Journal of Applied Botany and Food Quality 83, 37 - 43 (2009)

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Effects of heavy metals on antioxidant activities of *Atriplex hortensis* and *Atriplex rosea*

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(Received April 17, 2009)

Summary

In the present study, the effects of heavy metals generating antioxidative defense systems such as superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) glutathione reductase (GR, EC 1.6.4.2) and catalase (CAT, EC 1.11.1.6) were studied in the leaves of *Atriplex* plants grown in polluted soil with different heavy metals (Cu, Ni, Pb and Zn). The results showed that the exposure of plants to different levels of metals reduced the dry matter production and height of shoots. The decrease in root growth caused by the toxicity of metals was severe than the decrease in shoot growth. *Atriplex* showed gradual decrease in height following metal treatments, a four week exposure of *A. hortensis var. rubra L.* (red) to 25%, 50%, 75% and 100% contaminated soil gave a respective mean values of 21.4, 12.2, 9.3 and 6.5 cm which were lower in comparison to the plants of the control group. Of the antioxidant enzymes, the results showed that SOD and APX, were diminished by metal toxicity. However, the activity of CAT and GR were increased by the metal stress. Hence, the plants of the three annual arroach species or varieties used, all showed an intermediate level of tolerance according to the imposed treatments. The antioxidative activity seems to be of fundamental importance for adaptive responses of *Atriplex* plants against the metal toxicity.

Abbreviations: SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; MDHA, monodehydroascorbate; NBT, nitroblue tetrazolium; POD, peroxidase.

1. Introduction

Oxidative stress is induced by a wide range of environmental factors including heavy metal stress. Antioxidant resistance mechanisms, therefore, may provide a strategy to enhance the metal tolerance, and therefore, processes with the antioxidant responses to metal stress must be clearly understood. Heavy metal contamination of soils due to intensive industrial activities and agricultural development can usually cause environmental problems. Elevated levels of heavy metals not only decrease soil microbial activity and crop production, but also threaten human health through the food chain (MCLAUGHLIN et al., 1999). Phytoremediation, the use of plants to extract, sequester, and/or detoxify pollutants, has been reported to be an effective, non-intrusive, inexpensive, aesthetically pleasing, socially accepted technology to remediate the polluted soils (WEBER et al., 2001). Plants used for phytoextraction, i.e., metal removing plants, should have the following characteristics: (i) tolerant to high levels of metals, (ii) accumulate reasonably high levels of the ions, (iii) rapid growth rate, (iv) produce reasonably high biomass in the field, and (v) profuse root system (GARBISU et al., 2002).

In stress conditions such as metal toxicity, higher activities of antioxidant enzymes and higher contents of non-enzymatic antioxidant constituents are important for plants to tolerate the stress. These were initially thought to function as osmotic buffers. However, they also seem to play a key role in maintaining the natural state of macromolecules, probably by scavenging reactive oxygen species ROS (XIONG and ZHU, 2002). There is good evidence that the alleviation of oxidative damage and increased resistance to environmental stresses is often correlated with an efficient antioxidative system (CAKMAK et al., 1993). Strategies to minimize oxidative damage are a universal feature of plant defense responses. In some species, the effects of both biotic and abiotic stress on the antioxidant systems induces oxidative stress, resulting from the production and accumulation of toxic oxygen species such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) (FOYER et al., 1994). The ROS are strong oxidizing agents that cause oxidative damage to biomolecules such as lipids, proteins, carbohydrates, and nucleic acids and eventually lead to cell death (MOLASSIOTIS et al., 2006). Mechanisms for the generation of ROS in biological systems are represented by both non-enzymatic and enzymatic reactions. There is evidence that the tolerance of plants is correlated with increasing amounts of antioxidants and increasing activity of radical scavenging enzymes. The antioxidant defense system in the plant cell includes both enzymatic, such as (SOD), (CAT), (APX), and non enzymatic antioxidants such as ascorbate, glutathione and atocopherol. As a major scavenger SOD catalyzes the dismutation of superoxide (O^-_2) to hydrogen peroxide (H_2O_2) and oxygen (O_2). However, H_2O_2 is also toxic to cells and has to be further detoxified by CAT and/or (POD) to water and oxygen (ZHU et al., 2004). When plants are subjected to environmental stresses, the oxidative damage may happen due to the imbalances between the production of ROS and their detoxification by the antioxidative system (GOMEZ et al., 1999). Tolerance of damaging environmental stresses is correlated with an increased capacity to scavenge or detoxify activated oxygen species (SMIRNOFF, 1993). Among the halophyte flora, species belonging to the genus *Atriplex* may be of special interest because of their high biomass production associated with a deep root system which is able to cope with the poor structure and xeric characteristics of several polluted substrates. These species also naturally produce high amounts of oxalic acid, which may assume positive functions in tolerance mechanisms to heavy metal stress (SAYER and GADD, 2001). Among Chenopodiaceae the genus *Atriplex* is probably the most studied. These plants could be promising, since *Atriplex* species have special bladders in the leaves that act as salt sinks for the removal of the excess of salt (LAEUCHI and LUETTGE, 2002). The genus *Atriplex* have been proposed as possible candidates for the removal of Se (VICKERMAN et al., 2002). Additionally, recent studies have shown that *A. hortensis* (red orach), a salad green, has also a high salt tolerance as compared to other vegetables (WILSON et al., 2000). Uptake of Se could be greatly reduced due to the competitive inhibition in Na_2SO^{4-}dominant salts. *Atriplex spp.* is often grown as fodder plant in dry areas because of its great tolerance to drought and salt tolerance (ABOU EL NASR et al., 1996). Recent works have aimed to identify the role of antioxidative metabolism in heavy metal tolerance in *Thlapsi. caerulescens* (BOOMINATHAN and DORAN, 2003a; 2003b). Superior antioxidant defenses, particularly catalase activity, may play an important role in the hyperaccumulator phenotype of *T. caerulescens*.

