BIOLOGIA PLANTARUM 48 (4): 575-581, 2004

Effects of NaCl and mycorrhizal fungi on antioxidative enzymes in soybean

M. GHORBANLI*, H. EBRAHIMZADEH** and M. SHARIFI*[1]

*Department of Biology, Tarbiat Moalem University, No. 49, Dr Mofatteh Ave. Tehran, Iran**
*Department of Biology, Tehran University, Enghelab Ave., Tehran, Iran***

Abstract

The effects of different concentrations of NaCl on the activities of antioxidative enzymes in the shoots and roots of soybean (*Glycine max* [L.] Merr cv. Pershing) inoculated or not with an arbuscular mycorrhizal fungus, *Glomus etunicatum* Becker & Gerdemann, were studied. Furthermore, the effect of salt acclimated mycorrhizal fungi on the antioxidative enzymes in soybean plants grown under salt stress (100 mM NaCl) was investigated. Activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were increased in the shoots of both mycorrhizal (M) and nonmycorrhizal (NM) plants grown under NaCl salinity. Salinity increased SOD activity in the roots of M and NM plants, but had no effect on CAT and polyphenol oxidase activities in the roots. M plants had greater SOD, POD and ascorbate peroxidase activity under salinity. Under salt stress, soybean plants inoculated with salt pre-treated mycorrhizal fungi showed increased SOD and POD activity in shoots, relative to those inoculated with the non pre-treated fungi.

Additional keywords: ascorbate peroxidase, catalase, *Glycine max*, peroxidase, polyphenol oxidase, salinity, superoxide dismutase.

Introduction

There is accumulating evidence that production of reactive oxygen species (ROS) is a major damaging factor in plants exposed to different environmental stresses, including salinity (Hernandez *et al.* 1995). These oxygen species can seriously disrupt normal metabolism through oxidative damage of lipids, proteins (Jiang and Zhang 2001) and nucleic acids (Imlay and Linn 1988). Plants with high concentrations of antioxidants have been reported to have greater resistance to these oxidative damages (Spychalla and Desbough 1990, Dionisio-Sese and Tobita 1998, Jiang and Zhang 2002). Plants have evolved specific protective mechanisms, involving antioxidant molecules and enzymes in order to defend themselves against oxidants (Jiang and Zhang 2002, Núñez *et al.* 2003/4). Therefore, antioxidants and antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APOX) function to interrupt the cascades of uncontrolled oxidants in some organelles (Jiang and Zhang 2001, Harinasut *et al.* 2003).

Salinity results in higher activities of CAT (Dionisio-Sese and Tobita 1998), POD (Gossett *et al.* 1994) and SOD (Sudhakar *et al.* 2001). Also, increased APOX and SOD activities in response to salt stress has been reported (Benavides *et al*. 2000, Arbona *et al.* 2003). Mycorrhizal colonization is often thought to increase salinity tolerance of host plants (Hirrel and Gerdemann 1980, Al-Karaki *et al*. 2001). Arbuscular mycorrhizal (AM) fungi have been shown to promote plant growth and salinity tolerance mainly by enhancing nutrient acquisition (Al-Karaki and Al-Raddad 1997), producing plant growth hormones, improving rhizospheric and soil conditions (Linderman 1994), altering host physiological and biochemical properties (Smith and Read 1995), and defending roots against soilborne diseases (Dehne 1982). AM fungi are well known to bring about physiological changes in plants by increasing various enzymatic activities. Total POD and polyphenol oxidase (PPO) activities have been enhanced in mycorrhizal plants (Mathur and Vyas 1995, 1996).

