Effect of Aluminium on the Development of Poplar (Populus tremula L. × P. alba L.) in vitro and in vivo

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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 (1/2 and 1/4 MS) was used for multiplication and rooting of shoots. To the media, aluminium was added in the form of sulphate, at a concentration 50−70 mg Al dm⁻³. The culture was continued in vitro for more than 12 months. The cultures developed on media with aluminium (Al+) were more tolerant to aluminium in the medium during multiplication than those developed on media without aluminium (Al−). Rooted poplar cuttings obtained from cultures on media with aluminium (Al+) grew better in soil from an area polluted by a phosphate fertilizer factory (Luboń) than those from media without aluminium (Al−). This soil was characterized by a high Al level, low Ca/Al ratio and low pH, as compared to the control soil, from an area regarded as free from toxic pollution.

Keywords: Populus tremula × P. alba, in vitro, in vivo culture, development, aluminium, soil pollution

Introduction

Industrial and agricultural pollution leads to permanent and increasing acidification of soils, which accumulate toxic ions such as aluminium, copper, lead, zinc or cadmium. The ions hinder the development of plant roots and shoots, and at higher concentrations may lead to plant death [8, 18]. The mechanisms of defence against industrial pollution are still poorly understood. In general, broad-leaf trees are regarded as less sensitive to environmental pollution than conifers [9, 23]. However, the decline of some broad-leaf species was observed in experimental plots around some factories. This applies, for example, to silver birch (Betula pendula Roth.), which was earlier classified as relatively tolerant [9, 26]. The trees originating from different populations may vary in sensitivity to industrial pollution [25, 28]. Plant tolerance to toxic metal ions is conditioned by several mechanisms which are controlled by different genes but operate simultaneously and complement one another [1, 2, 16].

Tissue culture has been used in research on plant responses to toxic metal ions [13, 14, 33]. This method has enabled selection of plants tolerant to some metal ions: aluminium, copper, manganese or nickel [3, 17, 30, 32].

The main objective of this study was to assess the influence of aluminium on development of shoots and roots of poplar cultured in vitro and in vivo. This can broaden our knowledge of plant sensitivity to toxic ions and enable production of plants with a greater tolerance of industrial pollution.

Material and Methods

From a poplar clone (Populus tremula L. × P. alba L.), apical and lateral buds were collected as explants from June through September. Tissue culture was conducted with the use of a modified ½ or ¼ strength Murashige and Skoog medium [24], supplemented with hormones (BAP 0.25−0.5 mg·dm⁻³ and NAA 0.1 mg·dm⁻³), and solidified with Bactoagar. Except for control cultures, aluminium ions were added to the medium as aluminium sulphate (50−75 mg Al dm⁻³). The pH of the medium was stabilized at the level 4.5. Every 4−5 weeks, the cultures were
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passaged to fresh agar media with the same composition as during multiplication. Shoots of about 2 cm in length were rooted in perlite saturated with the same but liquid medium. Poplar culture on media with aluminium (Al+) and without it (Al−), was conducted continuously and after at least 12 months their development was controlled. The assessment included the following parameters: number and length of shoots as well as their quality, i.e. degree of chlorosis and browning on a scale of 0-3 (0 = lack of chlorosis/browning; 1 = very slight, 1−20% of chlorosis/browning; 2 = moderate, with 21-60% of chlorosis/browning; 3 = substantial, with 61-100% of chlorosis/browning).

Poplar cultures were kept in 300-ml jars at 22−23°C in a growth chamber illuminated with mercury-discharge lamps (7500 mW/m²) for 16 hours a day. All in vitro experiments were established in a randomized block design, with 4 replicates of 6 jars each. While testing differences between combinations, the Tuckey test was used at the significance level of 0.01 or 0.05.

