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Differential responses of oat genotypes: oxidative stress provoked by aluminum

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Abstract The phytotoxic effects of aluminum and the mechanisms of genetically-based Al tolerance have been widely investigated, as reported in many papers and reviews. However, investigations on many Al-sensitive and Al-resistant species demonstrate that Al phytotoxicity and Al-resistance mechanisms are extremely complex phenomena. The objective of the present study was to analyze the effects of aluminum on the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX). Also was evaluated the lipid peroxidation, H_2O_2 content, levels of ascorbic acid (ASA), non-protein thiols (NPSH) and Al content in three genotypes of oat, *Avena sativa* L. (UFRGS 930598, UFRGS 17, and UFRGS 280). The genotypes were grown in different concentrations of Al ranging from 90 to 555 μM for 5 days. The antioxidant system was unable to overcome toxicity resulting in negative effects such as lipid peroxidation and H_2O_2 content in UFRGS 930598. The results showed that UFRGS 930598 was the most sensitive genotype. UFRGS 17 and UFRGS 280 were more resistant to Al toxicity. These results suggest that UFRGS 17 has mechanisms of external detoxification and UFRGS 280 has mechanisms of internal detoxification. The different behavior of enzymatic and non-enzymatic antioxidants of the genotypes showed that aluminum resistance in UFGRS 17 and UFRGS 280 may be related to oxidative stress.

Keywords *Avena sativa* · Superoxide dismutase · Hydrogen peroxide · Ascorbate peroxidase · Catalase · Aluminum content · Oxidative stress

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Introduction

All aerobic organisms including plants require molecular oxygen for survival, which can be easily

reduced to reactive oxygen species (ROS) through various biochemical reactions (Morita et al. 2008). ROS include the superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), the hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) which are reactive species and can oxidize and damage cellular components (De Biasi et al. 2003). The cells possess a defensive system, consisting of various enzymes such as catalase (CAT—E.C. 1.11.1.6), ascorbate peroxidase (APX—E.C. 1.11.1.11), and superoxide dismutase (SOD—E.C. 1.15.1.1), that reduce ROS under normal conditions, but if complete reduction does not occur, the result may be a state of oxidative stress leading to the oxidation of biomolecules as lipids, proteins, and DNA (Mittler 2002). The antioxidative system falls into two general classes: (1) low molecular weight antioxidants, which consist of lipid-soluble membrane associated antioxidants such as a-tocopherol and b-carotene, and water-soluble reductants such as glutathione and ascorbate and (2) antioxidative enzymes: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Gratão et al. 2005).

The toxicity caused by aluminum in plants in acid soils is a well-known example of environmental stress (for a review, see Ma and Hiradate 2000; Matsumoto 2000; Ma et al. 2001). Although Al itself is not a transition metal and cannot catalyze redox reactions, the involvement of oxidative stress in Al toxicity has been suggested. Al^{3+}, the most toxic of the soluble forms of Al (Yamamoto et al. 2001) induces oxidative stress, and a number of studies have reported its participation in various processes, including an increase in enzyme activity (SOD) related to ROS and lipid peroxidation in soybeans (*Glycine max*) (Cakmak and Horst 1991) peas (*Pisum sativum*) (Yamamoto et al. 2001), changes in the expression of various genes induced by Al in *Arabidopsis* (Sugimoto and Sakamoto 1997; Richards et al. 1998), tobacco (Ezaki et al. 2000), and wheat (*Triticum aestivum* L.) (Snowden et al. 1995; Cruz-Ortega et al. 1997; Hamel et al. 1998). Comparative studies of Al tolerance in 22 species from seven families have established that plants can resist the toxic effects of Al. There are many proposed mechanisms of Al tolerance in plants involving external avoidance or internal tolerance (Kochian 1995). The phytotoxic effects of aluminum and the mechanisms of genetically-based Al tolerance have been widely investigated, as reported in many papers and reviews (Kochian 1995; Delhaize and Ryan 1995; Ciamporová 2002; Gonçalves et al. 2009). Evidence corroborating the relation between aluminum stress and oxidative stress in plants has been obtained with transgenic *Arabidopsis* plants (Ezaki et al. 2000). However, investigations on many Al-sensitive and Al-resistant species demonstrate that Al phytotoxicity and the mechanisms of Al-resistance are extremely complex phenomena. When comparing the physiology and biochemistry of genotypes that differ in Al tolerance, it is preferable that the genetic backgrounds be similar so as to eliminate differences that are unrelated to Al tolerance (Ezaki et al. 2000).

In this study, we have used three genotypes of oat (*Avena sativa* L.) that differ in Al tolerance. These genotypes, UFRGS 17, UFRGS 930598, and UFRGS 280, were previously classified as tolerant, sensitive, and intermediate to aluminum, respectively in accordance with morphological characteristics suck as root length (Federizzi et al. 2000). Measurements of the effects of Al were carried out after 5 days of exposure, and allowed us to reveal whether there are any biochemical differences between UFRGS 17, UFRGS 930598 and UFRGS 280 and their classification as tolerant, sensitive, and intermediate, respectively in accordance with previous morphological analysis was confirmed. It was also shown that aluminum resistance may be related the oxidative stress.