Received 26 November 2003, *accepted* 15 April 2004.
Abbreviations: AM - arbuscular mycorrhizae; APOX - ascorbate peroxidase; CAT - catalase; CFU - colony forming unit; LSD - least significant difference; M - mycorrhizal; NBT - nitroblue tetrazolium; NM - non mycorrhizal; POD - peroxidase; PPO - polyphenol oxidase; ROS - reactive oxygen species; SOD - superoxide dismutase.
Acknowledgements: This work was supported by Tarbiat Moalen University of Tehran. Also the authors acknowledge Biology Department of Tarbiat Moalem University and Department of Plant Physiology of Tehran University.
[1] Corresponding author; fax: (+98) 21 6400997, e-mail: msharifi@khayam.ut.ac.ir

There is little information about the effect of NaCl salinity on enzymatic antioxidant defense system using mycorrhizal inoculation. Therefore in this study, an effort was made to assess the influence of AM inoculation on the SOD, POD, CAT, APOX, and PPO activities in soybean plants grown under different NaCl concentrations. Also, we investigate the effect of salt pre-treated (salt acclimated) AM fungi on the enzymatic activities of the host plant after exposure to salt stress. The aim of this study was to discover a possible inter-relationship between AM inoculation and antioxidative enzymes in soybean plants exposed to different concentrations of NaCl. In the second experiment, we expected that exposure of AM fungi to low salinity conditions prior to salt stress, may affect these activities as a result of salt acclimation of AM fungi.

Materials and methods

Mycorrhizal inoculation and soybean culture under different NaCl concentrations: The AM fungus (*Glomus etunicatum* Becker & Gerdemann) was isolated from a soybean (*Glycine max* [L.] Merr. cv. Pershing) field in the north of Iran and multiplied in pot cultures using maize as a host plant. A mixture of soil, root fragments and spores (15 spores per g soil) obtained from the pots was used as AM inoculum. For plant growth, a 4:1 mixture of autoclaved sand and soil was dispensed into plastic pots using 1.5 kg per pot. Half of the pots received the AM fungus *G. etunicatum* by adding 100 g of the inoculum, while the other half received 100 g of the autoclaved inoculum, as NM controls.

Soybean seeds were germinated in moistened vermiculite. Three 7-d-old seedlings, uniform in size, were soaked in 20 cm^3 of 1.66×10^7 CFU cm^{-3} soybean *Rhizobium* culture for 5 min, then were transplanted into each pot. To achieve a uniform stand, the plants were thinned to one seedling per pot after 7 days. The mycorrhizal and nonmycorrhizal treated seedlings were grown for 4 weeks after the transplantation, then subjected to four NaCl concentrations: 0, 50, 100 and 150 mM. The plants were watered with the salt solutions once a week to get different salt treatments, after the pots were completely washed by distilled water to avoid build-up of salinity in the pots. At the initiation of the salinity treatment, NaCl concentration was gradually increased by 50 mM at 1-day intervals until reaching the required salinity of NaCl for each concentration. Plants received 50 cm^3 pot^{-1} wk^{-1} of half-strength Hoagland's solution (Hoagland and Arnon 1950) until harvest and were watered daily if necessary by 50 cm^3 of tap water.

Salt pre-treated AM fungi for inoculation of soybean plants grown under salt stress: In the first step of this experiment, to obtain salt adapted mycorrhizal fungi, maize plants were used as a host for the fungus. Maize seeds were germinated in moist vermiculite, and three seedlings were transplanted into each pot containing a 4:1 mixture of autoclaved sand and soil plus either 100 g of the *G. etunicatum* inoculum or of the autoclaved inoculum. Four wk after transplantation, half of the M and NM maize plants were exposed to NaCl with a gradual increase in concentration (salt adaptation). The salt concentration was increased by 25 mM at 3-d intervals, reaching the maximum of 100 mM after 12 d. The remaining M and NM plants received no salt as non saline control. Thus, there were four treatments (+salt+AM, +salt−AM, −salt+AM, −salt−AM) with three replications per treatment. Pots were irrigated daily by 50 cm^3 tap water if necessary, and received 50 cm^3 pot^{-1} $week^{-1}$ of the half-strength Hoagland's solution (Hoagland and Arnon 1950). Plant shoots were cut 13 d after the highest salt concentration (100 mM) was reached. Roots from the M and NM treatments were extracted from the soil and were used as salt pre-treated and non salt pre-treated inocula in the second step of this experiment. Root colonization for salt pre-treated and non salt pre-treated mycorrhizal maize was not statistically different and were 59 and 61 %, respectively.

