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Arbuscular mycorrhizal fungi differentially affect the response to high zinc concentrations of two registered poplar clones

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Inoculation with arbuscular mycorrhizal fungi can improve poplar tolerance to heavy metals in phytoremediation programmes.

Abstract

The effects of a high concentration of zinc on two registered clones of poplar (*Populus alba* Villafranca and *Populus nigra* Jean Pourtet), inoculated or not with two arbuscular mycorrhizal fungi (*Glomus mosseae* or *Glomus intraradices*) before transplanting them into polluted soil, were investigated, with special regard to the extent of root colonization by the fungi, plant growth, metal accumulation in the different plant organs, and leaf polyamine concentration. Zinc accumulation was lower in Jean Pourtet than in Villafranca poplars, and it was mainly translocated to the leaves; the metal inhibited mycorrhizal colonization, compromised plant growth, and, in Villafranca, altered the putrescine profile in the leaves. Most of these effects were reversed or reduced in plants pre-inoculated with *G. mosseae*. Results indicate that poplars are suitable for phytoremediation purposes, confirming that mycorrhizal fungi can be useful for phytoremediation, and underscore the importance of appropriate combinations of plant genotypes and fungal symbionts.

Keywords: Arbuscular mycorrhizae; Phytoremediation; Polyamines; Poplar; Zinc

1. Introduction

Phytoremediation is an emerging branch of science and technology, which uses plants for this purpose. It is of special interest for those pollutants, such as metals, that can be immobilized (phytostabilization) or taken up by the roots and transferred to above-ground organs of the plant (phytoextraction). It is an environment-friendly approach and has reduced costs when compared to traditional physical–chemical methods (Faison, 2004). In addition, only phytoremediation permits the restoration of the microbial soil community, an important aspect from an ecological point of view.

Abbreviations: AMF, arbuscular mycorrhizal fungi; DW, dry weight; *NoMet*, plants grown in soil unsupplemented with zinc; PA(s), polyamine(s); Put, putrescine; Spd, spermidine; Spm, spermine; TF_L, translocation factor from roots to leaves; TF_S, translocation factor from roots to shoots; *Zn*, plants grown in soil supplemented with zinc; *ZnGi*, plants inoculated with *G. intraradices* and grown in soil supplemented with zinc; *ZnGm*, plants inoculated with *G. mosseae* and grown in soil supplemented with zinc.

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Plants used for phytoremediation should be fast-growing, have a wide-spreading root system and large biomass, should be easy to propagate, and able to store large amounts of the metal of interest (Kramer and Chardonay, 2001). Although most true metal hyper-accumulators have been found among herbaceous plants (Macek et al., 2004), some trees, such as poplar and willow, possess several of the features mentioned above. Amongst these, the incomparably larger biomass make their use very favourable, in spite of a reduced efficiency of accumulation (Pulford and Watson, 2003). The use of poplars has been proposed for the phytoremediation of various pollutants, including hydrocarbons, herbicides, and metals (Di Baccio et al., 2003).

The natural ability of plants in the removal of contaminants can be integrated and improved by arbuscular mycorrhizal fungi (AMF), which are naturally present in the roots of most plant species where they form a mutualistic association (Smith and Read, 1997). Besides improving plant mineral nutrition and health, they are themselves involved in the uptake and detoxification of various pollutants, including heavy metals (Turnau et al., 2005). The mycobiont thereby enhances plant growth as well as tolerance towards biotic (Lingua et al., 2002; Berta et al., 2005) and abiotic stresses (Leyval et al., 1997; Volante et al., 2005). In some cases, increased tolerance towards heavy metals observed in mycorrhizal plants has been explained by reduced metal translocation to the above-ground organs of the plant (Schutzendubel and Polle, 2002). However, different fungal–plant associations can provide different responses, and, therefore, further information is required to understand whether mycorrhizal plants, and specific fungal taxa or strains, enhance the bio-accumulation or bio-stabilization of pollutants and through what mechanisms.