Oxidative stress can lead to inhibition of the photosynthesis and respiration processes and, thus, plant growth. Plants have evolved enzymatic and non enzymatic systems to scavenge active oxygen species. In enzymatic systems, for example, SOD catalyses the dismutation of O^{-}_2 to H_2O_2 and O_2. CAT and APX can break down H_2O_2. GR also can remove H_2O_2 via the ascorbate glutathione cycle to maintain a high level of reduced ascorbate within chloroplasts. Hydrogen peroxide is eliminated by CAT and ascorbate peroxidases (CHEN and ASADA, 1989). These enzymes rapidly destroy the vast majority of H_2O_2 produced by metabolism, but they allow low steady state levels to persist presumably to maintain redox signaling pathways (NOCTOR and FOYER, 1998). Several enzymes are involved in the detoxification of ROS. SOD converts superoxide to H_2O_2. SOD, which is the most effective anti-oxidative enzyme in preventing cellular damage, catalyzes the conversion of the superoxide anion to H_2O_2, Hydrogen peroxide is scavenged by CAT and different classes of peroxidases (BOWLER et al., 1992). APX plays a key role in the ascorbate-glutathione cycle by reducing H_2O_2 to water at the expense of oxidizing ascorbate to MDHA (ASADA, 1994). Heavy-metal can cause many toxic symptoms, such as the inhibition of growth and photosynthesis and the activation or inhibition of enzymes. The present paper discusses the toxic symptoms and defense mechanisms induced by heavy metal in *Atriplex* plants.

2. Materials and Methods

2.1. Plants

Seeds of *A. hortensis* were obtained from a botanic garden: Denmark House, Pymoor, Ely, Cambridgeshire (CN seeds). The seeds of *A. rosea* were collected from the site of Usinor and, the plants were then grown in sand cultures at the "Conservatoire Botanique" Laboratory of the "Tête d'Or", in Lyon.

2.2. Collection and preparation of soil from field sites and plants

Soil was collected from a station located near Saint Etienne (Rhône Alpes, France), the site is contaminated with heavy metals, the soil Usinor "U" is the location of one of the largest metallurgical plant in France. The soil with different concentrations of metals was incubated in plastic pots for 1 month. Soil was maintained as 70% in field capacity, and weighted with water every day. Plants were grown in 10 cm diameter pots in a growth chamber at a thermoperiod and a photoperiod of 22 °C/16 h the day, and 20 °C/8 h the night (150 µmol photons m^{-2} s^{-1}).

Seeds of *A. hortensis* and *A. rosea* were germinated for 3 days on a Petri dish filled with water-soaked sponge. Seedlings were transported to the 10 cm-pots in diameter and filled with soils having different concentrations of metals, and incubated one month in a greenhouse, five seedlings were planted per pot. After harvest, tissues were separated into shoots and roots. Shoots and roots were washed with distilled water, and then dried at 50°C for 72 h.