In the second step, pots containing sterilized sand and soil (4:1) were inoculated with the above mentioned maize root inocula, 33.3 g fresh root per pot. This step consisted of four treatments, including salt pre-treated mycorrhiza, non salt pre-treated mycorrhiza, salt pre-treated nonmycorrhiza and non salt pre-treated nonmycorrhiza, with three replications for each treatment. Three 7-d-old soybean seedlings were soaked in 20 cm^3 of 1.66×10^7 CFU cm^{-3} soybean *Rhizobium* culture for 5 min then were transplanted into each pot. After 7 d the plants were thinned to one seedling per pot to achieve a uniform stand. Plants were established for 4 weeks before being subjected to 100 mM NaCl, which was found in the first experiment as a salt stress condition for soybean, that does not inhibit the mycorrhizal colonization (data not published). All the plants were grown under 100 mM NaCl salinity for 4 weeks, and shoots and roots were then used for the assays.

In both experiments, the greenhouse conditions for plant growth were: 16-h photoperiod, irradiance of 500 μmol m^{-2} s^{-1} (high-power sodium vapour lamps), day/night temperature of 29/22 °C.

Enzyme extraction and assays: Extract for determination of SOD, POD, CAT and PPO were prepared from 1 g of plant material homogenized in 5 cm^3 of 50 mM Tris-HCl buffer, pH 7.5. Because APOX is labile in the absence of ascorbate, 5 mM ascorbate was

included for the extraction of this enzyme with the above procedure. The homogenate was filtered and then centrifuged in a refrigerated centrifuge at 13 000 *g* for 20 min, and the supernatant obtained was used for the assays. All the steps were carried out at 4 °C.

POD activity was determined as described by Kar and Mishra (1976). The reaction mixture contained 100 mM Tris-buffer (pH 7.0), 0.005 M H_2O_2, and 10 mM pyrogallol. 50 μm^3 of the enzyme extract was added to initiate the reaction which was measured spectrophotometrically at 425 nm.

CAT activity was assayed in the extract by measuring the decrease in absorption at 240 nm (Kar and Mishra 1976) in a reaction medium containing 60 μm^3 of the enzyme extract, 50 mM Tris-buffer, pH 7.0, and 5 mM H_2O_2.

Total SOD activity was determined by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) as described by Beauchamp and Fridovich (1971). The reaction mixture consisted of 100 μm^3 of enzyme extract, 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, and 2 μM riboflavin. Test tubes were shaken and placed 30 cm from a light bank consisting of three 30 W fluorescent lamps. The reduction in NBT was measured by reading absorbance at 560 nm. Blanks and controls were run in the same manner but without illumination and enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced a 50 % inhibition of NBT reduction under the assay conditions (Giannopolitis and Ries 1977).

APOX activity was measured immediately in fresh extracts and assayed as described by Nakano and Asada (1981) using reaction mixture containing 50 mM K-phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM ascorbate, and 0.1 mM EDTA. The hydrogen peroxide-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm.

PPO activity was assayed using the method of Kar and Mishra (1976). The reaction mixture containing 100 mM Tris-buffer (pH 7.6), 10 mM pyrogallol and enzyme solution was incubated at 25 °C for 5 min. The absorbance of the purpyrogallin formed was taken at 420 nm.

Protein determination: Protein content was evaluated by the method of Bradford (1976) using bovine serum albumin as a standard.

Statistical analysis: The experiment was randomized in complete blocks with four salt concentrations, two AM inoculum treatments and three pots as replications, and was terminated by harvesting the plants after 4 weeks of growth under saline conditions. Shoots and roots were used for analyses. Data were analyzed statistically using analysis of variance with *MSTATC*. Probabilities of significant difference between treatments and interactions, and LSDs ($P \leq 0.05$) were used to compare means within and between treatments.

Results

Effect of different NaCl concentrations on the enzyme activities of soybean inoculated or not with mycorrhizal fungi: SOD activity in shoots of M and NM plants grown under 50 and 100 mM NaCl was significantly greater than those plants that were grown under non saline conditions (Table 1). No significant difference was observed for shoot SOD activity between 150 mM NaCl and the non saline treatments (Table 1). In contrast to the shoot, SOD activity in roots of M and NM plants were not significantly different under 50 and 100 mM NaCl compared to the non saline control, but it was significantly increased at 150 mM relative to the other treatments (Table 1). Under the all treatments, SOD activity in shoots of M plants was significantly greater than NM plants, the percent increases were 50 % at control, 29 % at 50, 33 % at 100 and 39 % at 150 mM NaCl (Table 1). While, root SOD activity was significantly higher in M plants than in NM plants only at 50 and 100 mM NaCl (Table 1).