Zinc is an essential micronutrient for plants, but when present in excess it can produce noxious effects, similar to those due to toxic, non-essential elements, like cadmium or lead. Macroscopic effects concern plant growth (Rout and Das, 2003), especially root development, via profound alterations of mitotic activity and cell expansion (Eun et al., 2000; Liu et al., 2003) and via genotoxic damage (Borboa and de la Torre, 1996). The mechanisms of zinc toxicity are extremely diverse and they take place through various biochemical processes. Amongst these, there is the interaction with various functional groups of proteins, mainly with SH groups, resulting in the alteration of the reactive centre of many enzymes; in addition, the reduction of photosynthetic rate and chlorophyll content (Di Cagno et al., 1999), alterations in membrane permeability (Hernández and Cooke, 1997), oxidative damage (Briat and Lebrun, 1999), and increases in the cell polyamine pool (Sharma and Dietz, 2006) have been described.

Polyamines (PAs) are low molecular-weight aliphatic amines that are present in all living organisms. In higher plants, the main PAs, spermidine (Spd) and spermine (Spm) and their diamine precursor putrescine (Put), are essential for growth and development, by stabilizing nucleic acids and favouring transcription and translation (Bagni et al., 1993). PAs can be conjugated to hydroxycinnamic acid derivatives to produce hydroxycinnamoylamides (also known as soluble conjugated PAs), or to high molecular-mass compounds, such as cell wall components (insoluble conjugated PAs), which can be regarded as defense- or stress-related compound (Flores and Martin-Tanguy, 1991). Up-regulation of PA metabolism has been reported in response to several environmental stress conditions (Urano et al., 2003), including heavy metals in a variety of herbaceous plant species (Pirintsos et al., 2004; Scoccianti et al., 2006). In *in vitro*-grown microcuttings of *Populus alba* Villafranca, a concentration-dependent zinc-induced accumulation of free and conjugated Put and Spd has been reported (Franchin et al., 2007).

This study is part of a broader project on the selection of poplar clones suitable for phytoremediation purposes, and it complements field investigations on a contaminated site in which the main metal pollutants are copper and zinc (Lingua et al., 2005), as well as *in vitro* (Castiglione et al., 2007; Franchin et al., 2007) or other greenhouse (Todeschini et al., 2007) experiments carried out in parallel. In the present paper, the effect of treatments with a high concentration of zinc on two registered clones of poplar, inoculated or not with two AMF, *Glomus mosseae* or *Glomus intraradices*, was investigated. Special attention was paid to: (i) the extent of colonization by AMF; (ii) plant growth; (iii) metal accumulation in the different plant organs; and (iv) leaf PA concentration.

2. Materials and methods

2.1. Biological material

Two registered poplar clones, Villafranca (*P. alba* L.) and Jean Pourtet (*Populus nigra* L.), provided by the C.R.A. – Istituto di Sperimentazione per la Pioppicoltura (Casale Monferrato, AL, Italy) as 20-cm-long cuttings, were used for all the experiments. They were collected in February 2003 and stored at 4 °C until use.

Inocula of the two AMF, *Glomus mosseae* (Gerd. and Nicol.) Gerdemann and Trappe BEG 12, and *G. intraradices* (Schenck and Smith) BB-E, were supplied by Biorize (Dijon, France) on a sand carrier; they contained a minimum of 60,000 propagules kg^{-1}. Neither of the fungal species originated from contaminated soil.

2.2. Plant growth and fungal inoculation

The cuttings were removed from cold storage and placed overnight under running tap water. They were then put into 20-cm-high plastic pots (750 mL), containing a 3:1 mixture of heat sterilized (160 °C, 4 h) quartz sand: autoclaved soil (two 1-h treatments under flowing steam). Average sand particle diameter was 3–4 mm; soil came from an agricultural area (Tortona, AL, Italy), it was a sandy loam soil (according to USDA), and it had the following chemical features: organic matter = 2.24% DW; $N < 0.01\%$ DW; $K = 0.0237\%$ DW; $P = 0.0026\%$ DW; pH = 6.21; and $Zn = 47.5$ mg kg^{-1} DW. The chemical analyses (by Idrocons s.r.l., Rivalta Scrivia, Tortona, Italy) were carried out by ICP-OES, as described below (Section 2.6).

Eight cuttings of each clone were inoculated separately with one of the two AMF by adding 25% (v/v) inoculum to the substrate (pre-inoculated samples). Eight cuttings per clone were kept axenic (non-inoculated controls). The plants, irrigated three times a week with tap water, were maintained in a growth chamber (16-h photoperiod, $t = 24$ °C, 150 mE m^{-2} s^{-1} irradiance at pot height) for 40 days, at the end of which four pre-inoculated plants of each clone per treatment (see below) were harvested to evaluate the extent of colonization in newly formed adventitious roots.