The Arbuscular Mycorrhizal Status of Poplar Clones Selected for Phytoremediation of Soils Contaminated with Heavy Metals

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The aim of this work was to study the colonization of indigenous arbuscular mycorrhizal fungi (AMF) species in fine-roots of poplar clones. Roots of 7 poplar clones were sampled from a 1-year-old trial established at an industrial site strongly polluted with heavy metals at Balatonfűzfő, Hungary. The poplar clones have shown variable degrees of colonization by AMF, suggesting differential host susceptibility or mycorrhizal dependency. After outplanting the percentage of poplar survival was strongly correlated with the frequency of AMF infection. Two clones that survived at the lowest ratio after outplanting had not been colonized by AMF in contrast to those which survived to a much higher extent.

Key words: Arbuscular Mycorrhizal Fungi, *Populus* ssp., Stress Tolerance

Introduction

Pollution of the biosphere with toxic metals and other chemicals due to anthropogenic activities poses a major environmental and human health problem (Kádár, 1995). High concentration of trace elements in soils may be toxic to soil microorganisms and inhibit their functioning, especially in the case of long-term loadings. Traditional physico-chemical methods of clean-up are often difficult, expensive and inefficient. Phytoremediation, an environment friendly technology, includes several methods (*e.g.* phytodegradation, phytoextraction, phytostabilization, rhizofiltration) which use plants for removal, transfer, stabilization and degradation of contaminants in soil, sediments and water. Plants species used for phytoremediation include large trees such as poplar species (*Populus* sp.), large annual or perennial herbs (*Brassica juncea*, *Helianthus annuus*) and grasses (Brooks, 1998; Chaudhry *et al.*, 1998).

Mycorrhizal fungi as symbiotic partners of most terrestrial plants form a direkt link through their hyphae between soil and roots, and therefore they can markedly influence the availability and toxicity of contaminants including heavy metals to host plants (Leyval *et al.*, 1997; Khan *et al.*, 2000). The most ancient type of mycorrhiza is the arbuscular mycorrhiza (AM) which belongs to the endomycorrhiza group. Arbuscular mycorrhizal fungi (AMF) are the most widespread mycorrhizal and soil fungi (Gerdemann and Nicolson, 1963), most angiosperms, some gymnosperms, pteridophytes and bryophytes can form symbiosis with AM fungi (Harley and Harley, 1987; Smith and Read, 1997). AMF should be regarded as a vital component of the terrestrial ecosystems. They can significantly enhance the uptake of water and nutrients by host plants and influence plant health but also the ability of plants to tolerate environmental stresses (drought-, salt-, toxic elements etc.) (Marschner, 1997; Vosatka, 2001; Takács and Vörös, 2003). Poplar roots are usually mycorrhizal and some species are able to form arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM) (Vozzo and Hacskaylo, 1974). AM predominate in early stages of poplar plant life. The aim of this work was to study the colonization of indigenous AMF species in fine-roots of poplar clones from a 1-year-old phytoremediation trial established at an industrial site highly polluted with heavy metals at Balatonfűzfő, Hungary and from a control site with unpolluted soil.

Materials and Methods

Plant and soil samples

Seven poplar clones were investigated; six of them: 'Agathe F', 'Aprólevelű', 'Koltai', 'Kornik-21', 'Pannónia', and 'Rábamenti' were developed from *Populus* × *euramericana* (*Populus nigra* × *Populus deltoides*), and the 'Durvakérgű' from *P. deltoides.* One-year-old plants of these 7 poplar clones, originating from the Nursery of Forest Research Institute at Bajt, Hungary were planted at an industrial site with polluted soil at Balatonfűzfő, close to the lake Balaton, in Hungary. Samples of mycorrhizosphere soil, roots and leaves of the clones were collected six months after transplantation.

Fresh biomass and dry matter of poplar leaves, as well as plant metal concentrations were determined. Leaves at the 4–6th leaf positions from the apex were collected and dry matters were determined after drying at 80 °C until constant weight. Soil and root samples were taken at a depth of 15–30 cm from the 1-year-old trial established at the polluted site at Balatonfűzfő and at the unpolluted soil at Bajt. The soil samples were taken randomly from 10 points of rhizosphere of poplars, in seven replicates.

Chemical analyses

Total metal contents of soils were determined from air-dried soil samples digested with concentrated HNO_3 at 80 °C in a microwave oven. Available soil metal-concentrations were determined in extracts obtained with a 0.5 M ammonium-acetate solution containing 0.02 M EDTA (Lakanen and Erviö, 1971). The plant metal concentrations were assessed after wet digestion of the air-dried plant samples in a 5:1:19 mixture of cc. HNO_3, cc. H_2O_2 and distilled water. Metal contents of plant and soil samples were measured by inductively-coupled plasma atomic emission spectrometry (ICP-AES).

Mycorrhizal parameters

Samples of 1–2 g fresh fine lateral roots of poplars were randomly taken from 1 cm segments and dispersed in water (in three replicates). They were cleared and stained with acid glycerol aniline blue according to Phillips and Hayman (1970). The frequency (F%) and the quantity of the arbuscules (a%) in the roots of the host were estimated by rating the density of infection on 30 cm root segments using the five class system (Trouvelot *et al.*, 1986).