POD activity was significantly enhanced in shoots of both M and NM plants at all NaCl concentrations compared to the non saline control (Table 1). As NaCl was increased up to 100 mM, POD activity of M and NM plants was elevated directly proportional to the salt concentration. A significant decrease in POD activity was observed for M plants when NaCl increased from 100 to 150 mM, while the value was unaltered in NM plants. M plants had higher POD activity than NM plants under the saline and non saline treatments, except for 150 mM NaCl. The increments were 48 % at 0, 39.7 % at 50 and 28.9 % at 100 mM NaCl (Table 1)

CAT activity in the shoots of M and NM soybean was significantly enhanced when the plants were grown under 100 and 150 mM NaCl relative to the non saline control, but there was no significant difference between 0 and 50 mM treatments (Table 1). The levels of CAT activity in the shoots and roots were not altered in response to mycorrhizal inoculation, and there were no significant differences between M and NM plants at any NaCl treatment. Among the various NaCl treatments, the level of CAT activity in roots of M and NM plants was not altered (Table 1).

Mycorrhizal colonization caused a significant increment in APOX activity in soybean shoots when the plants were exposed to NaCl at any concentration (Table 1). The increments induced by mycorrhizal

inoculation were 17.5 % at 50, 17.9 % at 100 and 66.5 % at 150 mM NaCl. Among M plants, APOX activity in shoots was not significantly altered due to NaCl, whereas, a significant decrease in APOX activity was detected for NM plants by increasing the salinity to 150 mM (Table 1).

Among NM plants there were no significant changes in PPO activity of shoots in response to different saline conditions, although a relative increase was observed in PPO activity at 150 mM NaCl (Table 1). Change in the enzyme activity for M plants was significant only at 150 mM NaCl compared to the other treatments. No significant alteration in PPO activity was found between M and NM plant shoots at all NaCl treatments (Table 1). Mycorrhizal roots had relatively higher PPO activity than nonmycorrhizal roots under all salinity treatments, but the variations were not significant (Table 1). Change in PPO activity in response to various NaCl concentrations was not significant in the roots of M plants, whereas NM plants subjected to salinity had significantly higher PPO activity than the non saline control (Table 1).

Table 1. The activities of SOD [U g^{-1}(protein)], POD [ΔA_{425} mg^{-1}(protein) min^{-1}], CAT [ΔA_{240} mg^{-1}(protein) min^{-1}], APOX [ΔA_{290} mg^{-1}(protein) min^{-1}] and PPO [ΔA_{420} mg^{-1}(protein) min^{-1}] in soybean shoots and roots in presence (M) and absence (NM) of mycorrhizae at NaCl concentrations 0, 50, 100, 150 mM. Means in each row indicated by different letter are significantly different ($P \leq 0.05$) according to LSD test. Figures in parentheses in each row indicate percent of the non saline control.

