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Krystyna Bojarczuk, Krystyna Szczygieł

Effect of aluminium and copper on the development of birch (*Betula pendula* Roth.) cultured *in vitro* and *in vivo*

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Abstract: Adventitious bud cultures were established by using buds of a selected birch clone (*Betula pendula* Roth.) resistant to industrial pollution. The Murashige and Skoog medium (1/2 and 1/4 MS) was used for multiplication and rooting of shoots. Aluminium was added to the medium, in the form of aluminium sulphate (50–100 mg Al dm^{-3}), and birch culture was continued *in vitro* for over 12 months. The shoots developed on media with aluminium (Al+) proved to be more tolerant to aluminium and copper (added to the medium as nitrates or sulphates, at a concentration of 0.05–2.0 mM) during multiplication and rooting than those developed on media without aluminium (Al–). Rooted birch microcuttings obtained from cultures on media with aluminium (Al+) grew better in the soil from an unpolluted area (Zwierzyniec, Z) and from an area polluted by a phosphate fertilise factory (Luboń, L) than those from media without aluminium (Al–).

Additional key words: soil pollution, toxic metals

Addresses: K. Bojarczuk, Polish Academy of Sciences, Institute of Dendrology, Parkowa 5, 62-035, Kórnik, Poland, e-mail: bojark@man.poznan.pl
K. Szczygieł, Forest Research Institute Department of Genetics and Physiology of Forest Trees, Bitwy Warszawskiej 1920 roku 3, 00-973, Warszawa, Poland, e-mail: k.szczygiel@ibles.waw.pl

Introduction

The emission of toxic pollutants into the environment has recently decreased in Poland and in the world. However, the toxic ions accumulated in the soil, such as copper, lead, zinc, cadmium or aluminium, will continue to inhibit plant development for many years in the habitats degraded by industry (Boudot et al. 1996; Mc Laughlin 1998).

Broadleaved trees are regarded as less sensitive to environmental pollution than conifers. However, recently some broadleaves, such as silver birch (*Betula pendula* Roth.), have been observed to decline around industrial plants, although this species is generally considered as relatively tolerant to pollution (Rachwał and Wit-Rzepka 1989; Boudot et al. 1994). The tolerance of plants to toxic metal ions is genetically controlled (Anioł and Gustafson 1990; Ernst et al. 1998). Scientific research showed that even trees of the same species but belonging to different populations may vary in sensitivity to industrial pollution (Reich et al. 1994; Oleksyn et al. 1996).

In vitro culture is used in research on plant reactions to toxic metal ions in the substrate (Arnold et al. 1994; Samantaray et al. 1999 b). Plant cultures from explants collected in polluted areas have proved to be more tolerant to metal ions in media than cultures of the same plant species growing in unpolluted areas (Samantaray et al. 1999 a). Plant culture *in vitro* has enabled selection of plants tolerant to ions of some metals, such as copper, manganese, nickel or aluminium (Van Sint Jan et al. 1997; Rout et al. 1998; Gori et al. 1998).

At the Institute of Dendrology, Polish Academy of Sciences, Kórnik, Poland, were conducted research on effects of the toxic aluminium and copper ions on development of birch shoots and roots. Rooted microcuttings produced *in vitro* were then transferred to a greenhouse, and planted in a polluted or a control substrate. This study aimed to improve our understanding of the mechanisms of plant sensitivity to toxic ions in the substrate, and to produce plants with an increased tolerance to high concentrations of toxic ions in the soil.

Material and methods

The selected birch clone K-03-144 (*Betula pendula* Roth.) was resistant to environmental pollution (Rachwał and Wit-Rzepka 1989). We collected its apical and lateral buds as explants from June till September. *In vitro* culture was performed on a modified 1/2 or 1/4 strength MS medium (Murashige and Skoog 1962) 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}). In some experiments, aluminium and copper were added to the medium as sulphates or nitrates, at a concentrations ranging from 0.05 to 3.00 mM, to test the tolerance of birch cultures to metal ions.

