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Early responses to cadmium of two poplar clones that differ in stress tolerance

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ABSTRACT

Soil cadmium (Cd) contamination is becoming a matter of great global concern. The identification of plants differentially sensitive to Cd excess is of interest for the selection of genotype adaptive to grow and develop in polluted areas and capable of ameliorating or reducing the negative environmental effects of this toxic metal. The two poplar clones I-214 (*Populus* × *canadensis*) and Eridano (*Populus deltoides* × *maximowiczii*) are, respectively, tolerant and sensitive to ozone (O_3) exposure. Because stress tolerance is mediated by an array of overlapping defence mechanisms, we tested the hypothesis that these two clones differently sensitive to O_3 stress factor also exhibit different tolerance to Cd. With this purpose, an outdoor pot experiment was designed to study the responses of I-214 and Eridano to the distribution of different Cd solutions enriched with $CdCl_2$ (0, 50 and 150 μM) for 35 days. Changes in leaf area, biomass allocation and Cd uptake, photosynthesis, chlorophyll fluorescence, leaf concentration of nutrients and pigments, hydrogen peroxide (H_2O_2) and nitric oxide (NO) production and thiol compounds were investigated. The two poplar clones showed similar sensitivity to excess Cd in terms of biomass production, photosynthesis activity and Cd accumulation, though physiological and biochemical traits revealed different defence strategies. In particular, Eridano maintained in any Cd treatment the number of its constitutively wider blade leaves, while the number of I-214 leaves (with lower size) was reduced. H_2O_2 increased 4.5- and 13-fold in I-214 leaves after the lowest (L) and highest (H) Cd treatments, respectively, revealing the induction of oxidative burst. NO, constitutively higher in I-214 than Eridano, progressively increased in both clones with the enhancement of Cd concentration in the substrate. I-214 showed a more elevated antioxidative capacity (GSH/GSSG) and higher photochemical efficiency of PSII (F_v/F_m) and de-epoxidation degree of xantophylls-cycle (DEPS). The glutathione pool was not affected by Cd treatment in both clones, while non-protein thiols and phytochelatins were reduced at L Cd treatment in I-214. Overall, these two clones presented high adaptability to Cd stress and are both suitable to develop and growth in environments contaminated with this metal, thus being promising for their potential use in phytoremediation programmes.

Abbreviations: A, antheraxanthin; A_{max}, photosynthetic rate measured at light saturation; C_a, ambient CO_2 concentration; C_i, intercellular CO_2 concentration; DEPS, de-epoxidation index; F_o, minimum chlorophyll fluorescence yield in 'dark-adapted' leaves; F_m, maximum chlorophyll fluorescence yield in 'dark-adapted' leaves; F_v/F_m, ratio of variable and maximal fluorescence; g_s, stomatal conductance to H_2O; ΦPSII, quantum efficiency of PSII photochemistry; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; LAR, leaf area ratio; LMA, leaf mean area; LMR, leaf mass ratio; LN, leaf number; MLA, mean leaf area; NO, nitric oxide; NPQ, non-photochemical quenching; NPTs, non-protein thiols; O_3, ozone; PCs, phytochelatins; qP, photochemical quenching; ROS, reactive oxygen species; SLA, specific leaf area; SMR, stem mass ratio; V, violaxanthin; Z, zeaxanthin.

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Introduction

Soil pollution with heavy metals and their accumulation in biotic systems as a consequence of human activities are becoming matters of growing global concern. Together with lead, mercury, chromium, nickel, zinc, arsenic and selenium, cadmium (Cd) represents a real threat for human health and the environment (Lado et al., 2008). Of all non-essential heavy metals, Cd has attracted most attention in soil science and plant nutrition due to the mobility in the soil–plant system and high toxicity to human health (McLaughlin and Singh, 1999). Cadmium is a trace metal naturally present in the environment, occurring as a minor constituent of phosphate rock ore, and it is released into the environment by many industrial activities, waste incinerators, urban traffic and as by-product of phosphate fertilizers (Sanità di Toppi and Gabbrielli, 1999). Cadmium accumulation in soil can result in a decrease of microbial activity, biodiversity and soil fertility with consequent damages on growth and development of crops up to animal and human health through the food chain (Sanità di Toppi and Gabbrielli, 1999; DalCorso et al., 2008; Baize et al., 2009).

Excess Cd in plants causes leaf roll and chlorosis, growth inhibition both in shoots and roots, early senescence and eventually plant death (Sanità di Toppi and Gabbrielli, 1999; Benavides et al., 2005). These effects are due to direct or indirect interferences with a series of physiological, biochemical and molecular processes, including photosynthesis, transpiration, water and nutrients balance, enzymatic activities, functions of transporter, antioxidants and several biomolecules, gene expression and regulation (Benavides et al., 2005; DalCorso et al., 2008). Despite the numerous studies on plant responses to Cd in the last decades, the mechanisms of Cd toxicity and, consequently, the plant capacity to tolerate Cd excess are not completely understood yet. However, we know that heavy metals and Cd accumulation in plants depends on physical–chemical characteristics of soils (pH, organic matter and water contents, cation exchange capacity) and biotic factors such as plant species, root activity and rizosphere-associated microrganisms (McLaughlin and Singh, 1999; Greger and Landberg, 2008; Baize et al., 2009). In particular, many plant species or cultivars differ in their Cd sensitivity and uptake capacity (Hart et al., 2006; Baize et al., 2009; Polle et al., 2013). The identification of properties (physiological processes, biochemical patterns and morphological traits) that make plants more or less sensitive to Cd excess in the environment is still an ongoing objective for the selection of genotype adaptive to grow and develop in polluted areas and/or capable to ameliorate or reduce the negative effects of this pollutant (Sebastiani et al., 2004, 2014; Greger and Landberg, 2008; Durand et al., 2010; Castagna et al., 2013; Cocozza et al., 2014).