	Enzymes	Treatments	0	50	100	150
Shoots	SOD	NM	4.50 ± 0.28c	6.30 ± 0.25b (140)	5.92 ± 0.24b (131)	4.80 ± 0.20c (106)
		M	6.75 ± 0.20b	8.14 ± 0.40a (120)	7.90 ± 0.46a (117)	6.71 ± 0.20b (99)
	POD	NM	5.41 ± 0.57e	7.55 ± 0.62d (139)	10.07 ± 1.24bc (186)	10.36 ± 0.36b (191)
		M	8.03 ± 0.63cd	10.55 ± 0.43b (131)	12.99 ± 0.59a (161)	10.10 ± 0.84b (125)
	CAT	NM	3.28 ± 0.10c	3.35 ± 0.14c (102)	4.50 ± 0.15b (137)	6.17 ± 0.60a (188)
		M	3.35 ± 0.04c	3.46 ± 0.02c (103)	4.80 ± 0.32b (143)	6.53 ± 0.29a (194)
	APOX	NM	16.37 ± 1.13b	16.77 ± 0.63b (102)	17.25 ± 0.72b (105)	12.03 ± 0.53c (73)
		M	18.15 ± 1.00ab	19.72 ± 0.22a (108)	20.35 ± 0.54a (112)	20.03 ± 0.43a (110)
	PPO	NM	2.03 ± 0.15b	2.01 ± 0.16b (99)	2.07 ± 0.14b (101)	2.31 ± 0.06ab (113)
		M	2.23 ± 0.13b	2.31 ± 0.06ab (103)	2.31 ± 0.09ab (103)	2.67 ± 0.20a (119)
Roots	SOD	NM	1.72 ± 0.24d	1.71 ± 0.15d (99)	2.24 ± 0.12cd (130)	3.58 ± 0.20b (208)
		M	2.10 ± 0.47cd	3.05 ± 0.61bc (145)	3.03 ± 0.40bc (144)	4.96 ± 0.28a (236)
	CAT	NM	1.65 ± 0.15a	1.69 ± 0.28a (102)	2.10 ± 0.18a (127)	1.90 ± 0.03a (115)
		M	1.71 ± 0.14a	2.00 ± 0.21a (116)	2.10 ± 0.09a (122)	2.10 ± 0.15a (122)
	PPO	NM	4.20 ± 0.30b	5.60 ± 0.60a (135)	5.30 ± 0.20ab (127)	5.50 ± 0.50a (133)
		M	5.40 ± 0.50ab	6.10 ± 0.50a (112)	6.00 ± 0.30a (111)	6.20 ± 0.50a (114)

Effect of salt pre-treated AM fungus on the enzyme activities under salt stress: Under salt stress (100 mM NaCl), no significant differences were observed in activity of each studied enzyme in shoots of soybean inoculated with salt pre-treated nonmycorrhizal inoculum compared to those inoculated with non salt pre-treated one (Table 2). Shoots of soybean plants inoculated with salt pre-treated mycorrhizal inoculum showed significantly greater SOD and POD activity than those inoculated with non salt pre-treated mycorrhizal inoculum. The increments caused by this salt pre-treated inoculom were 34 % for SOD and 15 % for POD. The activities of CAT, APOX and PPO were not different in the shoots of soybean plants inoculated either with salt pre-treated or with non salt pre-treated mycorrhizal inoculums. In this experiment greater SOD, POD and APOX activities were observed in presence of mycorrhizal inocula than of nonmycorrhizal inocula regardless the salt pre-treatment of the inocula (Table 2). At 100 mM NaCl, the assay for SOD, CAT and PPO in the roots showed salt pre-treated mycorrhizal inoculum to induce relatively higher SOD activity than other three inocula (Table 2). Increase in root SOD activity in this treatment was not significantly different relative to non pre-treated mycorrhizal inoculum, while it was different compared to nonmycorrhizal inocula whether salt pre-treated or not. CAT and PPO activities were not significantly altered in soybean roots in response to salt pre-treatment of mycorrhizal fungi, where their activities were nearly same at all treatments (Table 2).

Table 2. The activities of SOD [U g^{-1}(protein)], POD [ΔA_{425} mg^{-1}(protein) min^{-1}], CAT [ΔA_{240} mg^{-1}(protein) min^{-1}], APOX [ΔA_{290} mg^{-1}(protein) min^{-1}] and PPO [ΔA_{420} mg^{-1}(protein) min^{-1}] in soybean shoots and roots under 100 mM NaCl in presence of two non mycorrhizal and two mycorrhizal inoculums (salt pre-treated or nonsalt pre-treated). Means ± SE in a row followed by a different letter are significantly different ($P \leq 0.05$) according to LSD test.

	Enzymes	NM		M	
		non pre-treated	pre-treated	non pre-treated	pre-treated
Shoots	SOD	6.46 ± 0.69c	6.53 ± 0.43c	8.56 ± 0.53b	11.50 ± 0.40a
	POD	10.32 ± 0.92c	11.34 ± 0.49c	13.43 ± 0.4b	15.50 ± 0.46a
	CAT	4.22 ± 0.14b	4.10 ± 0.01b	4.36 ± 0.27b	4.43 ± 0.28b
	APOX	17.43 ± 0.65b	17.30 ± 0.40b	20.82 ± 0.59a	21.00 ± 1.04a
	PPO	2.08 ± 0.16a	2.06 ± 0.17a	2.21 ± 0.05a	2.23 ± 0.30a
Roots	SOD	2.19 ± 0.14b	2.16 ± 0.14b	2.93 ± 0.16a	3.25 ± 0.15a
	CAT	1.79 ± 0.11a	1.74 ± 0.31a	1.76 ± 0.12a	1.90 ± 0.14a
	PPO	4.60 ± 0.20a	4.70 ± 0.30a	4.90 ± 0.40a	4.90 ± 0.50a