2.3. Analyses

SOD was assayed by the nitroblue tetrazolium (NBT) method as described by GONG et al. (2005). The reaction mixture (3 mL) contained 50 mM K-phosphate buffer, pH 7.3, 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4 mM riboflavin and enzyme extract (0.2 mL). Riboflavin was added last, and the glass test tubes were shaken and placed under fluorescent lambs (60 mmol m^{-2} s^{-1}). The reaction occurred for 5 min and was then stopped by switching off the light. The absorbance was measured at 560 nm. Blanks and controls were run in the same manner, but without illumination and enzyme extract, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under the assay conditions.

Tab. 1: Soil characteristics and physiochemical properties

	Soil nature	
Composition	Sand	Soil of Usinor
Mineral elementals	**<----- Concentartion (ppm) ----->**	
Cao	6.76	3.74
Fe_2O_3	1.33	43.62
K_2O	2.32	0.66
MgO	0.79	1.69
MnO	0.02	0.55
Na_2O	1.23	0.63
P_2O_3	0.06	0.19
SiO_2	76.56	32.22
Heavy metals	**<----- Concentration (ppm) ----->**	
Ni	3.3	1673.7
Pb	14.2	1333.5
Cu	2.3	501
Zn	22.4	3587.9

Fig. 1: View of the metal-tolerant plant communities (mainly *Atriplex rosea*) over the base-metal mine near St Etienne, France.

APX activity was determined by following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 1 min in 2 mL of a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 1 mMEDTA–Na_2, 0.5 mM ascorbic acid, 0.1 mMH_2O_2 and 50 mL of crude enzyme extract at 25°C. APX was determined according to NAKANO and ASADA (1981). The decrease in ascorbate concentration was followed as a decline in the optical density at 290 nm, and activity was calculated using the extinction coefficient (2.8 mM^{-1} cm^{-1} at 290 nm) for ascorbate.

CAT activity was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of H_2O_2 (CAKMAK et al., 1993). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2 and 50 mL of crude enzyme extract at 25 °C. The activity was calculated using the extinction coefficient (40 mM^{-1} cm^{-1}) for H_2O_2 (KATO and SHIMIZU, 1987).

GSH was assayed by the enzymatic recycling procedure in which it is sequentially oxidized by 5,5'-dithiobis (2-nitrobenzoic acid)

(DTNB) and reduced by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of glutathione reductase according to GRIFFITH (1980). The ground tissue (approximately 1 g fresh wt.) was, homogenized in 4 ml 5% sulfosalicyclic acid and centrifuged at 10 000 x *g* for 10 min. A 330 µl aliquot was removed and neutralized by addition of 18 µl 7.5 *M* triethanolamine. One 150 µl sample was then used to determine concentrations of sure GSH plus (GSSG). Another was pretreated with 3 µl 2-vinylpyridine for 60 min at 20°C to mask the GSH by derivatization and to allow the subsequent determination of GSSG alone.

2.4. Statistical Analysis

The pot experiment was set up in randomized complete block design replicated with five replications. Differences were analyzed using one-way ANOVA followed by post-hoc comparisons. ANOVA (Statistica V6.1) was employed for statistical analysis of data. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Plant growth

The present study provided evidence that the responses of the three species of *Atriplex* to metal stress differed, as shown by growth and stress parameters.

In this study, alleviation of metal toxicity stress by the addition of polluted soil was assessed by measuring the shoot and root growth as well as the measurement of antioxidant enzymes. Metal toxicity significantly decreased the shoot and root growth of *Atriplex*. The reduction in growth that resulted from metal toxicity was significantly alleviated by 100% polluted soil. Compared to shoot growth, root growth was the more sensitive endpoint. The plant growth expressed as shoot height and dry weight of shoots and roots (Tab. 2) was adversely inhibited when exposed to metal stress. Inhibition of plant growth by metal combinations was much severe than that of 100% Usinor alone treatment for both taxa, indicating the existence a highly concentration of heavy metals in the soil.

