Journal of Plant Physiology and Breeding
2017, 7(1): 27-39 ISSN: 2008-5168

# *Trichoderma*-Induced Enhancement of Soybean Seedling Performance in Response to Salt Stress

**Saeid Khomari[1*] and Mahdi Davari[2]**

Received: June 14, 2016 Accepted: May 20, 2017
[1]Department of Agronomy and Plant Breeding, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[2]Department of Plant Protection, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
[*]Corresponding author; Email: saeid.khomari@gmail.com

**Abstract**
In this experiment soybean seeds were pre-treated with salt tolerant isolate of *Trichoderma harzianum* to evaluate the different aspects of seedling growth and metabolism in response to different concentrations of NaCl. *Trichoderma* isolate was more effective in improving dry weight and root volume of seedlings during mild salt stress. Seedlings obtained from bioprimed seeds had significantly higher leaf greenness, chlorophyll fluorescence, net photosynthesis and stomatal conductance than the control at all stress levels. NaCl-induced membrane damage was alleviated by *Trichoderma*, especially at 3 dS/m. The biopriming treatment showed lower accumulation of malondialdehyde (MDA) content under saline condition. Highest MDA content was recorded in the control (unprimed) seeds at salinity level of 9 dS/m. A common factor that adversely affects plants under saline conditions is generation of reactive oxygen species (ROS) and we tested the hypothesis that seed biopriming alleviated damages resulting from ROS attack in the stressed plants. Greatest catalase activity was detected in the bioprimed seeds at the salt stress level of 9 dS/m. The activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX) were significantly increased in response to NaCl stress. Seed biopriming enhanced SOD and APX activity averaged over all salinity levels. It could be concluded that seed biopriming with *Trichoderma harzianum* certainly ameliorated harmful impacts of mild salinity mainly through promotion of early seedling development and antioxidative defence system.

**Keywords:** Antioxidative enzymes; *Glycine max*; NaCl; Photosynthesis; Seedling vigour

## Introduction

Soil salinity is one of the major constraints for most non-halophyte plants in arid areas of the world. Higher salt concentrations cannot be tolerated by most field crops, a fact that severely limits the cultivation in salt affected soils (Wang *et al.* 2013). Saline soil is progressively being aggravated by agronomic practices such as inappropriate irrigation and fertilization, especially in arid regions (Parida and Das 2005). Saline soil water inhibits plant growth by an osmotic effect, which reduces the ability of the plant to take up water by ion-excess, which affects the plant cells (Munns 2005). This implicates that all the physiological disorders associated with the water stress can also be invoked by salt stress. Recent reports show that the area affected by salt in the world is over 800 million hectares, of which 34 million are found in Iran (Qadir *et al.* 2008; Amini *et al.* 2016).

The plant growth is eventually reduced by salinity stress in spite of the fact that plant species vary in their tolerance to salts (Manchanda and Garg 2008). Ideal field establishment depends on the water status of seedbed as well as on the seed germinability and subsequent seedling growth until osmotic potential drop under the threshold (Kiani *et al.* 2015). High levels of salt in soil can hinder seed germination and seedling growth, due to the combined effect of high osmotic potential and specific ion toxicity (Mahajan and Tuteja 2005).

Salt effects are the combined result of the complex interaction among different morphological, physiological and biochemical processes. Salt stress affects many aspects of plant metabolism and, as a result, growth is reduced (Maathuis 2006). Photosynthesis is reduced because it is affected by leaf expansion rate, leaf area and leaf duration, as well as by photosynthesis and respiration per unit leaf area (Manchanda and Garg 2008). Electroconductivity (EC) above 2 dS/m at 25 °C in the saturated-soil extract is considered to be hazardous for salt sensitive plants and injurious to emerging seedlings of most crops (Wahid *et al.* 1999).

Poor germination and non-uniform seedling establishment are the results of soil salinity, which adversely influences growth and development of field crops and results in low agricultural production (Khajeh-Hosseini *et al.* 2003; Rameeh 2012; Wu *et al.* 2015). Soybean is considered as a moderately salt-tolerant industrial crop and the reported salinity threshold is about 5 dS/m (Mass and Hoffman 1977; Rao and Reddy 2010). The deleterious impacts of salinity at early seedling development of soybean range from the loss in emergence and dry weight of seedling to the hampered uptake of nutrient elements. It is assumed that the inhibitory effect of salinity stress on seed germination could be related to a decline in endogenous levels of phytohormones (Javid *et al.* 2011). Nevertheless, application of *Trichoderma* in seed pre-treatments of the most widely grown grain and oilseed crops has resulted in increased levels of plant growth regulators and improved seedling vigor (Harman 2006; Schuster and Schmoll 2010). Biopriming as a biological seed pre-treatment refers to a combination of seed moistening and inoculation with beneficial organisms to protect seed. The technique improves seed germination uniformity even under stressful conditions (Reddy 2013). Some experiments have indicated that root inoculation by *Trichoderma harzianum* results in elevated level of antioxidative enzymes, including various peroxidases to provide substantial tolerance against stress (Harman 2006; Shoresh *et al.* 2010; Hamilton *et al.* 2012). Production of malondialdehyde (MDA), which is an indicative of lipid peroxidation, rises as salinity stress increases in plant and serves as a sign of oxidative damage. Consequently, peroxidation damage of the plasma membrane leads to leakage of contents, rapid desiccation and cell death (Bailly 2004).