Discussion

It has been reported that the content of ROS was elevated with increased salinity, due to the imbalance in the production and destruction of ROS (Asada 1994). The metabolism of ROS is dependent on various functionally interrelated antioxidant enzymes, such as SOD, POD, CAT and APOX. A strong correlation between the antioxidant defense system and salt tolerance in many plants are reported (Benavides *et al.* 2000, Garratt *et al.* 2002). A constitutively high antioxidant capacity under stress conditions can prevent damages due to ROS formation (Harinasut *et al.* 2003).

SOD is reported to play an important role in cellular defense against oxidative stress, because it converts superoxide to hydrogen peroxide (Smirnoff 1993). There are accumulating reports that shown greater SOD activity in salt tolerant plants (Benavides *et al.* 2000, Sudhakar *et al*. 2001).

Our results showed that low and moderate salinity caused a significant increase in SOD activity in shoots of both M and NM plants, whereas root SOD activity was increased under high salinity. This result is similar in part to results obtained by Garratt *et al.* (2003) who found enhanced SOD activity under salinity in cotton. Based on the induced SOD activity in the shoots and roots of soybean grown under NaCl salinity it could be concluded that SOD is important for soybean to tolerate the salinity. Furthermore, enhanced SOD activity in M plants compared to NM plants supports this view that increased antioxidative enzyme activities could be involved in the beneficial effects of mycorrhizal colonization on the performance of plants grown under semi-arid conditions (Alguacil *et al.* 2003). Gradual exposure of the AM fungus to salinity enhanced it's ability to increase SOD activity in the host plants. The great SOD activity in M plants could increase the capacity of shoots and roots to scavenge superoxide radicals. Enhancement of SOD activity under salt stress by salt adapted fungi may correlate with increased infectivity of the adapted fungi compared to non adapted fungi.

Plant posses hydrogen peroxide scavengering enzymes: POD and CAT. Detoxification of the reactive oxygens protects cells against harmful concentration of hydroperoxides (Castillo 1992). The increased POD in response to salinity have been reported (Harinasut *et al.* 2003). In tolerant plants, POD activity was found to be higher to protect plants against the oxidative stresses (Sreenivasulu *et al.* 1999). In the present study greater POD activity in shoots of M plants than in NM plants grown at NaCl treatments indicates that POD could be involved in mycorrhizal mediated enhancement of host plant growth under salinity. POD activity increased significantly in response to salinity in both M and NM plants, suggesting that in soybean plants POD may play an important role in detoxification of H_2O_2 generated by SOD. Since salt pre-treatment of the AM fungus caused a significant increase in POD activity following exposure to 100 mM NaCl, we concluded that it might be related to increased root length colonized by the salt adapted fungi (data not published).

CAT activity remained unaltered in shoots and roots of mycorrhizal colonized plants compared to non colonized plants, and not affected by salt pre-treatment of the fungi. These results may suggest that CAT is not responsible for detoxification of that H_2O_2 which generated due to mycorrhizal induced SOD activity. We conclude that no relationship exists between CAT activity and beneficial effects of mycorrhizal colonization on soybean growth (data not published) under salinity. However, Alguacil *et al.* (2003) reported that mycorrhizal inoculation increased CAT activity in *Olea europaea* grown under semi-arid conditions. On the other hand, since CAT is involved in decomposition of H_2O_2 in

peroxisomes, similar increases in CAT activity of M and NM plant shoots at moderate and high NaCl indicate that under these conditions H_2O_2 is probably produced in higher concentrations in the peroxisome. However the level of CAT activity in soybean roots was not affected by salinity or by mycorrhizal treatments.