Materials and methods

Plant material and growth conditions

Avena sativa L. seeds of three genotypes (UFRGS 17 Al-tolerant, UFRGS 930598 Al-sensitive, and UFRGS 280 Al-intermediate), commonly known as oat, provided by Programa de Melhoramento Genético de Aveia from the Universidade Federal do Rio Grande do Sul (UFRGS) were germinated in plastic recipients (1000 ml) containing 250 ml of medium with $Al_2(SO_4)_3$ diluted in a 0.5% agar solution. No nutritive solution was added to the agar. The seedlings made use of the seed nutrition in the initial stage of development, and in a previous experiment, it was verified that up to the tenth day the plants did not suffer any nutrient deficiency (data not shown). Five different $Al_2(SO_4)_3$

treatments (0, 90, 185, 370, and 555 μM) were applied randomly. The medium pH was adjusted to 4.0. Each experimental unit consisted of 30 seeds, totalizing 15 replicates per treatment. After germination, the seedlings were maintained in a growth chamber with controlled temperature (25 ± 1°C) and photoperiod (16 h light; light intensity of 35 μmol/m^2/s at plant level). On the fifth day, the seedlings were separated into root and shoot and prepared for homogenization.

Metal determination

Al content was determined in the roots and shoot of 5-day-old oat seedlings. Approximately 50 mg of roots and shoot were digested with 4 ml HNO_3 utilizing the following stages of heating: (a) 50°C for 1 h; (b) 80°C for 1 h; and (c) 120°C for 1 h in a digester block (Velp, Italy). The samples were then diluted to 50 ml with high-purity water. Al concentrations were determined using a Model AAS 5 EA atomic absorption spectrometer (Analytic Jena, Germany) equipped with a transversely heated graphite furnace and an autosampler (MPE 5). The content absorbed was expressed in mg/kg dry weight.

Antioxidant enzyme activities

Catalase

For the CAT assay, the oat root and shoot seedlings were prepared by the homogenization of fresh tissue material in a solution containing 50 mM KH_2PO_4/K_2HPO_4 (pH 7.0), 10 g/l PVP, 0.2 mM EDTA, and 10 ml/l Triton X-100, at a ratio of 1:5 (w/v). After the homogenate was centrifuged at 12,000×*g* at 4°C for 20 min, the supernatant was used to determine CAT activity, which was assayed according to the modified method of Aebi (1984) by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm in a reaction mixture with a final volume of 2 ml containing 15 mM H_2O_2 in 50 mM KPO_4 buffer (pH 7.0) and 30 μl extract. CAT activity was expressed as ΔE/min/mg protein.

Ascorbate peroxidase

For determination of APX activity, oat root and shoot were homogenized in a 50 mM potassium phosphate buffer containing 1 mM EDTA and 2% PVP, pH 7.8, at a ratio of 1:3 (w/v). The homogenate was centrifuged at 13,000×*g* for 20 min at 4°C, and the supernatant was used for enzyme activity, which was assayed according to the modified method of Zhu et al. (2004). The reaction mixture, at a total volume of 2 ml, consisted of 25 mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2, and 100 μl extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in absorbance at 290 nm [$E = 2.8$/(mM cm)]. APX activity was expressed as μmol oxidized ascorbate/min/mg protein.

Superoxide dismutase

The activity of SOD was assayed according to (Mc Cord and Fridovich 1969). About 200 mg fresh tissues were homogenized in 5 ml of 100 mM potassium phosphate buffer (pH 7.8) containing 0.1 mM EDTA, 0.1% (v/v) Triton X-100, and 2% (w/v) polyvinyl pyrrolidone (PVP). The extract was filtered and centrifuged at 22,000×*g* for 10 min at 4°C, and the supernatant was utilized for the assay. The assay mixture consisted of a total volume of 1 ml, containing glycine buffer (pH 10.5), 60 mM epinephrine, and enzyme material. Epinephrine was the last component to be added. The adrenochrome formation over the next 4 min was recorded at 480 nm in a UV–vis spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation under the experimental conditions. SOD activity was expressed as U SOD/mg protein. This method is based on the ability of SOD to inhibit the autoxidation of epinephrine at an alkaline pH. Since the oxidation of epinephrine leads to the production of a pink adrenochrome, the rate of increase of absorbance at 480 nm, which represents the rate of autoxidation of epinephrine, can be conveniently followed. SOD has been found to inhibit this radical-mediated process.

Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 contents of both control and treated seedlings were determined according to Loreto and Velikova (2001). Approximately 100 mg of root and shoot were homogenized at 4°C in 2 ml of 0.1% (w/v)

trichloroacetic acid (TCA). The homogenate was centrifuged at 12,000×*g* for 15 min and 0.5 ml of 10 mM potassium phosphate buffer pH 7.0 and 1 ml of 1 M KI. The H_2O_2 content of the supernatant was evaluated by comparing its absorbance at 390 nm with a standard calibration curve. The H_2O_2 content was expressed as μmol/g fresh weight.

Estimation of lipid peroxidation

The levels of lipid peroxides in the seedling were determined by measuring malondialdehyde (MDA) content from the thiobarbituric acid (TBA) reaction as described by El-Moshaty et al. (1993). The root and shoot were homogenized in 0.2 M citrate–phosphate buffer, pH 6.5, at a ratio of 1:20 (w/v). The homogenate was filtered through two layers of paper filter and then centrifuged at 20,000×*g* at 4°C for 15 min. One milliliter of the supernatant fraction was added to an equal volume of 20% TCA containing 0.5% TBA. Tubes were placed in a 95°C water bath for 40 min, and then immediately cooled on ice for 15 min. Samples were centrifuged at 10,000×*g* for 15 min. The absorbance of the supernatant at 532 nm was read and corrected for unspecific turbidity by subtracting the value at 600 nm. MDA values were expressed in nmol MDA/mg protein.

Non-protein thiol

Non-protein thiol (NPSH) content in root and shoot (mg) was measured spectrophotometrically with Ellman's reagent (Ellman 1959). The reaction was read at 412 nm after the addition of 10 mM 5-5-dithio-bis (2-nitrobenzoic acid) (DTNB) (0.05 ml). Treated seedlings were homogenized in 10 mM Tris/HCl, pH 7.4, centrifuged at 4,000×*g* at 4°C for 10 min, and supernatants were used for total thiol group determination. NPSH groups were determined in the fraction obtained after mixing one volume of supernatant with one volume of 4% TCA followed by centrifugation and neutralization (to pH 7.5) with 1 M Tris as described by Jacques-Silva et al. (2001). A standard curve using cysteine was used to calculate the content of thiol groups in samples, and was expressed as μmol SH/g fresh weight.

Ascorbic acid

Ascorbic acid (ASA) determination was performed as described by Jacques-Silva et al. (2001). Briefly, root and shoot were homogenized in 10 mM Tris/HCl, pH 7.4, centrifuged at 4,000×*g* for 10 min and protein was removed by dilution with one volume of 4% TCA followed by centrifugation. An aliquot of the sample was incubated at 37°C in a medium containing 4.5 mg/ml dinitrophenylhydrazine, 0.6 mg/ml thiourea, 0.075 mg/ml $CuSO_4$, and 0.675 mol/l H_2SO_4 (final volume 1 ml). After 3 h, 1 ml of 65% H_2SO_4 was added and samples were read at 520 nm and were expressed as μg ASA/g fresh weight. A standard curve was constructed using ASA.

Statistical analysis

The analyses of variance were computed on statistically significant differences determined based on the appropriate *F*-tests. The results are the means ± SD of at least three independent replicates. The mean differences were compared utilizing Tukey's range test.

Results

Metal determination

The roots of all genotypes showed the highest concentration of aluminum. The roots of the sensitive (UFRGS 930598) and intermediate (UFRGS 280) seedlings showed an increase of aluminum content at 90, 185, 370, and 555 μM when compared to control (Table 1). In the tolerant seedling root (UFRGS 17), there was also a significant increase at all concentrations, but this increase was lower than that observed in the sensitive seedling root. The maximum accumulation of Al was 8950 mg/kg dry weight in roots treated with 555 μM in the sensitive seedling. More aluminum was accumulated in the roots than in the shoot. The content of aluminum was significant in all genotype shoots, but was greatest in the intermediate seedling with a dry weight of 7056 mg/kg at 555 μM. In the sensitive seedling shoot, the aluminum content was 6678 mg/kg dry weight at 555 μM. The tolerant seedling shoot accumulated the least amount of aluminum.

Table 1 Concentration of aluminum in root and shoots of three genotypes of oat (UFRGS 17 Al-tolerant, UFRGS 930598 Al-sensitive, and UFRGS 280 Al-intermediate)

Al concentration	Root (mg/kg dry weight) UFRGS 930598	Shoot (mg/kg dry weight) UFRGS 930598
0 μM	3648 ± 200[d]	1893 ± 200[d]
90 μM	4096 ± 250[c]	2993 ± 500[c]
185 μM	5870 ± 300[b]	4568 ± 450[b]
370 μM	6520 ± 500[b]	5194 ± 600[b]
555 μM	8950 ± 350[a]	6678 ± 500[a]
	UFRGS 17	UFRGS 17
0 μM	3700 ± 200[d]	1766 ± 290[d]
90 μM	4533 ± 290[c]	2394 ± 350[c]
185 μM	5006 ± 200[b]	3458 ± 380[b]
370 μM	6142 ± 300[a]	4083 ± 342[a]
555 μM	6370 ± 350[a]	4478 ± 300[a]
	UFRGS 280	UFRGS 280
0 μM	3756 ± 200[d]	1926 ± 200[e]
90 μM	4580 ± 350[d]	3478 ± 250[d]
185 μM	5089 ± 300[c]	3996 ± 200[c]
370 μM	6890 ± 290[b]	5720 ± 250[b]
555 μM	7145 ± 250[a]	7056 ± 190[a]

