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Research article

# Transcript analysis of stress defence genes in a white poplar clone inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and grown on a polluted soil

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ABSTRACT

In this study we investigated if the symbiosis with the arbuscular mycorrhizal fungus *Glomus mosseae*, which contributes to alleviate heavy metal stress in plants, may affect the transcription of genes involved in the stress defence in the white poplar clone 'AL35' grown on a multimetal (Cu and Zn) contaminated soil. The results obtained showed that the symbiosis with *G. mosseae* reduced transcript abundance of genes involved in antioxidant defence in leaves and roots of 'AL35' plants grown on the heavy metal-polluted soil. Moreover, the interaction between this poplar clone and the arbuscular mycorrhizal fungus induced the gene coding for phytochelatin synthase in leaves, whereas the expression of genes involved in heavy metal homeostasis did not change in roots. The present results suggest that, in presence of high levels of heavy metals, inoculation with *G. mosseae* may confer to 'AL35' a more efficient control of the oxidant level. Moreover, in mycorrhizal plants heavy metal chelation pathways appear involved in the defence strategies in leaves, whereas in roots they do not seem to contribute to increase the plant tolerance of heavy metals.



## 1. Introduction

Many anthropogenic activities, such as mining, combustion of fossil fuels, industrial waste, vehicle emission and use of pesticides and fertilizers in agriculture, may cause heavy metals (HM) contamination of soils and aquatic sediments. HM cannot be degraded by biological or chemical processes, thus their accumulation at high concentrations in soils represents one of the main problems for human and environmental health. Common strategies for metal extraction rely on soil treatment with physicochemical agents, that may be employed *in situ* or after excavation [1]. Besides being highly expensive, these remediation methods dramatically inhibit soil fertility and can be used only for point source contaminations. In recent years, phytoextraction has been proposed as one of the most promising strategies for soil clean up. In this approach plants capable of accumulating high levels of metals are grown in contaminated soil and, at maturity, metal-enriched above-ground biomass is harvested [2,3]. This technique is carried out *in situ*, minimizes costs and preserves the biological and physical structure of the soil [4]. Poplar (*Populus* spp.) has shown to be a good candidate for phytoextraction purposes, since it displays fast rate of growth, large biomass, wide-spreading root system and the ability to tolerate and accumulate HM in the aerial part [5,6]. Moreover, the remarkable clonal variability characterizing this plant genus allows selecting genotypes with traits more suitable for metal extraction [7].

Arbuscular mycorrhizal fungi (AMF), belonging to the phylum Glomeromycota, colonize the root parenchyma of most land plants, establishing a mutualistic symbiosis [8]. It is well known that they may improve HM tolerance of plants [9], although the causes of this phenomenon are not well understood [10]. Some authors suggest that this effect may be due to an increased nutrient supply conferred to the plant [11], but also other mechanisms have been proposed, such as metal precipitation and/or absorption by extra-radical soil mycelium [12] and metal chelation in fungal cells through the production of chelating molecules [13,14]. AMF may also influence the fate of HM in the plant, enhancing [15] or reducing [16], the concentration in plant tissues; moreover AMF may promote a higher HM accumulation in a specific organ of the plant [17]. An interesting application of these findings is the possibility to use AMF symbiosis to integrate and improve the

*Abbreviations:* AMF, arbuscular mycorrhizal fungi; APX, ascorbate peroxidase; CAT, catalase; DHAR, dehydroascorbate reductase; ECS, γ-glutamilcysteine synthase; GR, glutathione reductase; GSH, glutathione; HM, heavy metals; MT, metallothioneins; MTP, metal transport protein; PC, phytochelatins; PCS, phytochelatin synthase; ROS, reactive oxygen species; RT-qPCR, real-time quantitative PCR; SOD, superoxide dismutase.

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efficiency of HM extraction from polluted soils in phytoextraction processes [9,17]. Different AMF species may affect in a different manner the accumulation and HM up-take in the plant partner of the symbiosis [17]. For this reason, it is important to identify plant–fungal associations more suitable for phytoextraction, also in relation to the metal that has to be removed. This aim may be highly facilitated by improving the present knowledge on the mechanisms promoting HM tolerance in mycorrhizal plants compared to non-mycorrhizal ones.

Toxic concentration of HM in soil may be highly harmful for plant health. HM-tolerant plants protect themselves against homeostatic disturbance and cellular damages through the induction of a wide range of defence molecules [18,19]. Some of them are involved in the antioxidant system, whereas other ones carry out a fundamental role in the maintenance of HM homeostasis. It is well known that HM may cause a dramatic increase in the cellular level of reactive oxygen species (ROS), thus damaging many important cellular components, such as lipids, proteins, DNA and RNA. The control of cellular oxidant level is performed by the antioxidant system, which includes antioxidant enzymes as well as lower molecular weight secondary products with antioxidant activity. The main scavengers of oxygen radicals are superoxide dismutase (SOD), catalase (CAT) and the enzymes of the ascorbate–glutathione pathway, such as ascorbate peroxidase (APX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) [20,21]. Additionally, a network of high-affinity ligands may allow plants to maintain HM concentration in the cytosol under toxic levels, through HM chelation and compartmentalization in the vacuoles. Potential ligands include two classes of peptides, the phytochelatins (PC) and the metallothioneins (MT) [19,22]. Phytochelatins are also involved in HM transport to the vacuole that takes place through specific metal transporters (MTP) located at the level of vacuolar membranes [18]. The effect of mycorrhizal symbiosis on transcription of genes involved in antioxidant defence and in metal homeostasis has been so far poorly studied and needs to be elucidated.