Results and Discussion

The unpolluted control area at Bajt, Hungary is characterized by continental climate, the annual mean precipitation is 650 mm and the mean temperature is 9.8 °C. The soil type is Luvisol according to the FAO Soil Classification. The soil analyses showed that the ammonium-lactate (AL)-soluble P_2O_5 and K_2O contents were (Egner *et al.*, 1960) 28–108 and 51–99 mg kg^{-1}, respectively. The average level of the ground-water table is around 200–400 cm. The polluted industrial site at Balatonfűzfő, Hungary has an annual mean temperature of 10.2–10.5 °C and an average precipitation of 550–600 mm; the climate is continental, semiarid region. According to the soil analyses the AL-soluble P_2O_5 and K_2O contents were 14–161 and 97–483 mg kg^{-1}, respectively. The brown forest soil (FAO: Luvisol) was formed on red sandstone.

The total copper (Cu) and zinc (Zn) contents of the Balatonfűqzfő soil samples were 2-fold higher than those of the uncontaminated Bajt soil. The total mercury (Hg), cadmium (Cd), lead (Pb), manganese (Mn), and nickel (Ni) content of the polluted soil were several fold higher than in the unpolluted Bajt soil (Table I). However, the arsenic (As) and the barium (Ba) contents of polluted soil were lower than metal concentrations in uncontaminated soil. Total Cd, Hg and Pb contents of the polluted soil exceeded the permissible limits by several fold. The Cr, Cu, Mn, Ni, Se and Zn contents in the metal polluted soil samples were above the average metal content of Hungarian soils. Herbicides residues, especially chlorophenol and acetochlor were also detected close to the environmental limit in the polluted industrial soil (data not shown). Their distribution was heterogenous similarly to the heavy metal pollution. The Cd, Mn, Pb and Zn availability was high in the polluted soil and this resulted in reduced poplar biomass production.

The poplar clones showed variable degrees of colonization by arbuscular mycorrhizal fungi, suggesting differential host susceptibility or mycorrhizal dependency (Fig. 1). The infection frequency (F%) of indigenous AMF was universally high at both sites (Fig. 1A). In 1-year-old plantations, AMF colonization ranged from 0% to 63% at unpolluted soil and from 46% to 60% at contaminated soil. The microscopic observation of the stained root samples found no root colonization by AM fungi neither in the 'Rábamenti' nor in the 'Kornik-21' poplar clones in unpolluted soil. However functional mycorrhizal structures, arbuscules and vesicules were found in the roots of the other clones (Fig. 1).

Table I. Total and available concentrations of contaminating elements in samples of mycorrhizosphere soil. Mean values ± SD are shown (n = 7).

Elements	Polluted soil at Balatonfűzfő		Unpolluted soil at Bajt	
	Total	Available	Total	Available
	of contaminating metals [mg kg^{-1}]			
As	2.30 ± 0.43	*	6.15 ± 1.80	*
Ba	24.00 ± 2.89	34.3 ± 7.3	159 ± 37.8	38.7 ± 7.10
Cd	12.29 ± 1.96	0.15 ± 0.02	0.07 ± 0.01	0.05 ± 0.006
Cr	6.52 ± 0.88	0.30 ± 0.08	56.18 ± 11.30	0.18 ± 0.04
Cu	29.0 ± 5.16	3.40 ± 0.10	22.4 ± 6.33	4.63 ± 1.42
Hg	1.69 ± 0.18	*	*	*
Mn	11448 ± 2359	239 ± 125	794 ± 167	124 ± 58
Ni	562 ± 337	1.10 ± 0.14	39.8 ± 9.6	2.47 ± 0.49
Pb	889 ± 137	5.90 ± 1.70	20.1 ± 0.5	3.63 ± 0.52
Se	16.90 ± 4.57	*	*	*
Zn	191 ± 55	44.2 ± 32.3	82.3 ± 14.7	3.73 ± 0.49

* Concentrations are below detection limit (detection limits in mg kg^{-1}: As, 0.066; Hg, 0.048; Se, 0.092).

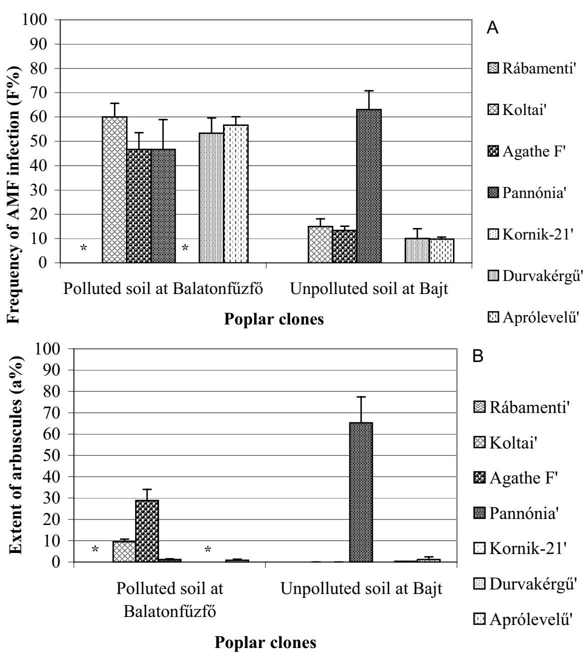

Fig. 1. Extent of the indigenous arbuscular mycorrhizal fungi infection (A) and arbuscularity (B) in roots of poplars.