Different letters are statistically different ($P < 0.05$)

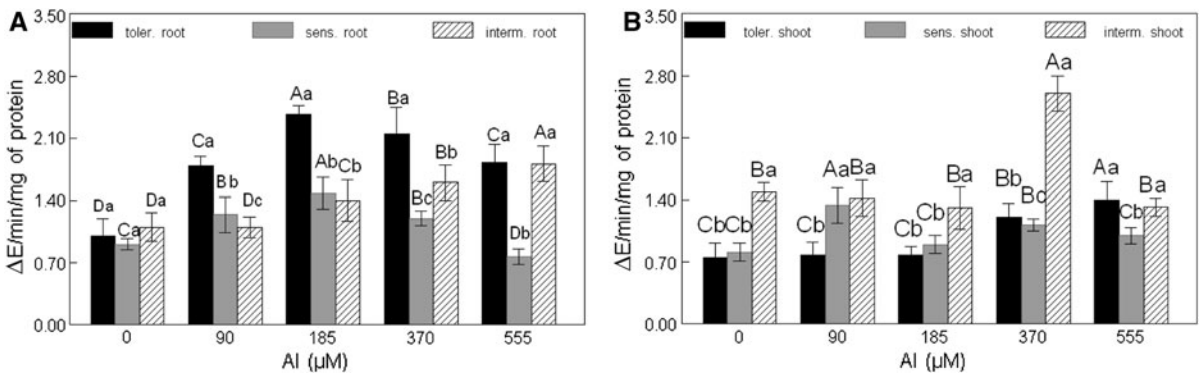

Fig. 1 Effect of increasing concentration of $Al_2(SO_4)_3$ on CAT activity, in root (**a**) and shoot (**b**) in 5-day-old oat seedlings of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

Activities of antioxidant enzymes

The activities of antioxidant enzymes, namely CAT, APX, and SOD were determined in 5-day-old roots and shoots of the sensitive, intermediate, and resistant genotypes (Figs. 1, 2, and 3, respectively). As a result of Al stress, root CAT activity increased in all three genotypes (Fig. 1a), while shoot CAT activity of the sensitive seedling was decreased at 370 and 555 μM when compared between the genotypes. By contrast, root CAT activity increased to a greater extent in the tolerant seedling than in the sensitive seedling. There was no significant change in shoot CAT activity in the resistant seedling (Fig. 1b). Shoot CAT activity in

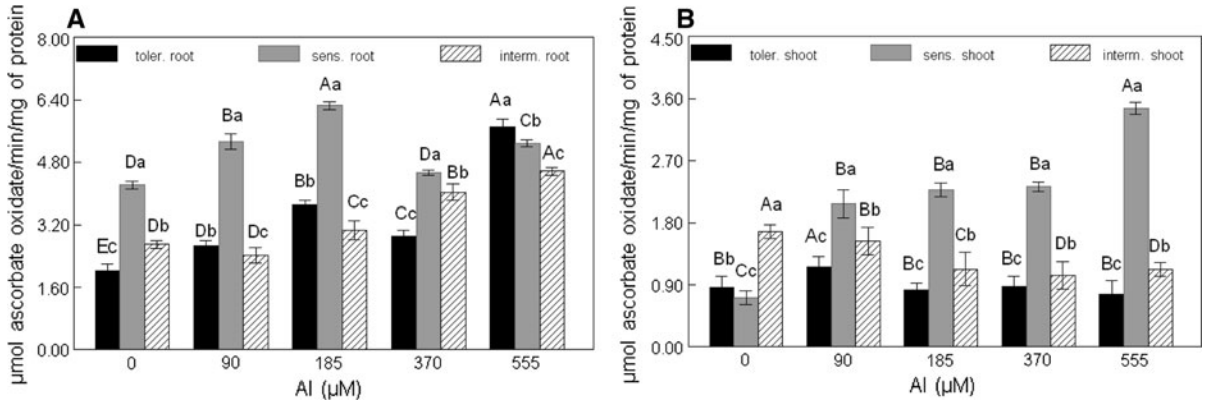

Fig. 2 Effect of increasing concentration of $Al_2(SO_4)_3$ on APX activity, in root (**a**) and shoot (**b**) in 5-day-old oat seedlings of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$) *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

