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Aluminum stress induces up-regulation of an efficient antioxidant system in the Al-tolerant maize line but not in the Al-sensitive line

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ABSTRACT

The response of the antioxidant enzymes, superoxide dismutase (SOD; EC 1.15.1.1) and peroxidase (POD; EC 1.11.1.7), to Aluminum (Al) stress was studied in roots of two inbred lines of maize (*Zea mays* L.) differing in their tolerance to Al. In addition, the production of malondialdehyde (MDA) was measured to evaluate the level of lipid peroxidation as well as the accumulation of proline (Pro) and carbohydrates under 72 h Al stress. Roots of Al (0, 120, 240, 360 and 480 μM, at pH 4.2) -treated plants were sampled at various times (12, 24, 48, 72 h) after commencement of stress. A major difference in the antioxidant enzymes between the two maize lines associated with Al tolerance was observed after 24 h of Al exposure. A gradual increase in the membrane lipid peroxidation in Al-stressed root of the susceptible maize line was accompanied by decreased activities of the antioxidant enzymes SOD and POD. In contrast, increased activities of the SOD and POD were found in Al-treated roots of the tolerant maize line, in which the level of membrane lipid peroxidation remained almost unchanged. After 72 h exposure to 480 μM Al the accumulation of Al in roots was almost from 90 times (tolerant) to 140 times (sensitive) than the control (without Al), while at the same time Al treatment resulted in 2.2 to 2.5-fold (at 240, 360 and 480 μM Al) increased Pro content in the roots of the tolerant line compared to 0 μM Al. Yet, 72 h exposure to 480 μM Al increased 1.7-fold the carbohydrate concentration in the roots of the Al tolerant maize line VA-22 while in the sensitive line $A_{4/67}$ remained almost unchanged. These data provide evidence of an internal mechanism of tolerance that increase the antioxidant system activity in order to limit cellular damages and possibly linked to the Al tolerance of the maize line VA-22. Analyses of the 12, 24, 48, and 72 h POD and SOD isoforms showed that in the Al-tolerant maize plants the anionic POD isoforms A_1, A_3 and A_4 and the SOD isoforms SOD_1 and SOD_2 were induced by increased Al-stress. It seemed that in the Al tolerant maize line, the anionic POD isoforms A_1, A_3 and A_4 and the SOD isoforms SOD_1 and SOD_2 were required for adaptation as the oxidant level increased by the increased Al stress. Our results suggest that Al toxicity may be mediated by oxidative stress and that the better protection of the Al tolerant maize roots from Al-induced oxidative damage results, at least partially, from the increased activity of their antioxidative system.

1. Introduction

Aluminum (Al^{3+}) toxicity limits plant productivity on acid soils and micromolar concentrations in the soil solution can rapidly inhibit root elongation and subsequently the uptake of water and nutrients, resulting in significant reduction of crop production on acid soils, which comprises 30–40% of the world's arable soils (von Uexküll and Mutert, 1995). Inhibition of root elongation is one of the most distinct and earliest symptoms of Al toxicity, which occurs within hours or even minutes of exposure to Al^{3+} (Kochian et al., 2005). In plants growing in fields with subsoil acidity, the capacity to explore the soil for nutrients and water is substantially restricted. As a result, plants may suffer from severe water stress after only a few days without rainfall (von Uexküll and Mutert, 1995). The reduced volume of the root system may not be the only reason for Al-induced water deficiency in plants (Barceló and Poschenrieder, 1990). In addition to inhibition of root elongation and cell division (Doncheva et al., 2005), plants suffered from Al toxicity also display symptoms such as formation of barrel-shaped cells (Gunsé et al., 1997) and swelling of root apex (Čiamporová, 2002). The primary mechanism underlying the Al^{3+}-induced inhibition of root growth remains to be deciphered (Barceló and Poschenrieder, 2002; Jones et al., 2006; Sun et al., 2007).

Abbreviations: Al, aluminum; GB, glycine betaine; ICP-AES, inductively coupled plasma atomic emission spectrometry; MDA, malondialdehyde; PCD, programmed cell death; Pro, proline; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloracetic acid.

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Some plant species have developed mechanisms to cope with Al toxicity both internally and externally (Kochian et al., 2005), which helps them to grow on acid soils. Understanding the nature of these tolerance mechanisms has been the focus of ongoing research in the area of stress physiology (Poschenrieder et al., 2008).

Several reports have shown that Al stress can increase the production of reactive oxygen species (ROS), and activate several antioxidative enzymes in plant cells (Cakmak and Horst, 1991; Ezaki et al., 1996, 2000; Richards et al., 1998; Yamamoto et al., 2002; Tamás et al., 2003, 2004, 2005, 2006; Meriga et al., 2004; Simonovicová et al., 2004; Jones et al., 2006; Corrales et al., 2008). Pan et al. (2001), Boscolo et al. (2003), Simonovicová et al. (2004) and Tamás et al. (2005), suggested that Al stress might induce cell death in plants through ROS-activated programmed cell death (PCD).

Oxidative stress is described as an unbalance between ROS, [represented predominantly by superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (–OH), and singlet oxygen ($^1O_2$)] and antioxidants in biological systems, and may be triggered by an enhanced formation of ROS and/or by a reduction in the antioxidant defenses (Munné-Bosch et al., 2001). In non-stressing conditions, the antioxidative defense system of cells provides adequate protection against ROS by the concerted action of both enzymic and non-enzymic antioxidants (Asada, 1999).

