in the soil may lead to production of plants that are better adapted to environmental pollution and can enable better management of degraded soil (e.g. industrial areas and highways).

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