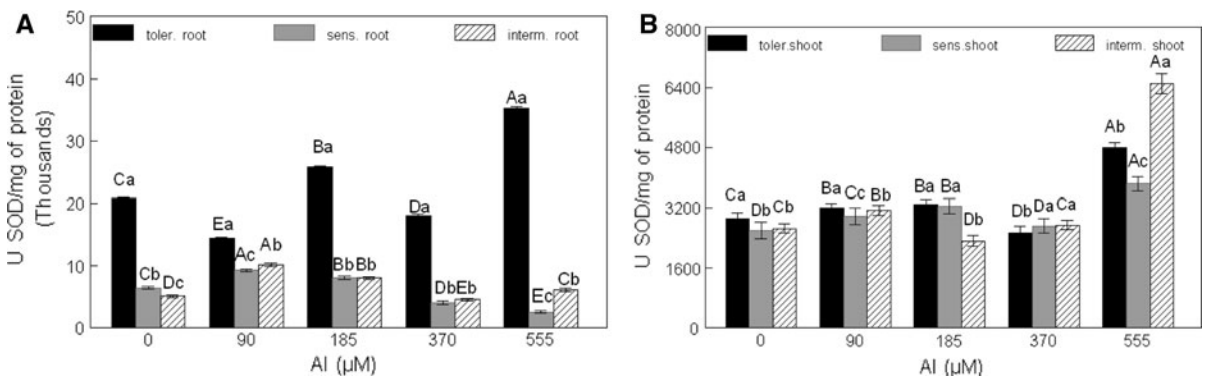

Fig. 3 Effect of increasing concentration of $Al_2(SO_4)_3$ on SOD activity, in root (**a**) and shoot (**b**) of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

the tolerant seedling was increased only at 370 and 555 µM. In the sensitive seedling shoot, CAT activity increased at 90 and 370 µM while in the intermediate shoots it was increased only at 370 µM.

Root APX activity was increased in all seedlings (Fig. 2a). Nonetheless, shoot APX activity was inhibited in the intermediate seedling in all treatments, while in the sensitive seedling shoots APX activity was increased in all treatments (Fig. 2b). The maximum APX activity was 3.46 µmol ascorbate oxidized/min/mg protein at 555 µM $Al_2(SO_4)_3$ in sensitive shoots (Fig. 2b) and 6.25 µmol ascorbate oxidized/min/mg protein at 185 µM $Al_2(SO_4)_3$ in sensitive root (Fig. 2a).

Figure 3 shows the SOD activity of oats seedlings. Root SOD activity was increased at 185 and 555 µM $Al_2(SO_4)_3$ in the resistant seedling (Fig. 3a) and decreased at 370 and 555 µM $Al_2(SO_4)_3$ in the sensitive and intermediate seedlings. Root SOD activity increased to a greater extent in the resistant seedling during Al stress. Nonetheless, shoot SOD activity in the intermediate seedling was increased at 555 µM $Al_2(SO_4)_3$ when compared with the shoot of resistant and sensitive seedlings (Fig. 3b). The maximum SOD activity was 35249 U SOD/mg protein at 555 µM $Al_2(SO_4)_3$ in resistant roots (Fig. 3a) and 6500 at U SOD/mg protein at 555 µM $Al_2(SO_4)_3$ in intermediate shoots (Fig. 3b).

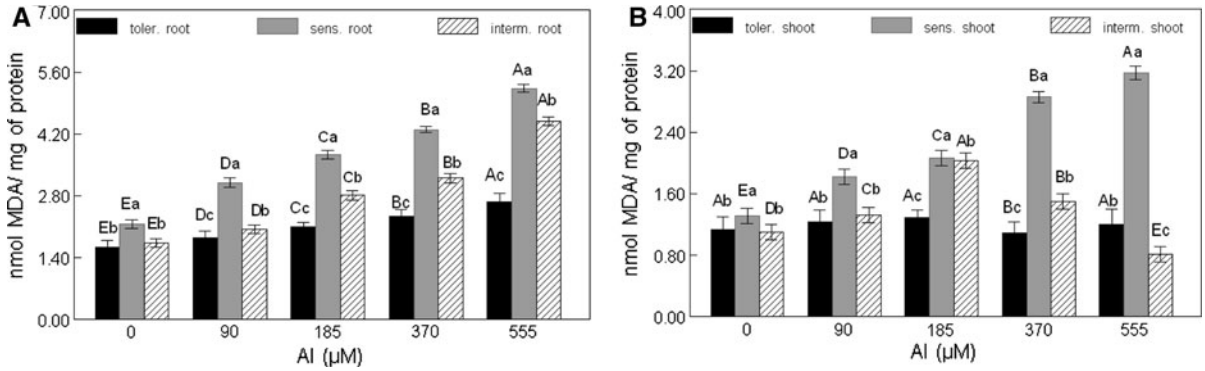

Fig. 4 Effect of increasing concentration of $Al_2(SO_4)_3$ on level of lipid peroxides, in root (**a**) and shoot (**b**) of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