Plants have defensive mechanisms and utilize several biochemical strategies to avoid damage caused by ROS (Mittler et al., 2004; Foyer and Noctor, 2005). Plant enzymatic defences include antioxidant enzymes such as peroxidases (POD; EC 1.11.1.7) and superoxide dismutases (SOD; EC 1.15.1.1), which, together with other enzymes, promote the scavenging of ROS (Alscher et al., 2002; Veljovic-Jovanovic et al., 2006). SOD catalyses the dismutation of O_2^- to H_2O_2 and molecular oxygen. POD is widely distributed in all higher plants and protects cells against the destructive influence of H_2O_2 by catalysing its decomposition through oxidation of phenolic and endiolic co-substrates. The biochemical defense system also includes the amino acid proline (Pro), an osmolyte and cellular protector largely accumulated in several plant species in response to abiotic stress, which might act as a ROS scavenger (Sharma and Dietz, 2006; Ashraf and Foolad, 2007).

Accumulation of proline and carbohydrates in response to environmental stresses including Al stress, water stress, salinity and metal toxicity has been reported by various workers (Hare and Cress, 1997; Hare et al., 1999; Girija et al., 2002; Sharma and Dietz, 2006; Ashraf and Foolad, 2007; Ali et al., 2008; Giannakoula et al., 2008; Silveira et al., 2009). Proline accumulation is regarded as a component of stress tolerance mechanism (Hare et al., 1999; Sharma and Dietz, 2006). Accumulation of Pro in plant tissue under stressful conditions has been suggested to be the result of a decrease in Pro degradation, increase in proline biosynthesis, a decrease in protein synthesis or Pro utilization and increased hydrolysis of proteins (Hare et al., 1999).

Aluminum (Al) stress, like other abiotic and biotic stresses in plants, may disturb the redox homeostasis and may even lead to oxidative stress. Peroxidase (POD) and superoxide dismutase (SOD) constitute the first line of defence against reactive oxygen species (ROS), and changes in their activity and amounts have been identified as an indicator of a redox status change under Al stress (Jan et al., 2001; Tamás et al., 2003; Meriga et al., 2004; Simonovicová et al., 2004; Ali et al., 2008). Investigation of this adaptation mechanism to Al stress may give new insights into the process of Al stress in plants or even help improve aluminum tolerance in crops. Over the years it has also become clear that free-radical scavenging systems are important components in the mechanisms of aluminum tolerance (Yamamoto et al., 2002; Simonovicová et al., 2004; Tamás et al., 2005, 2006; Jones et al., 2006; Giannakoula et al., 2008).

Since roots are the first part of the plant to sense the Al-stress and the first line of adaptation reactions, antioxidant responses were assessed by the expression and activity of different forms of SOD and POD in roots of two inbred lines of maize (*Zea mays* L.) differing in their tolerance to Al. The SOD and POD patterns were compared between Al-tolerant and -sensitive maize inbred lines stressed and non-stressed by Al. In addition, the production of malondialdehyde (MDA) was measured to evaluate the level of lipid peroxidation of tolerant and sensitive maize inbred lines under stress and non-stress conditions. We have previously shown (Giannakoula et al., 2008), that in the tolerant maize line a long period exposure to Al (7 days in hydroponic culture) has induced an internal mechanism of tolerance that minimizes accumulation of lipid peroxides through a higher Pro and carbohydrate content which was related to osmoregulation and membrane stabilization. In this study we investigated also, whether a short period exposure to Al (72 h) could induce differential Pro and carbohydrate accumulation in the tolerant and sensitive maize inbred lines. The aims of this study were to test the hypothesis that the antioxidant enzymatic systems are up-regulated and function to protect the Al-tolerant maize line, and to establish whether a higher Pro and carbohydrate content is involved to the adaptation of maize plants to Al stress conditions, under short time exposure to Al.

2. Materials and methods

2.1. Plant material and growth conditions

Seeds of two maize inbred lines (*Zea mays* L., VA-22 and $A_{4/67}$) provided by the Cereal Institute of Thessaloniki, Greece were sterilized with 0.1% $HgCl_2$, 0.03% EDTA and 0.1% KCl for 15 min, rinsed excessively with distilled water and germinated on moist filter paper in plates for 4 days in darkness at 23 °C. The seedlings were then placed in 2-L hydroponic pots and transferred to the growth chamber where they were grown on aerated hydroponic modified Hoagland nutrient solution (Moustakas et al., 1995). Al was supplied as $KAl(SO_4)_2.12H_2O$ at concentrations 0, 240, 360 and 480 μM. According to the GEOCHEM speciation programme (Parker et al., 1995) the free Al^{3+} activities in the nutrient solutions were 0, 44, 65 and 87 μM, respectively. The growth solutions were adjusted to pH 4.1 ± 0.1 and were replaced every 3 d.

All the experiments were carried out in a growth chamber (EF7, Conviron, Montreal, Canada) with the following conditions: 14 h photoperiod, photosynthetic photon flux density 220 ± 20 μmol m^{-2} s^{-1}, temperature 22 ± 1 °C/18 ± 1 °C day/night and relative humidity 65 ± 2%/75 ± 2% day/night.

Three replicates each consisting of 12 pots of seedlings from each maize inbred line (a total of 108 seedlings from each line) were included for both Al treatment and non-treated controls. The whole experiment was repeated three times. After root sample collection, each replicate (treated/control) of the three experiments was randomly combined to make one biological replicate, and six biological replicate samples were used for each analysis.

2.2. Aluminum concentrations in roots

After 72 h of Al treatment, roots of the two maize inbred lines were rinsed thoroughly with distilled water and oven dried for 24 h at 80 °C. For preparation of samples for inductively coupled plasma atomic emission spectrometry (ICP-AES analysis), root samples were digested in a nitric acid/perchloric acid solution (HNO_3/$HClO_4$) 4:1 (v/v). Aluminum was determined by ICP-AES analysis (PerkinElmer Optima 3300XL PerkinElmer, USA). Each sample was analyzed at least four times and mean values were used as one observation.