In a previous study the poplar clone 'AL35' (*Populus alba*) was selected in a field trial for its high level of HM tolerance and accumulation [7]. Moreover, the symbiosis between 'AL35' and *Glomus mosseae* improved plant biomass and promoted a higher accumulation of Cu and Zn in plant organs, thus confirming that this plant–fungal association may be efficiently employed for phytoremediation purposes [23].

In this paper the expression of genes involved in antioxidant defence and in HM homeostasis was investigated in leaves and in roots of 'AL35' grown on the HM polluted soil with the aim to deepen the knowledge about the effect of *G. mosseae* symbiosis on plant defence against HM. For this purpose, we used cDNA macroarray approach and confirmed the results obtained by real-time quantitative PCR (RT-qPCR).

## 2. Results

### 2.1. Expression profile analysis of stress defence genes

In this study cDNA macroarray was performed to analyze the expression profiles of genes involved in antioxidant defence and in HM homeostasis in leaves and roots of 'AL35' plants, mycorrhized or not with *G. mosseae* and subjected or not to HM stress (Table 4; Table 5). Since transcripts for tubulin β (*Tub*) displayed a weak hybridization signal (data not shown), only those for ubiquitin (*Ubi*) were used in the calculation of relative gene expression. Some transcripts, such as those of *Cat3* in samples derived from leaves (Table 4) and those of *Cat2*, *Cat3*, *MT2a* and *MT3a* in samples derived from roots (Table 5) did not show a hybridization signal appreciably above the background level. In each organ the genes analyzed showed a wide range of expression levels (Table 4; Table 5). Two of most abundant signals in leaves corresponded to the SOD genes *CuZnSodchl* and *CuZnSodcytb* (Table 4), whereas in roots the highest RNA level was observed for *CuZnSodcytb* (Table 5). Comparing the expression levels of the genes in leaves and roots no major differences in the two organs, even with some exceptions, were observed (Table 4; Table 5). In fact, RNA abundance of *FeSod1*, *FeSod2*, *CuZnSodchl* and *Apxper* was at least six fold higher in leaves than in roots. On the contrary, RNA abundance of *Mtp* and *MnSod2* was at least nine fold higher in roots than in leaves (Table 4; Table 5).

For the validation of the macroarray data, transcript abundance of three genes involved in antioxidant defence (*Apxper*, *Dhar*, *CuZnSodcytb*) and two genes involved in HM homeostasis (*MT1a* and *Pcs*) was analyzed by RT-qPCR. For each gene, the log2 of the expression ratio obtained by real-time PCR was plotted versus the log2 of the ratio obtained with the macroarray using the same total RNA. The high determination coefficient ($R^2 = 0.94$) observed between the two data sets confirmed the validity of the macroarray analysis (Fig. 1).

Two-way ANOVA was performed to evaluate the effect of HM, mycorrhization and their interaction on the expression of antioxidant genes in roots and leaves (Table 6). Both factors had a remarkable impact on antioxidant genes expression in both organs, even if an evident tissue-specificity was observed. In leaves, HM affected significantly the expression level of all the genes analyzed, with the exception of *Cat1* (Table 6). On the contrary, in

**Table 1**
Biomass of the plant organs, copper and zinc concentration in the various organs and mycorrhizal colonization of the poplar roots in the four treatments. Data were previous published (as graphs or tables) in Cicatelli et al. (2010), since the biological materials used for the above mentioned paper and the present experiments were exactly the same.

| | Non-polluted | | Polluted | |
|---|---|---|---|---|
| | Control | Gm | Control | Gm |
| Root biomass (dry weight, g)[a] | 5.32 ± 2.98 | 3.13 ± 1.04 | 0.79 ± 0.08 | 4.88 ± 1.78 |
| Stem biomass (dry weight, g)[a] | 10.31 ± 4.47 | 12.69 ± 3,13 | 1.31 ± 0.12 | 8.24 ± 2.83 |
| Leaf biomass (dry weight, g)[a] | 3.88 ± 0.59 | 0.76 ± 0.22 | 0.50 ± 0.06 | 2.82 ± 0.15 |
| Cu root concentration (mg/kg dry weight) | 37.13 ± 3.20 | 14.21 ± 1.20 | 97.56 ± 8.70 | 605.47 ± 54.30 |
| Cu stem concentration (mg/kg dry weight)[b] | 8.45 ± 0.69 | 5.71 ± 0.50 | 19.07 ± 1.74 | 7.72 ± 0.60 |
| Cu leaf concentration (mg/kg dry weight) | 13.76 ± 1.31 | 12.08 ± 0.90 | 20.16 ± 1.79 | 31.86 ± 2.70 |
| Zn root concentration (mg/kg dry weight) | 92.24 ± 8.20 | 43.79 ± 3.80 | 98.50 ± 8.80 | 212.11 ± 19.10 |
| Zn stem concentration (mg/kg dry weight)[b] | 82.09 ± 7.28 | 82.99 ± 7.50 | 126.96 ± 11.28 | 63.76 ± 5.70 |
| Zn leaf concentration (mg/kg dry weight) | 285.50 ± 60.87 | 269.22 ± 24.20 | 387.12 ± 34.95 | 532.63 ± 47.80 |
| Mycorrhizal colonization (M%)[c] | 0.35 ± 0.11 | 4.65 ± 1.01 | 0.26 ± 0.09 | 11.36 ± 5.98 |

[a] Dry weight was calculated as follows: Fresh weight of the plant parts was measured, a sample was taken for each organ and its weight was measured, then it was dried. The ratio between the dry and fresh weight of the sample was used to calculate the total dry weight.
[b] Stem did not include the cutting used for producing the plants, therefore it consisted only of newly formed biomass.
[c] Mycorrhizal colonization was measured according to Trouvelot et al. (1986).