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Responses of *Populus* × *euramericana* (*P. deltoides* × *P. nigra*) clone *Adda* to increasing copper concentrations

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Abstract

We investigated the effect of high copper (Cu) concentrations on poplar woody cuttings (*Populus* × *euramericana*) clone *Adda* in order to estimate the degree of metal tolerance. Metal accumulation was also investigated to determine where and to what extent Cu is stored within the plant. Plant responses to Cu were determined comparing biomass growth and photosynthetic potential measured at concentrations of 0.4 (control), 20, 100, 500 and 1000 μM of Cu supplied with Hoagland's solution in an aerated hydroponic system. Results obtained in this study show that increasing levels of Cu resulted in a general reduction of plant growth at concentrations equal or higher than 100 μM. At these concentrations of Cu, reductions of chlorophyll contents and photosynthetic parameters were also observed. The metal was mainly accumulated in the root system at all Cu levels. Since no significant differences were noticed in growth and photosynthesis between control plants and those treated with 20 μM of Cu, we conclude that *Adda* clone was able to tolerate quite high concentration of Cu and we propose it as a good candidate for screening Cu tolerance in the field.

Keywords: Growth analysis; Photosynthesis; Poplar

1. Introduction

Copper (Cu) is an essential micronutrient for plant, acting as a cofactor of enzymes, structural proteins and phytohormone receptors. However, when present in elevated concentrations it inhibits plant growth and development (Weng et al., 2005; Xu et al., 2006). Contamination of soils and ground water with Cu mainly results from human activities such as smelting, disposal of sewage sludge and agricultural practices (Nriagu and Pacyna, 1988; Epstein and Bassein, 2001; Brun et al., 2003). In addition, low price fertilizers mainly deriving from intensive farming (e.g. pig slurry) are widely used for non-food plantations and notoriously contain high amount of Cu (Giardini, 2002).

Plants have evolved different strategies to cope with heavy metal stress, including detoxification and exclusion (Hall, 2002). Hyperaccumulators that naturally absorb high levels of metal ions in their shoots, possess multiple mechanisms of resistance, and have a significant phytoremediation potential (Salt et al., 1998). Excluder species that prevent metal uptake into the roots, even though having low potential for metal extraction, can be successfully used to stabilize the soil and avoid further contamination by leaching and erosion (Peer et al., 2005). Responses of plants to high levels of Cu have been mainly investigated in herbaceous and ruderal species (Reichmann, 2002; Brun et al., 2001, 2003; Sgherri et al., 2001; Demirevska-Kepova et al., 2004; Cuypers et al., 2005), although the use of trees in managing contaminated sites has been attracting an increasing interest (Pulford and Watson, 2003). Multipurpose tree plantations bring environmental benefits in the process of reclamation of polluted soils (Coleman et al., 1995), and among them fast-growing hybrid poplars have been indicated as good candidates for stabilizing metal-contaminated sites (Djingova et al., 1999; Robinson et al., 2000; Sebastiani et al., 2004; Peuke and Rennenberg, 2005).

In this sense, most research works deal with accumulation capacity and biomass production of poplars exposed to high concentrations of pollutants while the knowledge of physiological mechanisms of heavy metal accumulation and tolerance is

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still in its infancy (Arisi et al., 2000; Rennenberg and Will, 2000; Koprivova et al., 2002; Di Baccio et al., 2003, 2005; Tognetti et al., 2004). The success of using poplars for environmental restoration will depend on several factors, including the extent of soil contamination, metal availability and plant ability to intercept, absorb and accumulate metals in shoots (Sebastiani et al., 2004). Factors such as the extent of metal tolerance are highly variable among *Populus* species and clones, due to abundant genetic variation throughout the genus (Brunner et al., 2004). Comparative studies on metal accumulation and growth demonstrated that different clones respond differently to the exposure to the same industrial waste or contaminants (Sebastiani et al., 2004; Tognetti et al., 2004).

The necessity for large-scale experiments in open conditions testing the degree of metal resistance in poplar plantations, reinforce the need for preliminary screenings in controlled environments to examine physiological processes of tolerance in specific genotypes. In the present study we investigated the effect of increasing Cu concentrations on growth traits and photosynthetic parameters in *Populus* × *euramericana* (Dode) Guinier (*Populus deltoides* × *P. nigra*), clone *Adda* commercialized in Northern Italy (Fossati et al., 2005) in order to evaluate the tolerance to the metal. Uptake of metal was also examined to determine where and to what extent Cu is stored within the plant. Our results indicate that the *Adda* clone was resistant to quite high Cu concentrations; hence we propose this clone for experimental plantations to evaluate its potential use in environmental restoration of metal-contaminated sites.