As sessile organisms, plants withstand a plethora of stress conditions during their life cycle in the natural and cultivated environment, including abiotic and biotic stresses; for this reason they have developed unique strategies for responding to environmental changes, monitoring their surroundings and adjusting their metabolic systems to maintain homeostasis (Pastori and Foyer, 2002). Many plant species are naturally less sensitive to some stresses in comparison with other plants, and this behaviour is one of the most relevant factors that drive the distribution and survival of vegetation worldwide. For example, in *Sedum alfredii* species Cd hyperaccumulator and non-hyperaccumulator ecotypes have been characterized (Sun et al., 2013), and it is well known that *Silene vulgaris* species includes Cd-sensitive and Cd-tolerant ecotypes. Two poplar clones with well known different sensitivity to ozone (O_3) stress (Diara et al., 2005; Rizzo et al., 2007) evidenced different responses to industrial heavy metal-enriched organic waste pollution (Sebastiani et al., 2004; Tognetti et al., 2004) and, in particular, to elevated Zn levels (Fernàndez et al., 2012), or to the combination of high Cd and O_3 concentrations (Castagna et al., 2013). Several authors support the thesis that different plant species make use of common pathways and components in the stress-response relationship, showing that plants that can tolerate a particular stress factor also exhibit co-tolerance to other stresses (Pastori and Foyer, 2002; Singh et al., 2010). Polle et al. (2013), for example, tested the tolerance capacity of *Populus euphratica* (salt tolerant species) and *Populus* × *canescens* (salt sensitive species) to Cd stress. *P. euphratica* was more sensitive to Cd than *P.* × *canescens*, but the former accumulated 10-times more Cd in the leaves than the latter.

Previous studies revealed that the two poplar clones I-214 (*Populus* × *canadensis*) and Eridano (*Populus deltoides* × *maximowiczii*) are differentially sensitive to O_3 stress, both under acute and chronic exposure (Diara et al., 2005; Di Baccio et al., 2008). Indeed, to our knowledge no study has ever tested how I-214 and Eridano poplar clones respond to high level of the single Cd stress factor in the substrate.

The aim of the work was to verify if the two hybrid poplars I-214 and Eridano have different sensitivity to Cd pollution; with this purpose, woody cuttings of both clones were rooted and young plants grown in a substrate contaminated with different Cd levels in an outdoor pot experiment. In addition to Cd uptake and distribution in leaves, stems and roots, growth and gas exchange parameters, chlorophyll fluorescence, pigment and the main cation and anion concentrations, H_2O_2 and nitric oxide (NO) production and the concentration of thiols in leaves were investigated. Moreover, the performance of the two poplar clones under Cd stress during early and more sensitive phase of life cycle (rooting and first weeks after leaf full expansion) is of great importance to evaluate the tolerance and the potential of fast growing tree species for their use in soil reclamation.

Materials and methods

Plant materials and Cd treatments

Woody cuttings of two poplar clones, I-214 (*Populus* × *canadensis*) and Eridano (*Populus deltoides* × *maximowiczii*), were planted in 4 L-plastic pots filled with a sandy soil:peat substrate mix (2:1, v:v) with pH (H_2O) 5.8. To simulate field conditions, the pot experiment was conducted outdoors under a waterproof roofing with a shade net (mean daily photosynthetic photon flux density of 500–600 μmol photons m^{-2} s^{-1}) at the experimental station of the University of Pisa (43°43′ N, 10°23′ E). After 2–3 days of acclimation, twenty four uniform cuttings (12 for each clone) were selected for the experiments and irrigated with half-strength Hoagland nutrient solution (pH 6.5) added with 0, 50 and 150 μM Cd concentrations in order to reach the following final total Cd amounts in the pot substrate, respectively: 0 mg Cd kg^{-1} soil DM or ppm (control), 54.3 ppm (the lowest Cd concentration, L Cd) and 163.0 ppm (the highest Cd concentration, H Cd). Cadmium was supplied as $CdCl_2$ totally dissolved in the nutrient solution and distributed every week in each pot, never exceeding the field capacity; the plants were grown in these conditions for 35 days. Each plant possessed 1–5 shoots (mean value: 2.2), which were maintained until the end of the experiment. During the experiment no growth restriction due to limited pot volume was observed. Meteorological data for the experimental period (March–May) were as follows: average air temperature, 13.2 ± 4.0 °C; relative humidity, 71 ± 10%.

Sample collection and growth analysis

After 35 days of Cd treatment, poplar plants (n = 4) of I-214 and Eridano from each Cd treatment were harvested, the roots washed free of growth substrate particles and all the organs (leaves,