Shoot and root dry matter production was always significantly reduced ($p \leq 0.05$) in contaminated soil (Tab. 2). *A. hortensis* had the highest shoot and root biomass at the end of the experiment in uncontaminated soil. In soil Usinor the difference between 25, 50, 75% concentration was significant, the large difference was probably due to the difficulty for plants to grown in soil highly contaminated with heavy metals. The effects of the heavy metals over the shoot growth were different as compared to the effects on root growth. The biomass of shoots and roots of the *A. rosea* and *A. hortensis* in the control treatment were significantly higher than that in the metal treatment ($P < 0.05$). This indicates that high levels of heavy metal in the soil inhibited the growth of those two plant species. The most general visible symptom of heavy metal stress is growth inhibition, which has been investigated in many plants, including *Atriplex* (Tab. 2).

3.2 Activies of Antioxidant enzymes

Under controlled conditions, varietal differences in glutathione reductase activity (Fig. 3) were similar to those observed for catalase. *A. rosea* expressed glutathione reductase at high levels, glutathione reductase activity in *A. hortensis* increase under metal-stress conditions (276 U/gFwt with 100% "U" respectively). The assay for glutathione reductase in control tissue showed *A. hortensis* (red) to have significantly lower glutathione reductase activity than the other two cultivars (Fig. 3). After metal stress, no significant changes in catalase activity were observed for *A. hortensis* (green) and *A. rosea* after 25 and 50% polluted soil treatment (Fig. 2). A 4-week exposure of *A. hortensis* (green) to metal stress gave a respective mean values of 11.86, and 12.85 U/gFM with 25 and 50% contaminated soil. These values were significantly ($p \leq 0.05$) higher than the 9.37 U/gFM observed for the control. However, increases in catalase activity were recorded in stressed plant of annual *Atriplex*.

There were no significant differences in ascorbate peroxidase activity of *Atriplex hortensis* (red) grown under any conditions. However, in the 25% soil contaminated treatment, APX activity gave a respective

Tab. 2: Height, stems and root biomass production (g/plant) of *Atriplex hortensis* (green and red) and *A. rosea* after 4 weeks in the pot experiment, with the "U" soil.

Taxon	Type of soil	Height (cm)	Dry weight (g/plant)	
			Stems	Roots
	Sand (S)	26.4 b	0.16 b	0.04 ab
Atriplex	S + 25% Usinor	22.8 c	0.14 b	0.04 ab
hortensis	S + 50% Usinor	15.5 d	0.10 c	0.07 a
Green	S + 75% Usinot	12.0 e	0.05 d	0.01 b
	100% Usinor	8.6 f	0.04 d	0.01 b
	$LSD_{0.05}$	3.2	0.03	0.035
	Sand (S)	39.0 a	0.29 a	0.13 a
Atriplex	S + 25% Usinor	21.4 b	0.13 c	0.06 b
hortensis	S + 50% Usinor	12.2 c	0.11 cd	0.03 c
Red	S + 75% Usinot	9.3 d	0.09 d	0.02 c
	100% Usinor	6.5 e	0.03 e	0.01 d
	$LSD_{0.05}$	2.6	0.03	0.01
	Sand (S)	21.0 b	0.08 b	0.004 ab
Atriplex	S + 25% Usinor	16.0 c	0.07 bc	0.005 a
rosea	S + 50% Usinor	13.7 d	0.06 c	0.005 a
	S + 75% Usinot	10.8 e	0.06 c	0.003 b
	100% Usinor	11.5 e	0.07 bc	0.003 b
	$LSD_{0.05}$	1.8	0.02	0.002

Data followed by different letters are significantly different LSD at $P < 0.05$.

mean values 6.60 U/gFM in *A. hortensis* (green) and 6.69 U/gFM in *A. rosea* (Fig. 5). No significant changes in ascorbate peroxidase activity were observed for *A. hortensis* (green) after 25 and 50% polluted soil treatment.

The activity of SOD was significantly lower in the *A. hortensis* controls than in the controls of the *A. rosea* (Fig. 4). No significant increases in SOD activity were observed when *A. hortensis* or *A. rosea* were subjected to the metal treatment, but SOD activity decreased significantly in *A. hortensis* and *A. rosea* (0.06 U/gFM with 100% "U" respectively).