Up to date, there is little information about the interaction between *Trichoderma* and salinity on photosynthesis and antioxidative enzymes of crop plants. The aim of this research was to evaluate the effects of seed biopriming with salinity tolerant isolate of *Trichoderma harzianum* on soybean growth performance, photosynthesis and activity of antioxidative enzymes under normal and saline conditions. In addition, in the present study we have considered and compared the chlorophyll, MDA and membrane stability index in seedlings of the treated and untreated soybean seeds with varying levels of NaCl in order to exploit salt-tolerant isolate of *Trichoderma* in relation to salinity stress tolerance in soybean.

## Materials and Methods

### Experimental design and treatments

The present experiment was conducted during 2014-15 in the Department of Agronomy and Plant Breeding, University of Mohaghegh Ardabili, Ardabil, Iran. The seed lot of soybean (*Glycine max* L.) cv. Williams was obtained from SPCRI (Seed and Plant Certification and Registration Institute, Karaj, Iran), with final germinability of

99%. Before use, seeds were surface sterilized in 1% sodium hypochlorite for 5 min, then thoroughly rinsed with double distilled water and air-dried.

A set of several *Trichoderma* isolates, collected from different salt affected soils located in Ardabil plains and maintained in the repository of Plant Pathology Laboratory, were considered for this study. The best isolate was selected as a salinity tolerant *Trichoderma harzianum* on the basis of growth at different NaCl concentrations for further investigation to test its capability to gain salt stress tolerance in soybean. After pre-soaking of seeds in water for 12 hours, soybean seed lot was mixed with salt tolerant isolate of *T. harzianum* for 10 g/kg of seeds. Seed lot was then incubated under warm (30±1 °C) and moist conditions just before radicle protrusion. The heap containing soybean seeds and *Trichoderma* hyphae were covered with a moist jute sack to maintain high humidity (Reddy 2013).

Non-and bio-primed seeds were sown in plastic pots containing perlite (previously tested for not releasing any salt) and placed on open-air vegetation yard in four blocks. Such conditions secured natural temperature and light conditions. Average Max and Min temperatures in the early spring (June) were 14 and 29 °C, respectively. Mean radiation reached to the canopy of seedlings was about 19 MJ/$m^2$/day during the experiment (28 days). Each pot was filled with 1 kg perlite. The plants were exposed to non-saline condition (control) and three levels of salinity viz. 3, 6 and 9 dS/m, 5 days after sowing. For salinity treatments, NaCl was directly added into half-strength Hoagland nutrient solution to set the different preferred ECs. The pH was always checked and adjusted around 6.7. Hoagland solutions with varying salinities were added to the pots in accordance with the treatments to achieve 100% FC. During the growth period, the pots were weighed and the losses were made up with Hoagland solution. The nutrient solutions were renewed every week. No agrochemical was applied during the experiment.

The experiment was laid out in a completely randomized design with five replications. Twenty seeds per pot were sown. After seedling establishment, each pot was thinned to eight plants. At the end of the experiment, the EC of the perlite in plastic pots was tested at regular intervals and final values were recorded to be 0.09, 3.13, 5.92 and 9.05 dS/m in pots with 0, 3, 6 and 9 dS/m salinity treatments, respectively. All observations were recorded at 28 days after sowing (DAS) using the two youngest fully expanded leaves. Leaf samples were collected from four plants per treatment per replicate.

Dry weight and root volume of 4-week old seedlings were measured through destructive sampling and reported as mg and $cm^3$ per seedling, respectively. Total leaf chlorophylls were extracted with 80% acetone and determined according to the equation proposed by Arnon (1949). The chlorophyll a fluorescence was determined on the youngest fully expanded leaf using a portable OS-30P fluorometer (Opti-Science, USA). The initial (F0) and maximum (Fm) fluorescences were analyzed and then quantum yield (Fv/Fm) was calculated. The leaves were previously adapted to the dark for 40 min so that all the primary acceptors of photosystem II were oxidized. The F0 was obtained with low intensity modulated light (<0.1μmol $m^{-2}$ $s^{-1}$) and the Fm was recorded by 0.3 s pulses of saturating light of 20,000 Hz. The variable fluorescence (Fv) was calculated from the difference between Fm and F0. Gas-exchange

measurements including net photosynthesis and stomatal conductance were measured using a LCi-SD photosynthesis system (ADC Bioscientific Ltd., UK) on intact leaves under full sunlight according to Burzynski and Klobus (2004).