It has been reported that APOX is involved in detoxification of H_2O_2 produced in chloroplasts of some salt stressed plants (Lopez *et al.* 1996, Benavides *et al.* 2000). The results observed with respect to APOX showed that M plants had greater APOX activity compared to NM plants, whereas the levels of APOX activity was unaltered when soybean plants were subjected to NaCl salinity. Based on these results it could be concluded that mycorrhizal inoculation induces the production of H_2O_2 in chloroplast of soybean plants. On the other hand we found that the ability of AM fungi to increase of APOX activity was not affected by gradual salt pre-treatment of the fungi.

PPO is a component of the defense mechanism of plants against pathogens (Mathur and Vyas 1995). It has been reported that mycorrhizal inoculation increased PPO activity in root system (Mathur and Vyas 1996). In present study, PPO activity in shoots and roots were not affected by AM inoculation or by salinity. It could be concluded that AM inoculation may had contrary effects on PPO activity in different plant species.

In conclusion, the present data reveal that exposure of soybean to NaCl salinity result in induction of antioxidative enzyme activities such as SOD, POD and CAT which could help the plants to protect themselves from oxidative effects of the ROS. Whereas, APOX and PPO have not an important role in the antioxidative defense induced by salinity in *Glycine max*. On the other hand, mycorrhizal colonized plants had higher fresh and dry masses under salinity and control conditions (data not published). Hence, the improved growth of M plants under salinity could be related to enhanced SOD, POD and APOX activities which protect plants from oxidative stresses. The results observed for the enzymes responsible for H_2O_2 detoxification (POD, CAT and APOX) lead us to conclude that when the NM plants were subjected to NaCl salinity, POD and CAT are more important than APOX in this detoxification, whereas in presence of mycorrhizal fungi APOX also help the plants to protect themselves from oxidative stresses. M plants probably had higher capacity to remove H_2O_2 by having greater POD and APOX activity. When the AM fungus were exposed to a low concentration of NaCl, greater SOD and POD activity was found in shoots of the host plant following exposure to salt stress, this result suggested that although greater SOD, POD, and APOX was achieved by mycorrhizal inoculation only SOD and POD activities were altered in response to salt adaptation of the AM fungus.

References

Alguacil, M.M., Hernandez, J.A., Caravaca, F., Portillo, B., Roldan, A.: Antioxidant enzyme activities in shoots from three mycorrhizal shrub species afforested in a degraded semi-arid soil. - Physiol. Plant. **118**: 562-570, 2003.

Al-Karaki, G.N., Al-Raddad, A.: Effect of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. - Mycorrhiza **7**: 83-88, 1997.

Al-Karaki, G.N., Hammad, R., Rusan, M.: Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. - Mycorrhiza **11**: 43-47, 2001.

Arbona, V., Flors, V., Jacas, J., García-Agustín, P., Gómez-Cadenas, A.: Enzymatic and non-enzymatic antioxidant responses of *Carrizo citrange*, a salt-sensitive citrus rootstock, to different levels of salinity. - Plant Cell Physiol. **44**: 388-394, 2003.

Asada, K.: Production of active oxygen species in photosynthetic tissue. - In: Foyer, C.H., Mullineaux, P.M. (ed.): Causes of Photoxidative Stress and Amelioration of Defense Systems in Plants. Pp. 77-104. CRC, Boca Raton 1994.

Beauchamp, C., Fridovich, I.: Superoxide dismutase: improved assay and an assay applicable to acrylamide gles. - Anal. Biochem. **44**: 276-287, 1971.

Benavides, M.P., Marconi, P.L., Gallego, S.M., Comba, M.E., Tomaro, M.L.: Relationship between antioxidant defence systems and salt tolerance in *Solanum tuberosum*. - Aust. J. Plant Physiol. **27**: 273-278, 2000.

Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the preinciple of protein-dye binding. - Anal. Biochem. **72**: 248-254, 1976.

Castillo, F.J.: Peroxidases and stress. - In: Penel, C., Gaspar, T., Greppin, H. (ed.): Plant Peroxidases. Topics and Detailed Literature on Molecular, Biochemical, and Physiological Aspects. Pp. 187-203. University of Geneva, Geneva 1992.

Dehne, H.W.: Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. - Phytopathology **72**: 1115-1119, 1982.