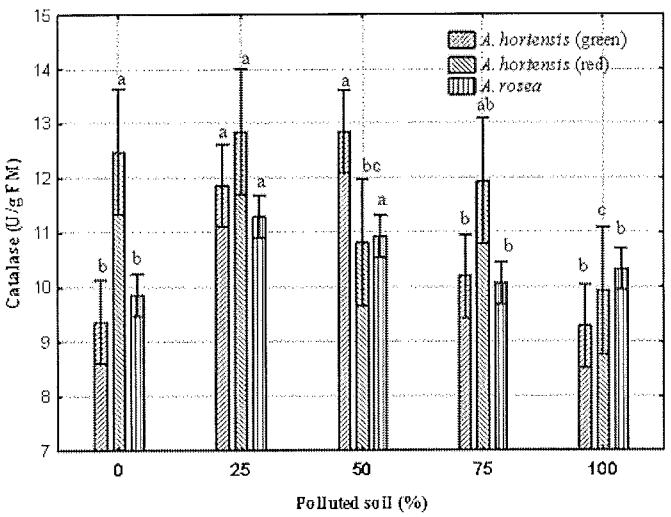

Fig. 2: The activity of catalase (CAT) related to the fresh mass (FM) in the leaves of *Atriplex* plants cultured under no stressed (0% polluted soil e.g. 100% sand) and stressed (25, 50, 75 and 100% pooluted soil) conditions. Values ± SE are average of five independent experiments, each with three replicates. Letters indicate a sig-nificant difference from the representative control value at $p = 0.05$.

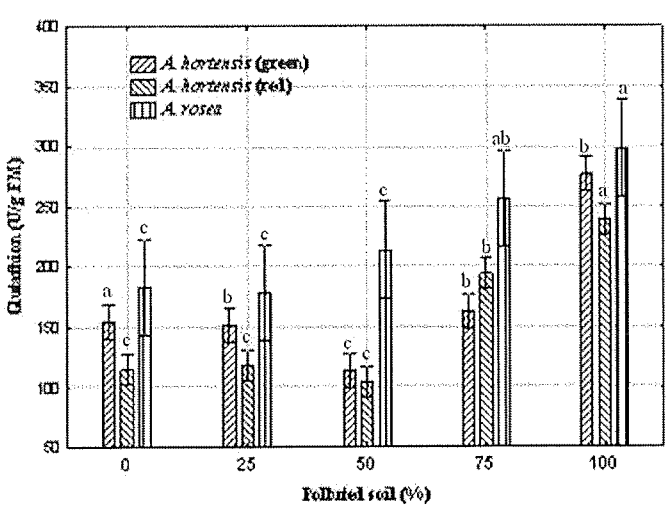

Fig. 3: The activity of reduced glutathione (GR) related to the fresh mass (FM) in the leaves of *Atriplex* plants cultured under no stressed (0% polluted soil e.g. 100% sand) and stressed (25, 50, 75 and 100% pooluted soil) conditions. Values ± SE are average of five independent experiments, each with three replicates. Letters indicate a significant difference from the representative control value at $p = 0.05$.

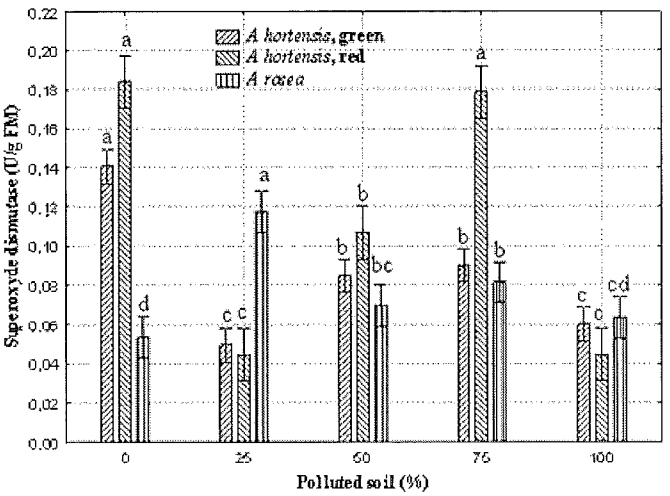

Fig. 4: The activity of superoxide dismutase (SOD) related to the fresh mass (FM) in the leaves of *Atriplex* plants cultured under no stressed (0% polluted soil e.g. 100% sand) and stressed (25, 50, 75 and 100% pooluted soil) conditions. Values ± SE are average of five independent experiments, each with three replicates. Letters indicate a significant difference from the representative control value at $p = 0.05$.