(F% infection frequency of indigenous AMF; a% arbuscularity of poplar roots with colonized indigenous AMF; * poplar breed that did not survive the transplanting).

The frequency of infection (F%) in poplar roots was significantly higher (LSD = 0.05) at 'Koltai', 'Agathe F', 'Aprólevelű' *Populus* × *euramericana* and 'Durvakérgű' *Populus deltoides* clones at higher metal rates in the polluted soil (Fig. 1A).

However, arbuscularity (a%) (Fig. 1B) tended to be a more reliable indicator of the functioning and effectivity of AM fungi under varying environmental conditions than the frequency of infection (F%). The amount of arbuscules (a%) as indicator of symbiotic effectiveness of endomycorrhizal fungi was also slightly enhanced in the polluted soil. High arbuscularity was found in roots of 'Agathe F' and 'Koltai' clones at the polluted industrial site (Fig. 1B). However, arbuscules were found only in root samples of 'Pannónia' and 'Aprólevelű' poplar clones at Bajt. Probably the mycorrhizal dependency of poplar host increased after transplanting.

The occurence of the most common mycorrhizal types of selected poplar clones was investigated in a 5-year-old plantation by Khasa *et al.* (2002). The selected poplars showed variable degrees of colonization by both ectomycorrhizal and arbuscular mycorrhizal fungi. Variations in the frequency of infection have been observed in different cultivars or lines of single species. In this 5-year-old plantation, AMF colonizations ranged from 20% to 50% and it was lower than ectomycorrhizal colonization. However, Aguillon and Garbaye (1989) found that in 5-month-old poplars the percentage

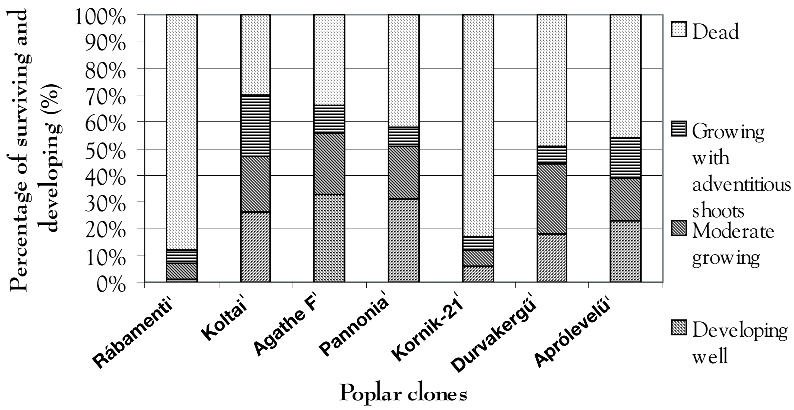

Fig. 2. Mortality rate (%) of poplar clones after planting.

of AMF root colonization was higher than ectomycorrhizal colonization, when *Glomus mosseae* (AMF) and *Paxillus involutus* (EMF) inoculations were applied. Mycorrhizal symbiosis is a dynamic process between fungi partners and changing root systems of the developing, growing host (Smith and Read, 1997). The ratio of ectomycorrhizal and arbuscular mycorrhizal colonizations changes during development of poplars, AM predominate in early stages of poplar plant life.

After transplantation the percent of poplar survival was in strong correlation with the frequency of AMF infection (Fig. 2). Two clones ('Rábamenti' and 'Kornik-21') that survived at the lowest ratio after outplanting had not been colonized by AMF in contrast to those that survived to a much higher extent (Fig. 2). We suggest that 'Rábamenti' and 'Kornik-21' clones are not or only slightly mycotrophic and due to the lack of AMF partner they were not able to tolerate the soil conditions at the polluted site.

Weak correlations have been found among AMF root colonization and biomass production or element content of poplar leaves. Dry matter accumulation of leaves at the unpolluted site (5.2 g ± 1.005 g/10 leaves) were significantly higher than at the polluted Balatonfűzfő soil (2.1 g ± 0.5 g/10 leaves).

After outplanting the poplar plants from unpolluted soil could not tolerate the higher level of heavy metal concentrations and acclimatization stress condition. No significant differences were detected between the foliar metal contents of poplar clones grown at the polluted and unpolluted sites (data not shown). Many papers reported the favourable effect of AMF on the uptake and transport of mineral nutrients, mainly the mobile phosphate forms of P to plants. In our study, we have found only a slight correlation between root colonization and the phosphorus content of leaves at Bajt, where at higher rates of AMF colonization higher P-concentrations were observed.

We conclude that soil microorganisms, especially AMF are able to increase stress resistance and survival of host plant leading to improved ecosystem stability. The development of stress-tolerant plant-mycorrhizal associations could be a promising new strategy for phytoremediation.

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