Membrane stability index (MSI) of fresh leaves was determined according to the method approved by Bailly *et al.* (1998). The conductivity of solution was measured using an electroconductivity meter and the membrane stability index was determined by the following formula:

$$MSI= 1- [C_1/C_2]$$

where $C_1$= conductivity at 40 °C; $C_2$= conductivity at 100 °C. Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Kamal and Komatsu (2015).

All extraction procedures were carried out at 4 °C. Soybean leaf (0.1 g FW per sample) was ground in 2 ml of potassium phosphate buffer (100 mM, pH= 7.8) containing 0.2 mM dithiothreitol and 10 µM EDTA, and mixed for 15 min. The homogenate was centrifuged at 12000 g for 15 min, and then the supernatant was used for assays. Catalase (CAT, EC 1.11.1.6) activity was determined spectrophotometrically (two measurements per extract) following $H_2O_2$ consumption (extinction coefficient of 39.4 $mM^{-1}$ $cm^{-1}$) at 240 nm (Bailly *et al.* 1998). The reaction mixture contained 50 mM potassium phosphate buffer (pH= 7.0), 10 mM $H_2O_2$ and 200 µl of enzyme extract in a 3-ml volume. The results were expressed as enzyme *Unit*, i.e. as µmol $H_2O_2$ decomposed $min^{-1}$. Ascorbate peroxidase (APX, EC 1.11.1.11) was assayed by recording the decrease in optical density due to ascorbic acid (extinction coefficient of 2.8 $mM^{-1}$ $cm^{-1}$) at 290 nmfor 1 min in 1 ml of a reaction mixture (Nakano and Asada 1981). The 3-ml reaction mixture contained 50 mM potassium phosphate buffer (pH= 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM $H_2O_2$ and 0.1 ml enzyme. The results were expressed as enzyme *Unit*, i.e. µmol ascorbate decomposed $min^{-1}$. Total SOD (EC 1.15.1.1) activity was determined by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977) with small modification. The 3- ml reaction mixture contained 50 mM potassium phosphate buffer (pH= 7.8), 13 mM methionine, 75 µM NBT, 2 µM riboflavin, 0.1 mM EDTA, and 100 µl enzyme extract. The reaction mixtures were illuminated for 15 min at a light intensity of 5000 lx. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

**Statistical analysis**

Data were analysed using SAS 9.2 program (SAS Institute Inc., 1998). The Proc-GLM procedure was used to perform the two-way analysis of variance. Analysis of variance was carried out according to the factorial layout based on completely randomized design with five replications. Comparison of means was carried out using *F*-protected LSD test at 0.05 probability level. The experiment was repeated twice and then the data were pooled based on equality of variances according to the result of Bartlett's test.

## Results

Analysis of variance (Table 1) indicated significant effects of experimental factors and the two-way interactions for seedling growth and some physiological characters except leaf chlorophyll

content, chlorophyll fluorescence, net photosynthesis, stomatal conductance and SOD and APX activity for which only the main effects were statistically significant.

The seedling dry weight and root volume significantly decreased as salt stress increased (Figure 1a,b). The highest dry weight (Figure 1a) and root volume (Figure 1b) of soybean seedling were found in the bioprimed seed lot under normal non-saline condition. The difference between bioprimed and unprimed treatments was not significant in terms of seedling dry weight, but was significant with regard to root volume. Minimum seedling dry weight was recorded in the bioprimed seed lot at 9 dS/m (109 mg) followed by unprimed treatment at the same salinity level (111 mg). Under mild salt stress (3 dS/m), the seedlings from the bioprimed seeds were heavier and had greater root volume than the control (unprimed) seed lot)(Figure 1a,b). The seedling dry weight reduced to almost more than 45% from 204 mg at 0 dS/m in the bioprimed seeds to 109 mg at 9 dS/m. The root volume reduced about 65% from 1.25 $cm^3$ at 0 dS/m in the bioprimed seeds to 0.46 $cm^3$ at 9 dS/m in the unprimed seeds.