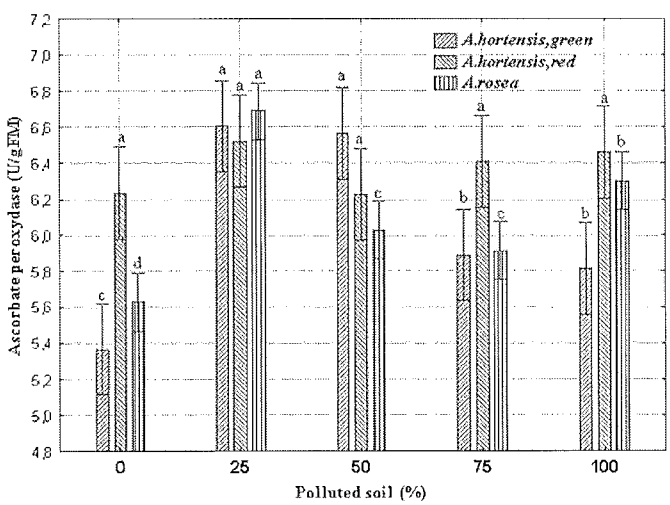

Fig. 5: The activity of ascorbate peroxidase (APX) related to the fresh mass (FM) in the leaves of *Atriplex* plants cultured under no stressed (0% polluted soil e.g. 100% sand) and stressed (25, 50, 75 and 100% pooluted soil) conditions. Values ± SE are average of five independent experiments, each with three replicates. Letters indicate a significant difference from the representative control value at $p = 0.05$.

4. Discussion

Recently, it has been reported that the root growth is a more sensitive endpoint of metal availability than chlorophyll assays (MORGAN et al., 2002). This growth inhibition was concentration-dependent and exhibited a positive correlation with the reduction in the viability of root cells (SIROKA et al., 2004). In conclusion, between the two species tested, *A. hortensis* plants grew much more rapidly and were able to yield higher biomass in comparison with *A. rosea*. The saltbush *Atriplex canescens* has been especially recommended for revegetation of mine sites and other harsh environments (NEWMAN and REDENTE, 2001).

The inhibition of root growth can be attributed in part to the inhibition of mitosis, the reduced synthesis of cell-wall components, damage to the Golgi apparatus, and changes in the polysaccharide metabolism, while browning is caused by suberin deposits (PUNZ and SIEGHARDT, 1993). An interaction of heavy metals with salini-ty factors in soils and plants is present under field conditions and a stronger soil salinity might increase the contents of heavy metals and specific metabolites in plant products considerably (HELAL et al., 1999). Since the phytoextraction of contaminants depends on shoot biomass production, also agronomic practices need to be developed to optimize growth. Furthermore, because absorption by roots could possibly be limited by reduced bioavailability, amendment strategies may need to be employed to increase metal bioavailability in the soil (EBBS et al., 1997). Such improvement in yield by Si under different oxidative stress conditions, such as salt stress in tomato (ROMERO-ARANDA et al., 2006), Al toxicity in barley (MORIKOWA and SAIGUSA, 2002), Mn toxicity in cucumber and cowpea (IWASAKI et al., 2002), As toxicity in rice (GUO et al., 2005), Cd toxicity in strawberry (TREDER and CIESLINSKI, 2005) and maize (LIANG et al., 2005) and drought stress in wheat (GONG et al., 2005) and sorghum (HATTORI et al., 2005) have been reported previously. A variety of abiotic stresses can cause molecular damage to plant cells either directly or indirectly through the formation of ROS (LIN and KAO, 2001).

However, in this study, the higher antioxidative activity was observed in *Atriplex* genus, indicating that the increased antioxidative activity might reflect a damage response to stress factors, which was in agreement with the report of MITTAL and DUBEY (1991), who presumed that high lipid peroxidation and antioxidative ability both were parts of a damage response to salinity in rice cultivars. Under metal toxicity, *A. hortensis* and *A. rosea* had not similar SOD activities (Fig. 4), which indicated that the dismutating capacities in C_3 and C_4 crops were different. However, LUNA et al. (1985) found that maize (C_3) had higher SOD activity than wheat. In maize leaves, increases were observed in the activity of peroxidase (KONG et al., 1999) and glutathione reductase (PAL et al., 2002) and decreases in that of superoxide dismutase and catalase (KONG et al., 1999), while the inhibition of guaiacol peroxidase was reported in the roots (PAL et al., 2002). Superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase activities as well as reduced and oxidized glutathione contents in all samples of leaves, roots and stolons were increased in the presence of Cd (ALKORTA and GARBISU, 2001). Cadmium causes a transient depletion of gluthathione and an inhibition of antioxidative enzymes, especially of glutathione reductase (SCHUTZENDUBEL and POLLE, 2002).