Birch culture *in vitro* was continued for over 12 months; passaging was repeated every 4–5 weeks, using new media of the same composition as mentioned above, with addition of aluminium sulphate $Al_2(SO_4)_3 \cdot 18\ H_2O$ (50–100 mg Al dm^{-3}). After this period, shoot fragments of ca. 2 cm in length were rooted in perlite saturated with a liquid medium, also with addition of aluminium sulphate. Medium pH was maintained at the level of 4.5 in all variants of experiments.

Birch cultures were kept in 300 ml jars (in each jar, 4 cultures of ca. 1 cm in diameter or 14 microcuttings) at 22–23°C in a culture room illuminated with mercury fluorescent lamps (7400mW/m^2) for 16 hours a day.

Birch cultures developed on media with aluminium (Al+) and without it (Al–) and on media with aluminium and copper were evaluated in respect of: number and length of shoots, number of leaves on the shoot, and culture quality. Culture quality was assessed as the level of chlorosis and browning on a scale of 0–5: 0 = no symptoms; 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%). On some media, birch shoot cultures formed root systems, whose development was estimated on a scale of 0–5: 0 = no roots; 1 = very poor development (1–20% roots in one jar); 2 = poor development (21–40%); 3 = moderate development (41–60%); 4 = strong development (61–80%); 5 = very strong development (81–100%). For birch microcuttings rooted in perlite, we assessed also the number and length of roots.

The number of shoots, shoot length, culture quality (chlorosis, browning), and level of root system development, were presented in figures and Table 1 as a mean value for 1 jar.

The *in vitro* experiments were established in randomised block designs with 4 replicates of 6 jars each.

In late May, birch cuttings cultured and rooted on a medium with Al (Al+) and without it (Al-) were transfered 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 Fertiliser Factory in Luboń (52°15'10"N and 16°50'31"E). Fertiliser production was started there in 1917 and since then the emission of toxic pollutants (mainly SO_2, NO_x, 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 (Karolewski et al. 2000).

Birch microcuttings were fertilized weekly with a multi-component fertilizer (pH 4.5). After 5 months of cultivation in the greenhouse, their biomass was assessed, i.e. fresh and dry weight of the whole plant, roots, shoot, and leaves. Moreover, concentrations of available forms of aluminium and calcium were measured in the soil (according to methods described by Bojarczuk et al. 2002).

In the greenhouse experiments, birch microcuttings were planted in 2 blocks with 3 replicates of 12 plants each. All results were analysed by means of Statistica 98 software. The significance of differences between combinations was assessed by the Tukey test, at the probability levels 0.01 and 0.05.

Results

The *in vitro* experiments on effects of aluminium and copper ions on birch development showed that copper ions are more toxic. Irrespective of their form (nitrate or sulphate, 0.5 mM), copper ions inhibited shoot and root development and lowered the quality of birch cultures, causing an increase in chlorosis and browning (Table 1, Fig. 2). Aluminium ions only at much higher concentrations (2–3 mM) inhibited birch development (Table 1 and Fig. 1). Very low concentrations of copper and aluminium did not inhibit shoot development, and 0.05 mM copper nitrate even stimulated root development (Table 1). As the concentration of the studied ions increased in the media, their toxic effects on culture development also increased (Table 1, Fig. 1).

Birch cultures were kept for over 12 months on media with aluminium (Al+). After transfer to the con-

Table 1. Effect of copper and aluminium on the development of birch (*Betula pendula* Roth.) cultured *in vitro*. Cu, Al, added to the medium as nitrates [Cu $(NO_3)_2 \cdot 3\ H_2O$ and $Al(NO_3)_3 \cdot 9\ H_2O$], concentration in mM. Values marked with the same letters are not significantly different from one another (P=0.05).