In late May, poplar cuttings cultured and rooted in a medium with Al (Al+) and without it (Al−) were moved to a greenhouse and planted in 14-cm pots (about 1.5 l in volume) filled with a relatively unpolluted soil (Z) from the Zwierzyniec Forest (52°15'N and 17°04'E) or a polluted soil (L) collected 2 km from the Poznań Phosphate Fertilizer Factory in Luboń (52°15’10"N and 16°50’31"E). Fertilizer production began there in 1917 and since then the emission of toxic pollutants

### Table 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 50 and 75 mg Al dm⁻³. Mean values marked with the same letters are not significantly different at P<0.05

<table>
<thead>
<tr>
<th>Treatment (mg Al dm⁻³)</th>
<th>No. of shoots</th>
<th>Shoot length (cm)</th>
<th>Degree of chlorosis (scale 0-3)</th>
<th>Degree of browning (scale 0-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al− cultures, control</td>
<td>7.0 b</td>
<td>3.6 c</td>
<td>1.3 bc</td>
<td>1.3 ab</td>
</tr>
<tr>
<td>Al+ cultures, control</td>
<td>8.4 b</td>
<td>4.4 d</td>
<td>0.5 a</td>
<td>1.1 a</td>
</tr>
<tr>
<td>Al− cultures, 50</td>
<td>4.3 a</td>
<td>1.4 a</td>
<td>1.7 cd</td>
<td>2.2 c</td>
</tr>
<tr>
<td>Al+ cultures, 50</td>
<td>6.8 b</td>
<td>3.0 c</td>
<td>0.9 b</td>
<td>1.2 ab</td>
</tr>
<tr>
<td>Al− cultures, 75</td>
<td>3.9 a</td>
<td>1.0 a</td>
<td>1.9 d</td>
<td>2.5 c</td>
</tr>
<tr>
<td>Al+ cultures, 75</td>
<td>6.9 b</td>
<td>2.2 b</td>
<td>0.9 b</td>
<td>1.7 b</td>
</tr>
</tbody>
</table>

### Table 2. Concentration of macro- and micronutrients (soluble forms, mg · dm⁻³) in control (Z) and polluted (L) substrates.

<table>
<thead>
<tr>
<th>Nutrients and Pollutants</th>
<th>Control substrate (Z)</th>
<th>Polluted substrate (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>254</td>
<td>153</td>
</tr>
<tr>
<td>Mg</td>
<td>65</td>
<td>69</td>
</tr>
<tr>
<td>Na</td>
<td>161</td>
<td>227</td>
</tr>
<tr>
<td>Cl</td>
<td>58</td>
<td>64</td>
</tr>
<tr>
<td>S-SO₄</td>
<td>61</td>
<td>101</td>
</tr>
<tr>
<td>Fe</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>Zn</td>
<td>1.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Mn</td>
<td>5.7</td>
<td>4.2</td>
</tr>
<tr>
<td>Cu</td>
<td>0.72</td>
<td>0.28</td>
</tr>
<tr>
<td>Pb</td>
<td>1.2</td>
<td>0.46</td>
</tr>
<tr>
<td>Cd</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Al</td>
<td>38.2</td>
<td>92.7</td>
</tr>
<tr>
<td>pH</td>
<td>5.1</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Fig. 1. Development of poplar cuttings, derived from in vitro cultures on media with aluminium (Al+) and without aluminium (Al−), after planting in a control substrate (Z) or a polluted substrate (L). (Experiment I, 1998).
Results

After more than 12 months of growth in media with aluminium (Al+) and without it (Al−), poplar cultures were transferred to a similar medium with aluminium sulphate at a concentration of 50 or 75 mg Al dm⁻³ (Table 1), to assess their sensitivity to aluminium ions. The Al+ cultures proved to be more tolerant to the toxic aluminium ions, as they produced stronger and more numerous shoots than Al− cultures. The greater tolerance of Al+ plants was also reflected in their markedly better quality (i.e. lower degree of chlorosis and browning), as compared to Al− plants.

For the greenhouse experiments, poplar shoots cultured in vitro for at least 12 months in media with aluminium (Al+) or without it (Al−) were rooted in perlite, and next transferred to a greenhouse and planted in a control soil (Z) and polluted soil (L). Although the level of pollution by the factory at Luboń had recently decreased (21), the soil from that area was characterized by a 2.5 times higher Al concentration, a lower Ca/Al ratio, and a lower pH than the control soil (Table 2). Poplar cuttings from Al− cultures grew slower in the polluted soil, forming a lower biomass of both aboveground parts and roots, than those planted in the control soil (Fig. 1). Moreover, the Al− plants growing in the polluted soil

(mainly SO₄, NO₃, HF) gradually increased till the late 1980s. In the 1990s the amount of emitted pollutants decreased considerably due to the termination of sulphuric acid and granulated superphosphate production [21].