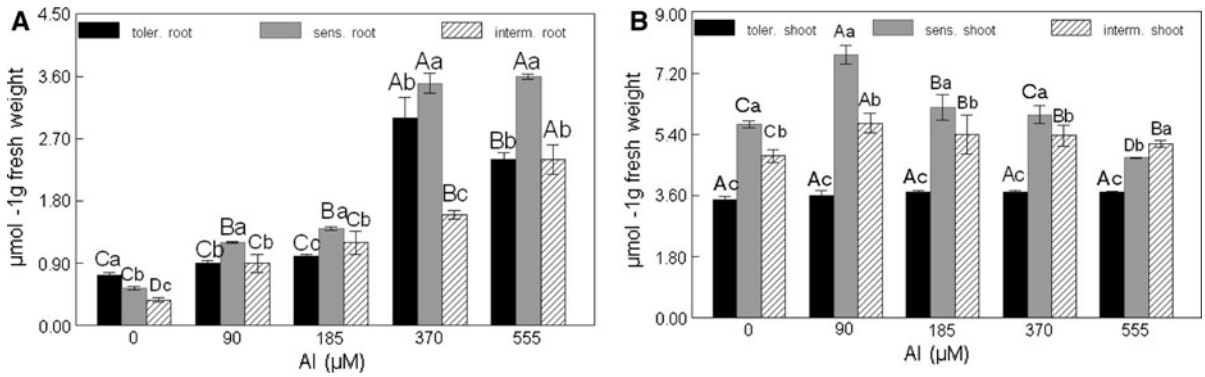

Fig. 5 Effect of increasing concentration of $Al_2(SO_4)_3$ on H_2O_2 content, in root (**a**) and shoot (**b**) of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

Lipid peroxidation and hydrogen peroxide levels

Al showed an increased root MDA content in all three seedlings when compared to control (Fig. 4a). The root MDA content increased to a greater extent in sensitive and intermediate seedlings if compared to the resistant seedling during Al stress. However, shoot MDA content of the resistant seedling did not change (Fig. 4b), while shoots MDA content showed significant changes in the sensitive and intermediate seedlings. At 555 μM $Al_2(SO_4)_3$, intermediate shoot MDA content was reduced (Fig. 4b).

The effect of $Al_2(SO_4)_3$ on H_2O_2 content is shown in Fig. 5. Root endogenous H_2O_2 increased by about 70% when compared to control in resistant and sensitive seedlings at 370 μM $Al_2(SO_4)_3$ (Fig. 5a). Moreover, shoot H_2O_2 content increased in sensitive and intermediate seedlings, but their no change was observed in the resistant seedling at any Al concentration (Fig. 5b).

ASA and NPSH group concentrations

The NPSH content increased upon Al exposure. However, at the highest Al concentrations [370 and 555 μM $Al_2(SO_4)_3$], root NPSH content was decreased in the resistant seedling (Fig. 6a). Al effects on shoot NPSH content are shown in Fig. 6b. Shoot NPSH content showed an increase in the resistant seedling at 90 and 185 μM $Al_2(SO_4)_3$ and a decrease at all concentrations in the sensitive seedling shoot.

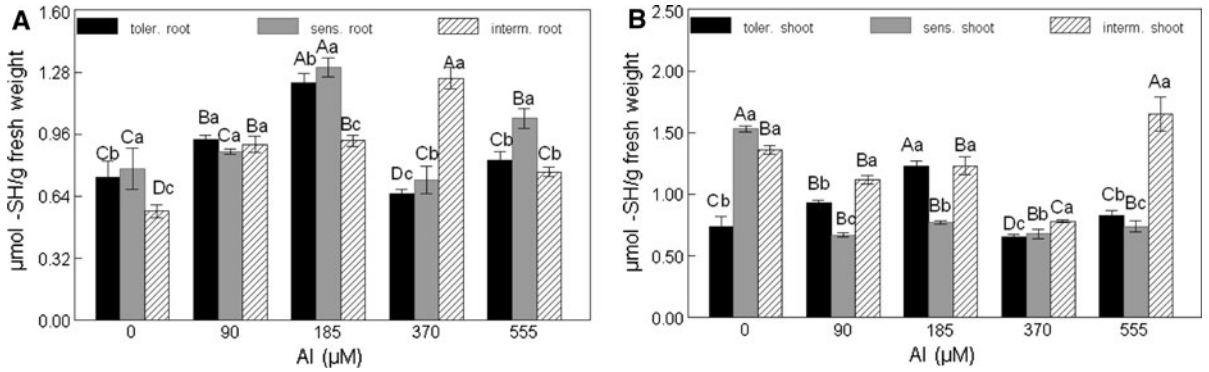

Fig. 6 Effect of increasing concentration of $Al_2(SO_4)_3$ on NPSH content, in root (**a**) and shoot (**b**) of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