Treatment (mM)	Shoot No.	Shoot length (cm)	Degree of root development (scale 0–5)	Degree of chlorosis (scale 0–5)	Degree of browning (scale 0–5)
Control	7.5 d	1.8 bc	1.2 bc	1.6 a	2.3 ab
Cu 0.05	5.6 c	2.3 c	2.5 d	2.1 ab	2.0 a
Cu 0.25	3.5 b	2.5 c	2.2 d	2.7 b	2.8 b
Cu 0.5	1.5 a	0.5 a	0.0 a	4.8 d	5.0 d
Al 0.5	5.8 cd	1.8 bc	2.1 cd	2.7 b	3.2 b
Al 2.0	3.0 ab	1.4 b	1.2 bc	3.8 c	3.9 c
Al 3.0	1.8 ab	0.5 a	0.7 ab	4.2 cd	4.5 cd

trol medium (0 – without toxic ions of copper or aluminium), they were characterized by a faster development than cultures kept on a medium without aluminium (Al–) (Fig. 1 and 2). Al+ cultures on the control medium (0) formed longer shoots with a higher number of leaves and produced more roots, as compared with Al– cultures (Fig. 1). Also after transfer to a medium containing toxic aluminium or copper ions, plants deriving from Al+ cultures were characterized by more intensive development of shoots and roots, and a higher quality of cultures (lower chlorosis and browning) than plants from Al– cultures. Al+ plants showed a particularly strong tolerance to toxic ions when high concentrations of those ions were applied (especially 0.5 mM copper sulphate), which was reflected in a much higher culture quality, as compared with Al– cultures (Fig. 2).

Rooted birch microcuttings deriving from Al+ cultures were moved to a greenhouse and planted in a polluted (Luboń – L) and a control (Zwierzyniec – Z) soil. Despite the reduction of emission of toxic pollutants by the factory at Luboń, the soil from that area