The potted plants were fertilized weekly with a commercial mixture of nutrients (pH 4.5). Biomass increments were measured weekly from June till September. The measurements included: fresh and dry weight of the whole plant, roots, shoot, leaves, secondary growth, and fallen leaves. The proportional biomass allocation to foliage (LWR) and roots (RWR) were calculated. With the use of a scanner, leaf area was measured, as well as area and length of roots in three classes of thickness, and numbers of root tips and branching points were assessed. At the beginning and end of the greenhouse experiment (late May and early October), also concentrations of available forms of micro- and macronutrients (Ca, Mg, Na, Cl, S- SO₄, Fe, Mn, Zn, Cu, Cd, Pb, Al) were measured in the soil with methods described in an earlier paper [8].

In the greenhouse experiments, poplar cuttings were planted in 2 blocks with 4 replications of 12 plants each. All presented results were analyzed statistically by IMP 3.1.6.2 software (SAS Institute, Cary, NC, USA).
Poplar cuttings from Al+ cultures grew very well in the unpolluted soil (Z), formed a greater biomass of above-ground parts (Fig. 1), and reached a higher proportional biomass allocation to foliage (LWR) than those from Al− cultures (Fig. 3). Also in the polluted soil, Al+ plants were characterized by a better growth of above-ground roots and plants than Al− plants (Fig. 1). Cuttings from Al+ cultures showed significantly higher LWR and lower RWR (Fig 3). It is noteworthy that the development of roots of Al+ cuttings was very good in the polluted soil. They were distinguished by greater root length, area, and number of root tips than Al− cuttings. Similar results were obtained in two years of experiments (Figs. 2 and 4).

Discussion

Plants vary in sensitivity to toxic compounds in the soil. Many studies showed that the sensitivity depends on many factors, such as physicochemical properties of the soil, concentration of organic matter and nutrients, but primarily on soil pH [19, 22, 29]. Aluminium is widespread in the Earth’s crust and its availability to plants increases with decreasing pH of the soil [7, 9, 10]. The biological activity of this element (its solubility and exchangeability) is related not only to the acidity of the soil solution but also to the presence of calcium or magnesium ions [12, 15, 31].

Poplar cuttings from in vitro cultures on media without aluminium (Al−) were characterized by poor growth of above-ground parts and roots in the polluted soil from Luboń (Figs. 1 and 2). That soil had a lower pH, a higher concentration of Al ions, and a lower Ca/Al ratio than the control soil (Table 2). The toxic Al ions present in the substrate can damage root cells, which results in limiting the transport of many nutrients and blocking their participation in important metabolic processes, such as photosynthesis and respiration [20, 27, 29]. Research on birch seedlings (Betula pendula Roth.) has shown that high concentrations of aluminium in the substrate decreased uptake of calcium, potassium, phosphorus, iron, and zinc, and consequently the growth of roots and shoots was limited [8, 18].

The majority of in vitro studies on plant tolerance to toxic ions (such as aluminium, copper, cadmium, and zinc), concerned herbaceous plants [11, 17, 30, 32]. Also cutt of Acer rubrum, from trees growing in a polluted area, was cultured in vitro and proved to be more tolerant to the activity of toxic metal ions (copper, nickel, cobalt) than cutt of trees growing in an unpolluted area [33]. As a result of repeated passaging of poplar and birch cultures to media containing aluminium, the produced plants were characterized by a better regeneration under stress conditions, i.e. in media with the addition of toxic ions of aluminium and copper [4, 5, 6]. Microcuttings, i.e. shoots of poplar and birch propagated in vitro on media with aluminium were also more tolerant to aluminium in the substrate during rooting than shoots cultured earlier without aluminium [4, 5].
Rooted cuttings from in vitro cultures with aluminium (Al+) in the present study were distinguished by a better development of roots and aboveground parts in vivo in a polluted soil from Luboń than control cuttings cultured without aluminium (Al–) (Figs. 1–4).

In the presented study, in vitro culture resulted in production of poplar cuttings characterized with a reduced sensitivity to the toxic aluminium ions, both in vitro and in a soil from a polluted area. Selection of plants tolerant to toxic ions contained in the soil can enable more effective management of degraded habitats.

Acknowledgements

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