2. Materials and methods

2.1. Plant material and treatments

Poplar woody cuttings (*P.* × *euramericana*, *Adda* clone) were rooted and grown in pots containing a universal loam:perlite mixture (1:1, v:v), and daily irrigated with deionised water for about 30 days. Plants were successively transferred in a growth chamber (temperature 23 °C; relative humidity 55–75% night–day; photoperiod 16-h light:8-h dark) and grown in pots filled with clay (diameter 13–15 mm, pH 7.0) suitable for hydroponic culture, and supplied with Hoagland's solution, pH 6.7 (Arnon and Hoagland, 1940). To prevent the death of roots the solution was completely replaced every 7 days and aerated by diffused air aeration systems. After 15 days of acclimation to the hydroponic system, 40 homogeneous plants were selected and randomly assigned to five groups of treatment with Hoagland's solution containing different concentrations of $CuSO_4{\cdot}5H_2O$: 0.4 (control), 20, 100, 500 and 1000 μM. Four plants randomly selected at the beginning of the experiment were used to determine the initial biomass (T_0).

2.2. Growth analysis

Fifteen (T_1) and 34 (T_2) days from the beginning of the experiment four plants from each of five groups of treatment were randomly selected and separated into leaves, petioles, stem, roots and woody cutting, weighted and oven-dried at 70 °C until constant weight. Samples of root were extensively rinsed with deionised water soon after harvesting. Stem length (SL) and basal diameter (SD) were measured weekly on each plant. Leaf area (LA) was determined by the regression equation $y = 0.7121x$ ($R^2 = 0.99$) forced through the origin, where (y) is leaf area and (x) the product of the two leaf main orthogonal diameters. The equation was previously determined by measuring the surface area of 215 randomly selected leaves and analysed using the software Scion Image 4.0.2. (Scion Corporation, MD, USA). Specific leaf area (SLA) was calculated as the ratio of leaf area to corresponding leaf dry mass. Relative growth rate (RGR) was calculated as $\mathrm{RGR} = [\log_e \mathrm{MT} - \log_e \mathrm{MT}_0]/T_x - T_0]$ where MT is the total dry mass at sampling time T_x (T_1 and T_2) and MT_0 is the initial (T_0) total plant dry mass average (Hunt, 1978).

2.3. Copper and nitrogen determinations

Samples of leaves, stem and roots were separately oven-dried at 70 °C and ground to a powder in a laboratory mill. 0.5 g of plant material was mineralised in HCl–HNO_3 (dilution factor 3:1), clarified with ultra pure water and used for measurements of total Cu in an atomic absorption spectrophotometer (Atomic Absorption Spectrometer 373, Perkin-Elmer, Norwalk, CT, USA).

Organic nitrogen (N) content was determined in leaves by using the Kijeldhal method.

2.4. Gas exchange and chlorophyll fluorescence measurements

The CO_2 response curves (A/C_i) were measured on the fourth and fifth leaf from the apex, using an infrared gas analyzer LI-6400 (LI-Cor, Lincoln, NE, USA). Leaf chamber temperature and humidity were adjusted to maintain a leaf-to-air vapour pressure difference of about 1.3 kPa. The CO_2 response curves were obtained by changing the CO_2 concentration entering the cuvette from 50 to 800 μmol mol^{-1} by means of an external CO_2 cartridge mounted on the LI-6400 console and automatically controlled by a CO_2 injector. Light intensity was maintained at 600 μmol photons m^{-2} s^{-1}. The response of leaf A to C_i was analysed according to the mechanistic model of CO_2 assimilation proposed by Farquhar (Farquhar et al., 1980). The biochemical model describing A calculates three parameters potentially limiting photosynthesis: $V_{c_{max}}$ (maximum carboxylation rate of ribulose-1,5-bisphosphate carboxylase–oxygenase, Rubisco), J_{max} (ribulose-1,5-bisphosphate, RuBP, regeneration capacity mediated by electron transport rate) and TPU (rate of triose phosphate utilization for sucrose and starch synthesis). Day respiration rate is designated R_{day} and results from processes other than photorespiration. The CO_2 compensation point (Γ_c) was calculated as the point where the model function crosses the x-axis ($A = 0$). The model was parameterised and fitted to the experimental data using Photosyn Assistant (Dundee Scientific, Dundee, Scotland, UK).

Contemporary to gas exchange determinations, chlorophyll fluorescence emissions in 30-min dark-adapted leaves were measured with a portable pulse amplitude modulation fluorome-