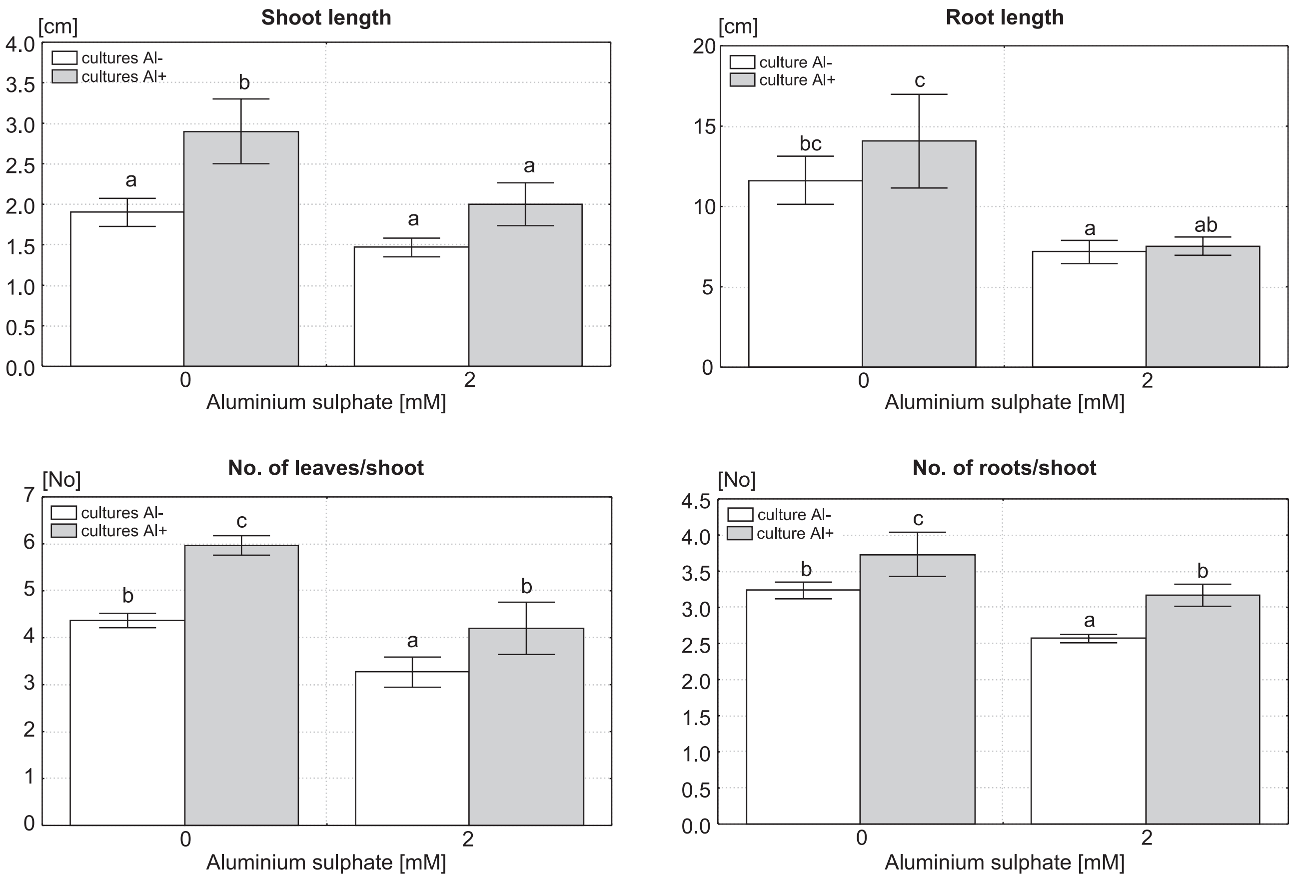

Fig. 1 Development of birch microcuttings obtained from cultures on media with aluminium (Al+) and without aluminium (Al–). The microcuttings were rooted in perlite saturated with a liquid medium (1/4 MS, pH 4.5) without aluminium (0) or containing aluminium sulphate [$Al_2(SO_4)_3 \cdot 18\ H_2O$] at a concentration of 2 mM