Beside SOD, glutathione is another important compound that may be involved in resistance to heavy metals. As an endogenous antioxidant molecule, it helps to reduce the effect of secondary oxidative stress resulting from the production of reactive oxygen species (NOCTOR et al., 2002). But it also constitutes the precursor of phytochelatins, which are small peptides binding to metal and accumulating in vacuoles (COBBETT and GOLDSBROUGH, 2002). Although glutathione has been found to increase in response to numerous environmental stresses in several species (NOCTOR et al., 2002), our results reveal that heavy metals induced an increase of both the reduced and the oxidized form of glutathione in the shoots of annual *Atriplex*. The results related to antioxidant responses under metal toxicity were in agreement with our previous work (GUNES et al., 2006) and the findings of MOLASSIOTIS et al. (2006), who reported increased CAT activity under B toxicity in grapevine and apple rootstocks. In contrast to this KARABAL et al. (2003) have shown increased SOD activity in tobacco leaves and barley, respectively, under B toxicity. We have shown that the activities of CAT and GR in metal stressed plants increased.

As the result of heavy-metal stress, changes occur in the lipid composition, and the membranes become rigid, thus resulting in changes in the activity of enzymes bound to membranes (FODOR et al., 1995). Plants synthesize numerous antioxidant molecules, such as glutathione, and enzymes, including catalase, superoxide dismutase, ascorbate peroxidase, glutathione-S-transferase, and glutathione reductase, as a defense response against oxidative stress. Many data in the literature confirm the increase in antioxidant activity in the course of heavy-metal stress (ZACCHINI et al., 2003). Above a certain heavy metal concentration, however, the antioxidant enzymes were found to be inhibited (STIBOROVA et al., 2004). A stressed plant (JING et al., 2003) is usually accompanied by a decrease in APX and GR activities in parallel with an increase of lipid peroxidation. In naturally senescing cucumber cotyledons, the GR activity decreased whereas APX activity increased.

Heavy metal ions reduce the efficiency of photosynthesis by inhibiting the key enzymes (Rubisco, phosphoenolpyruvate carboxylase) of the Calvin cycle (STIBOROVA et al., 1986a).

Plant peroxidases are oxidoreductive enzymes related to the metabolism of several organic contaminants (KOCHIAN and JONES, 1997). The level and isoenzyme pattern of peroxidases can be altered by environmental stress and these enzymes are frequently used as non-specific biomarkers of environmental pollution. The peroxidase activity has been used to evaluate contaminant exposure to terrestrial and aquatic plants. Increased peroxidase levels are thought to protect plant cells from free radical oxidation, allowing the plant to adapt to the stressor. The decay in peroxidase activity could be a result of the acute toxic effect produced on plants, as reflected by the ceasing of growth and other physiological parameters. Senescence is accompanied by an increasing generation of ROS and consequent oxidative damage (MUNNE-BOSCH and ALLEGRE, 2002).

5. Conclusion

The results of this study of *Atriplex* plant cultured in polluted soil show that varietal differences in metal tolerance of growth production are correlated with differences in antioxidant-enzyme activities. Notably *Atriplex* has been shown to exhibit metal tolerance, presenting no decrease in glutathione reductase and catalase activity. When exposed to heavy metals, the three taxa *A. hortensis* and *A. rosea*, all exhibited significant decreases in the activity of superoxide dismutase. Enzyme assays indicate that tolerance in *Atriplex* plant may be related to higher constitutive levels of glutathione reductase and catalase and a greater capacity to regulate ascorbate peroxidase activity.

In conclusion, it can be said that, although numerous questions remain to be clarified, a number of defense mechanisms capable of protecting plants from the effects of polluted soil have been discovered in recent years. Some of these mechanisms, such as the defense against heavy metals, while other processes, such as the functioning of the antioxidant system, are also involved in general stress tolerance. An important field for further research would be the tolerance mechanism of plants exhibiting metal hyperaccumulation. The knowledge gained in such investigations could facilitate both selection and the breeding of heavy metal-tolerant plants. Additional research is necessary to provide further insight concerning the specific relationship between metal stress and the antioxidant response.

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