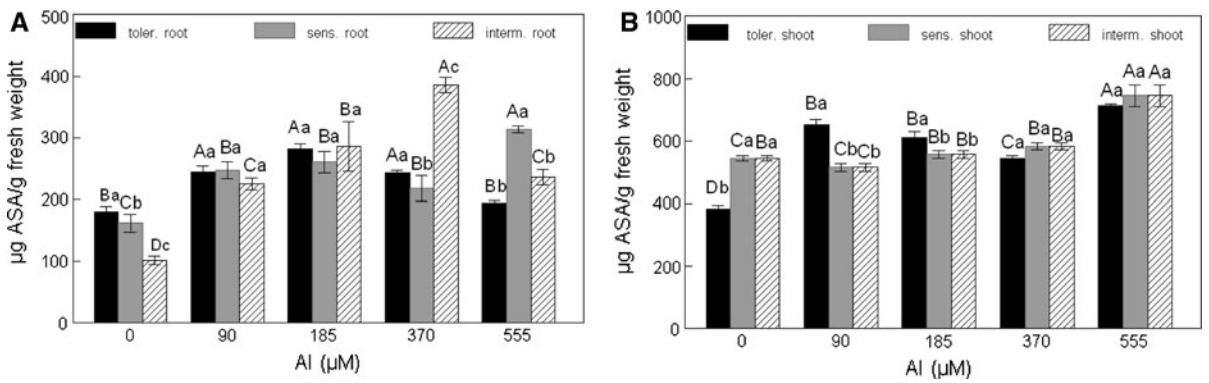

Fig. 7 Effect of increasing concentration of $Al_2(SO_4)_3$ on ASA content, in root (**a**) and shoot (**b**) of the three genotypes (tolerant, sensitive, and intermediate). Data represent the mean ± SD of three different experiments. Different letters are statistically different ($P < 0.05$). *A* difference into concentrations (0, 90, 185, 370, and 555) and *a* difference into genotypes in same concentration

The ASA content increased as a function of Al concentration in roots and shoots of all seedlings (Fig. 7a, b). ASA content increased to a greater extent in shoots than in root during Al stress. The maximum accumulation of ASA was 385.83 µg ASA/g fresh weight in intermediate seedling roots treated with 370 µM $Al_2(SO_4)_3$ (Fig. 7a) and 745.63 µg ASA/g fresh weight in sensitive seedling shoots treated with 555 µM $Al_2(SO_4)_3$ (Fig. 7b).

Discussion

In the present study, the sensitive, intermediate, and tolerant seedling genotypes showed a continuous increase in the concentration of Al^{3+} in roots and shoots with increasing $Al_2(SO_4)_3$ external concentration and accumulated significantly higher Al^{3+} concentration in roots. This finding has been reported by other researchers working with distinct plant species exposed to varied Al^{3+} concentrations (Yamamoto et al. 2003; Poschenrieder et al. 2008). This Al^{3+} accumulation in the root system may indicate that roots serve as a partial barrier to transport to the shoots. The root sensitive seedlings have the highest values of aluminum accumulated.

In the shoots of intermediate seedlings, there was a higher Al^{3+} concentration when compared with the shoots of tolerant and sensitive seedlings. These data suggest a genotypic difference among oat genotypes concerning the partitioning of Al^{3+}. In fact, differences in metal accumulation among the genotypes of

the same species have been observed in different plants (Tamás et al. 2006; Tria et al. 2007).

A common feature of stresses, such as Al toxicity, is perturbation of cell redox homeostasis and, as a consequence, enhanced production of ROS (Mittler 2002). During stress, the impairment of electron transport chains on injured membranes leads to the formation of ROS and to subsequent activation of the antioxidant defense system (Tamás et al. 2006). This study showed that CAT and SOD activities had a more protective effect in the resistant (UFRGS 17) and intermediate seedlings (UFRGS 280) than in the sensitive seedling (UFRGS 930598) (Figs. 1a, b, 3a, b). The sensitive seedling showed lower CAT activity than the other seedlings (Fig. 1a, b). However, APX had a more protective effect on the sensitive seedling than on the other seedlings (Fig. 2a, b).

APX, CAT, and SOD are major ROS-scavenging mechanisms in plants. The balance between these enzymes is crucial for determining the steady-state level of ROS excess. Different affinities of APX (μM range) and CAT (mM range) to H_2O_2 suggest that they belong to two different classes of H_2O_2-scavenging enzymes: APX might be responsible for the fine modulation of ROS in the sensitive seedling, showing that even small quantities of H_2O_2 caused oxidative stress in this genotype. This indicates that this genotype is, in fact, more sensitive than the resistant genotype, which is in accordance with Federizzi et al. (2000) who showed differences between seedling through the decreased root growth in the sensitive genotype (UFGRS 930598).