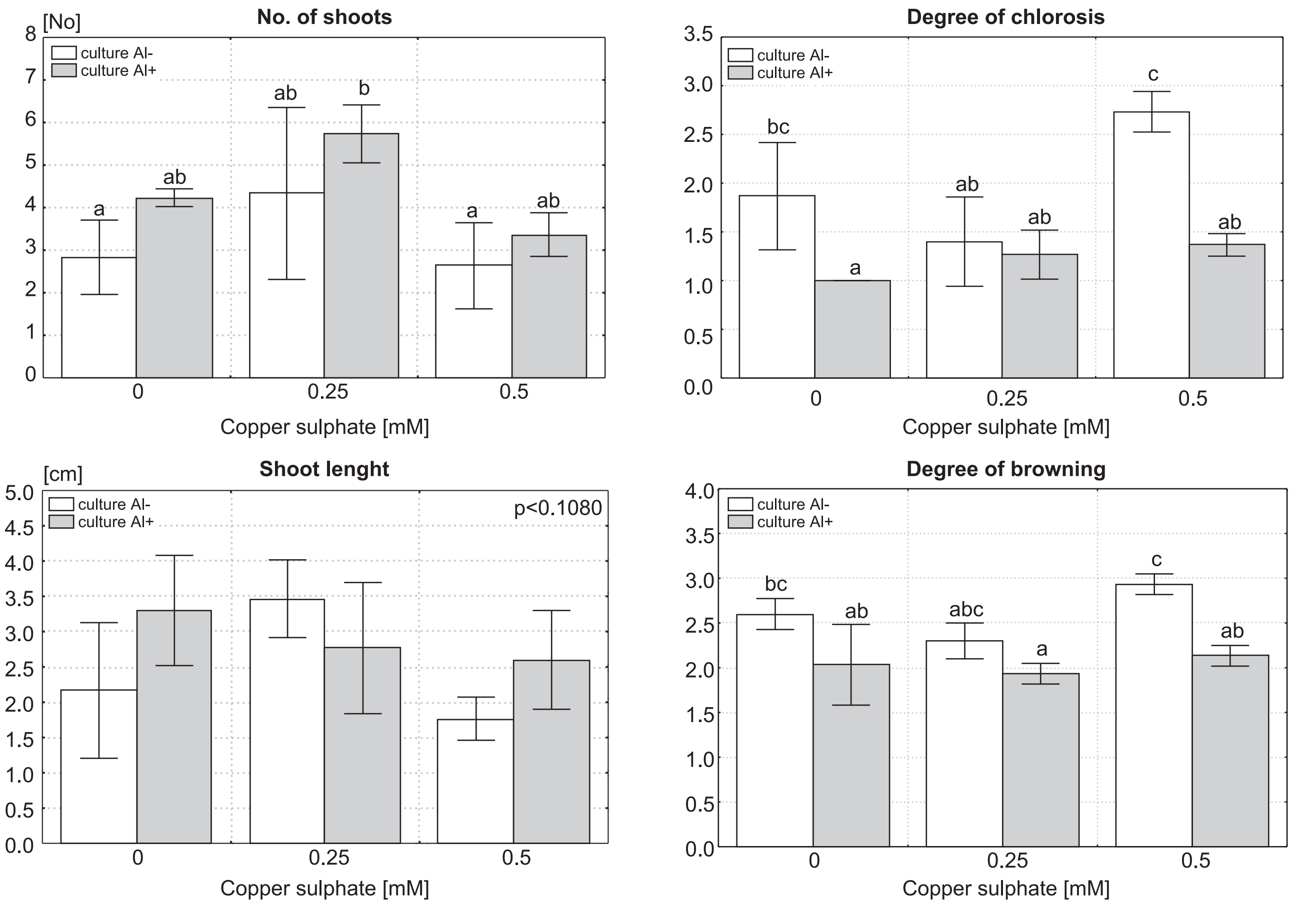

Fig. 2. Effect of copper on the development of birch cultures derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–). Concentration of copper sulphate [$CuSO_4 \cdot 5\ H_2O$]: 0 (control), 0.25 mM, and 0.5 mM

was characterized by a 2.4 times higher Al concentration, a lower pH and a lower Ca/Al ratio than the control soil (Table 2). Birch microcuttings from Al+ cultures grew much better than those from Al– cultures in both the polluted and the control soil (Fig. 3). Al+ microcuttings were distinguished by a strong growth of aboveground parts (dry weight of leaves and shoot) and developed larger root systems (dry weight of roots) than Al– plants. No differences in plant growth were observed between microcuttings from Al– cultures planted in polluted and unpolluted soil.

Table 2. Concentration of aluminium and calcium (soluble forms, mg·dm^{-3}) in control (Z) and polluted (L) substrates

Ca, Al and pH	Control substrate (Z)	Polluted substrate (L)
Ca	254.0	153.0
Al	38.2	92.7
Ca/Al	6.65	1.65
pH	5.1	4.5

Discussion

Experiments on effects of high concentrations of ions on regeneration of plants that are tolerant or sensitive to industrial pollution were earlier conducted *in vitro* by Wersuhn et al. (1994), El-Aref and Hamada (1998), Ramgareeb et al. (1999), and others. Callus of red maple (*Acer rubrum*) developed from explants collected from trees growing in a polluted habitat proved to be more tolerant to the activity of toxic metal ions (copper, nickel, cobalt) than callus obtained from trees from an unpolluted area (Watmough and Hutchinson 1997). Thus plant selection in natural conditions and *in vitro* may enable obtaining of plants tolerant to toxic ions present in a contaminated soil (Van Sint Jan et al. 1997; Gori et al. 1998, Barnabas et al. 2000).

In this study we used a birch clone selected in a polluted area and next cultured for over 12 months on media containing aluminium (Al+). The Al+ cultures were characterized by a better regeneration under stress conditions, i.e. on media containing a high concentration of Al ions (2 mM) than control cultures maintained on media without aluminium (Al–). Birch shoots from Al+ cultures formed more leaves and