Dionisio-Sese, M.L., Tobita, S.: Antioxidant responses of rice seedling to salinity stress. - Plant Sci. **135**: 1-9, 1998.

Garratt, L.C., Janagoudar, B.S., Lowe, K.C.: Salinity tolerance and antioxidant status in cotton culture. - Free Rad. Biol. Med. **33**: 502-511, 2002.

Giannopolitis, C.N., Ries, S.K.: Superoxide dismutases. 1. Occurrence in higher plants. - Plant Physiol. **59**: 309-314, 1977.

Gossett, D.R., Millhollon, E.P., Lucas, M.C.: Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. - Crop Sci. **34**: 706-714, 1994.

Harinasut, P., Poonsopa, D., Roengmongkol, K., Charoensataporn, R.: Salinity effects on antioxidant enzymes in mulberry cultivar. - Sci. Asia **29**: 109-113,

2003.

Hernandez, J.A., Olmos, E., Corpas, F.J., Sevilla, F., del Rio, L.A.: Salt induced oxidative stress in chloroplasts of pea plants. - Plant Sci. **105**: 151-167, 1995.

Hirrel, M.C., Gerdemann, J.W.: Improved growth of onion and bell peppers in saline soils by two vesicular arbuscular mycorrhizal fungi. - Soil Sci. Soc. Amer. J. **44**: 654-655, 1980.

Hoagland, D.R., Arnon, D.I.: The water culture method for growing plants without soil. - Calif. Agr. Exp. Sta. Circ. **347**: 1-38, 1950.

Imlay, J.A., Linn, S.: DNA damage and oxygen radical toxicity. - Science **240**: 1302-1309, 1988.

Jiang, M., Zhang, J.: Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. - Plant Cell Pysiol. **42**: 1265-1273, 2001.

Jiang, M., Zhang, J.: Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. - J. exp. Bot. **53**: 2401-2410, 2002.

Kar, M., Mishra, D.: Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. - Plant Physiol. **57**: 315-319, 1976.

Linderman, R.G.: Role of VAM fungi in biocontrol. - In: Pfleger, F.L., Linderman, R.G. (ed.): Mycorrhizae and Plant Health. Pp. 1-27. American Phytopathological Society, St. Paul 1994.

Lopez, F., Vansuyt, G., Casse-Delbart, F., Fourcroy, P.: Ascorbate peroxidase activity, not the mRNA level, is enhanced in salt-stressed *Rhaphanus sativus* plants. - Physiol. Plant. **97**: 13-20, 1996.

Mathur, N., Vyas, A.: Changes in isozyme patterns of peroxidase and polyphenol oxidase by VAM fungi in roots of *Ziziphus* species. - J. Plant Physiol. **145**: 498-500, 1995.

Mathur, N., Vyas, A.: Biochemical changes in *Ziziphus xylopyrus* by VA mycorrhizae. - Bot. Bull. Acad. sin. **37**: 209-212, 1996.

Nakano, Y., Asada, K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. - Plant Cell Physiol. **22**: 867-880, 1981.

Núñez, M., Mazzafera, P., Mazorra, L.M., Siqueira, W.J., Zullo, M.A.T.: Influence of brassinosteroid analogue on antioxidant enzymes in rice grown in culture medium with NaCl. - Biol. Plant. **47**: 67-70, 2003/4.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. - New Phytol. **125**: 27-58, 1993.

Smith, S.E., Read, D.J. (ed.): Mycorrhizal Symbiosis. Pp. 105-160. - Academic Press, New York 1995.

Spychalla, J.P., Desbough, S.L.: Superoxide dismutase, catalase, and alpha-tocopherol content of stored potato tubers. - Plant Physiol. **94**: 1214-1218, 1990.

Sreenivasulu, N., Ramanjulu, S., Ramachandra-Kini, K., Prakash, H.S., Shekar-Shetty, H., Savithri, H.S., Sudhakar, C.: Total peroxidase activity and peroxidase isoforms as modified by salt stress in two cultivars of fox-tail millet with differential salt tolerance. - Plant Sci. **141**: 1-9, 1999.

Sudhakar, C., Lakshmi, A., Giridarakumar, S.: Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. - Plant Sci. **161**: 613-619, 2001.