In the intermediate seedling, CAT and SOD activities were increased to a greater extent in shoots than in root at 370 and 555 μM, respectively when compared with shoots of others seedling. Hydrogen peroxide is a stable ROS and its synthesis has been shown to increase under a variety of stressful conditions (Dubey and Sharma 2004). It can be converted to more toxic molecules such as $^{\bullet}OH$, if it is not quickly removed from plant cells (Wang et al. 1999). The H_2O_2 content was increased in roots and shoots of both the sensitive seedling and the resistant seedling (Fig. 5a, b), but this increase was lower in the resistant seedling. These results suggest that the antioxidant system was more efficient in the resistant seedling, but that it was also activated in the sensitive seedling, although in a differential way.

Plants possess H_2O_2-scavenging enzyme APX that converts H_2O_2 to H_2O using ascorbate as an electron donor. A recent investigation revealed that ascorbate content regulates plant defense gene expression and modulates plant growth and development via phytohormone signaling (Pastori et al. 2003). ASA is involved in the regulation of photosynthesis, cell expansion, root elongation, and trans-membrane electron transport (Smirnoff 2000). ASA is found in millimolar concentration in leaves and plays an important role in plant tolerance to stresses as a component of the antioxidant system (Noctor and Foyer 1998). In line with this, our results show that the resistant seedling accumulated more ASA in shoot and root than did the sensitive seedling (Fig. 7a, b) Antioxidants such as ASA and glutathione, which are found at higher concentrations in chloroplasts and other cellular compartments (5–20 mM ASA and 1–5 mM glutathione) are crucial for plant defense against of oxidative stress (Noctor and Foyer 1998). The glutathione levels in oat were higher in the sensitive seedling than in the tolerant seedling (Fig. 6a, b). This suggests that aluminum activated the antioxidant system in the sensitive seedling. However, the antioxidant system may not have been efficient as greater lipid peroxidation was observed in this genotype (Fig. 4a, b)

Mutants with suppressed ASA levels and transgenic plants with altered content of glutathione are hypersensitive to stress conditions. It is generally believed that maintaining a high reduced per oxidized ratio of ASA and glutathione is essential for the proper scavenging of ROS in cells. In addition, the overall balance between different antioxidants has to be tightly controlled. Enhanced glutathione biosynthesis in chloroplasts can result in oxidative damage to cells rather than their protection, possibly by altering the overall redox state of chloroplasts (Creissen et al. 1999). In the present study, this hypothesis also may be valid for oats seedling sensitive to Al.

The cell plasma membrane, the ultimate obstacle for free access of Al ions into the symplast, is believed to be a primary target of rhizotoxic Al (Barceló et al. 1996). Aluminum ions bind with high affinity to negative charges on carboxyl and phosphate groups in the plasma membrane (Chaffai and Marzouk 2005). Membranes of root cells exposed to Al present several indications of substantial disturbance (Barceló et al.

1996). The most prominent indicator of plasma membrane damage is Al-stimulated lipid peroxidation. Aluminum ions bound to the membrane modify lipid packaging and structure, facilitating lipid peroxidation by Fe (Oteiza 1994). Lipid peroxidation levels in roots and shoots of sensitive seedlings were higher when compared to the resistant genotype (Fig. 4a, b). Root lipid peroxidation in the tolerant genotype may have been due to direct contact with the metal. In shoots of tolerant seedlings, lipid peroxidation was not detected. Regulation of lipid membrane composition and adjustment of the unsaturation level of membrane fatty acids are extremely important in dealing with metal toxicity and make a considerable contribution to plant survival. Changes in properties of cellular membranes occur to ensure the proper function of processes that take place within them, and lead to ameliorative growth contributing to plant adaptation (Chaffai and Marzouk 2005).

Through our measurements of Al effects on seedlings, it can be suggested, in agreement with Federizzi et al. (2000), that UFRGS 17 was more tolerant than UFRGS 930598. Al showed more toxic effects in UFRGS 930598, but this genotype also showed an activation of the antioxidant system, though less efficient if compared to that of the tolerant genotype (UFRGS 17).

UFRGS 280 (intermediate) showed some results that were similar to the tolerant genotype and others that were similar to the sensitive genotype. However, the intermediate seedling was the genotype that accumulated the most Al in shoots, although lipid peroxidation and H_2O_2 content were not the highest.

In conclusion, this result suggests the existence of mechanisms of internal detoxification for UFRGS 280, and mechanisms of detoxification by exudation of Al-ligating compounds for UFRGS 17. Therefore, there are biochemical differences between the three seedlings. However, the intermediate seedling may be more resistant than was shown by morphological evaluations, as it showed greater aluminum resistance. We suggest that the intermediate seedling is in fact a tolerant genotype, but in a transition stage. The different behavior of enzymatic and non-enzymatic antioxidants showed that Al-resistance in the resistant seedling (UFGRS 17) and the intermediate seedling (UFRGS 280) may be related to oxidative stress.

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