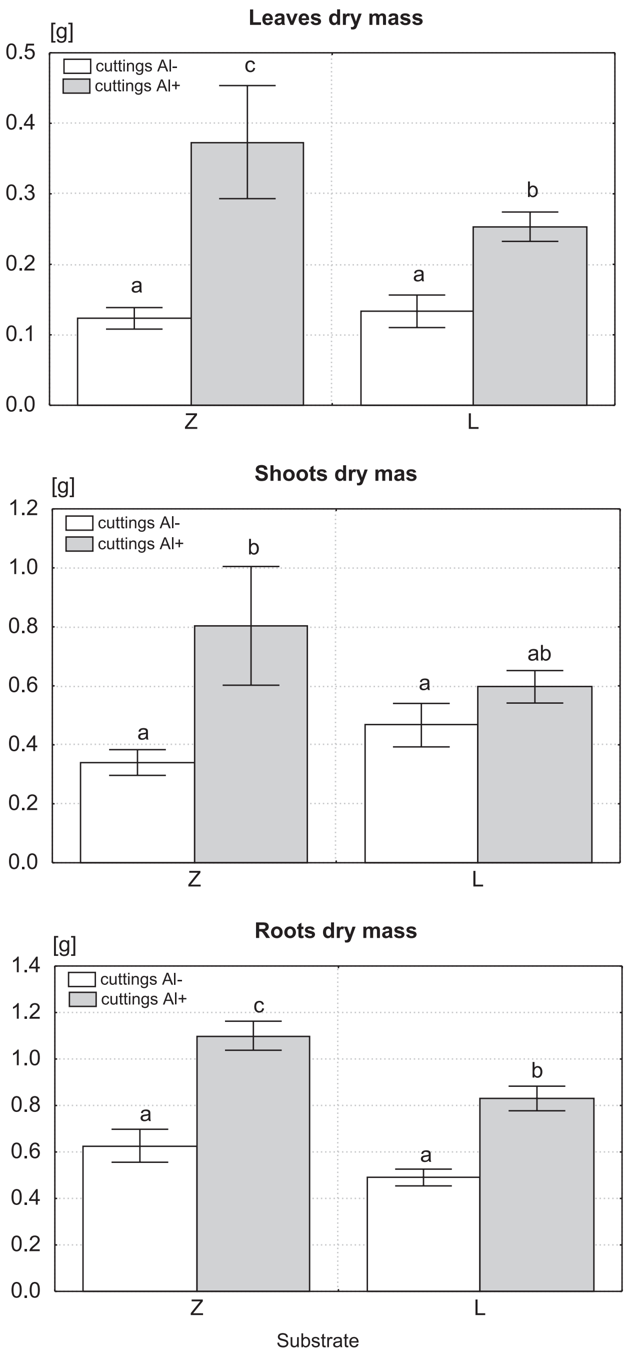

Fig. 3. Development of birch microcuttings derived from *in vitro* cultures on media with aluminium (Al+) and without aluminium (Al–) after planting in a control substrate (Z) or a polluted substrate (L)

roots than those from Al– cultures. A similar reaction to toxic ions present in media was observed also in poplar cultures *in vitro* and poplar microcuttings from media with aluminium (Bojarczuk 1999, 2000).

This study showed that birch cultures from media with aluminium (Al+) were tolerant not only to the toxic aluminium ions but also to high concentrations of copper ions (0.5 mM copper sulphate). Copper ions were more toxic to birch cultures than aluminium ions in this study. Chakravarty and Srivastava (1997) found that plants tolerant to zinc are characterized by very good growth *in vitro* in cultures on media containing not only zinc but also cadmium ions. Thus induction of tolerance to one toxic metal can result in reduced sensitivity to other metals.

Rooted birch microcuttings from *in vitro* cultures on media with aluminium (Al+), were characterized by a stronger development of leaves and roots after transfer to the soil – either polluted (L) or unpolluted (Z) – than seedlings from cultures on media without aluminium (Al–). No differences in plant growth were observed between microcuttings from Al– cultures grown on polluted and unpolluted soil. This was probably due to the fact that the explants for *in vitro* culture were obtained from a tree selected in a polluted area, so the tree was probably characterized by a high tolerance to toxic ions in the substrate. The microcuttings produced *in vitro* from these explants preserved the high tolerance to soil pollution, which could affect their growth on the polluted soil from Luboń (L).

Despite the reduced emission of pollutants into the air, the toxic aluminium ions present in the soil strongly limit the growth of plants, especially of their root systems (Göransson and Eldhuset 1995; Oleksyn et al. 1996; Bojarczuk et al. 2002). In this study, we produced microcuttings of birch – and earlier of poplar (Bojarczuk 2004) – that are characterized by an increased tolerance to aluminium contained in a polluted soil. Research on selection of plants with a higher tolerance to toxic ions in the soil can help to facilitate the management of habitats degraded by industry.

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