**Table 1. Analysis of variance of data for physiological and biochemical characteristics of soybean grown under four levels of salinity stress before and after seed biopriming with Trichoderma harzianum (n= 5)**

| Sources of variation | *df* | Mean squares[c] | | | | | |
|---|---|---|---|---|---|---|---|
| | | Seedling dry weight | Root volume | Leaf chlorophyll content | Chlorophyll fluorescence (Fv/Fm) | Net photosynthesis (*A*) | Stomatal conductance ($g_s$) |
| Salinity[a] (S) | 3 | 16152.3 ** | 1.014 ** | 4.037 ** | 0.311 ** | 113.397 ** | 8255.6 ** |
| Biopriming[b] (B) | 1 | 525.6 ** | 0.086 ** | 0.462 ** | 0.012 ** | 4.556 ** | 1265.6 ** |
| S×B | 3 | 244.0 * | 0.014 * | 0.001 ns | 0.0005 ns | 0.057 ns | 10.6 ns |
| Error | 32 | 68.7 | 0.004 | 0.120 | 0.001 | 0.207 | 77.7 |
| C.V. (%) | - | 5.14 | 7.16 | 7.02 | 4.85 | 3.69 | 11.47 |

| Sources of variation | *df* | Membrane stability index | Malondialdehyde content | Catalase activity | Superoxide dismutase activity | Ascorbate peroxidase activity |
|---|---|---|---|---|---|---|
| Salinity[a] (S) | 3 | 5599.9** | 62.806** | 0.734** | 0.327** | 0.014** |
| Biopriming[c] (B) | 1 | 297.0 ** | 3.906** | 0.053** | 0.033** | 0.004** |
| S×B | 3 | 45.7* | 0.540* | 0.015* | 0.0008ns | 0.00001ns |
| Error | 32 | 15.2 | 0.180 | 0.004 | 0.003 | 0.00008 |
| C.V. (%) | - | 6.48 | 9.61 | 11.07 | 9.46 | 8.44 |

[a]0 (0.54 dS/m), 3, 6 and 9 dS/m.
[b]*Trichoderma harzianum* isolate and unprimed (control).
[c]ns, * and ** indicate non-significance and significance, at 0.05 and 0.01probability levels, respectively.

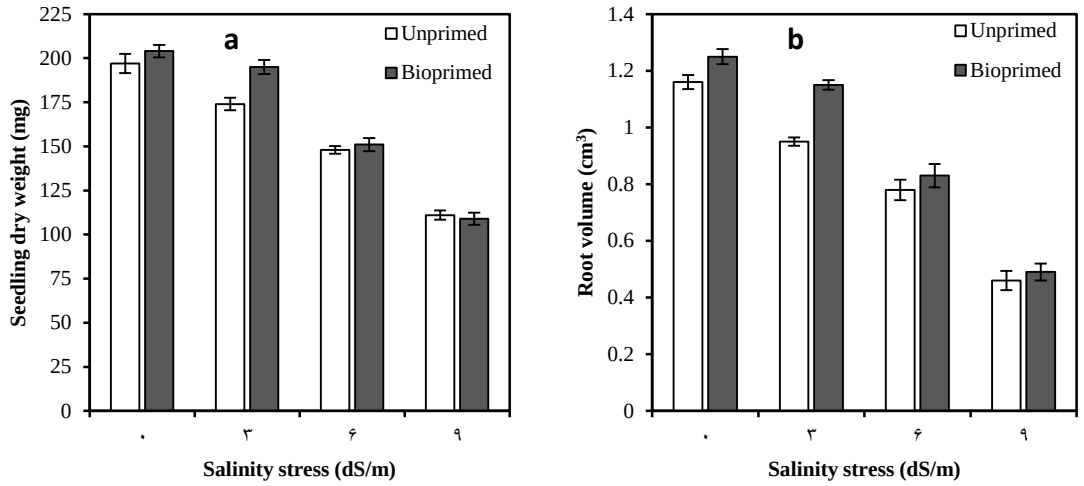


**Figure 1. Effect of seed biopriming with *Trichoderma harzianum* on a) dry weight and b) root volume of soybean seedlings grown under different saline conditions. The relevant *F*-protected LSDs at 5% probability level are 10.26 and 0.078, respectively. Data represent the mean ± SE of five replicates.**

Given the absence of significant interactions between salinity and biopriming for chlorophyll content, chlorophyll fluorescence (Fv/Fm), net photosynthesis and stomatal conductance, the effect of NaCl and *Trichoderma* for these variables were considered independently (Table 1). Physiological characteristics such as chlorophyll content, Fv/Fm, net photosynthesis and stomatal conductance were significantly decreased with increasing in salt concentration (Table 2). Pre-treatment with salt-tolerant *Trichoderma* repressed the decline in leaf greenness, chlorophyll fluorescence, photosynthetic rate and stomatal conductance. Percent drop in leaf chlorophyll content was significantly increased under salinity in soybean seedlings. Selected salt-tolerant *T. harzianum* isolate showed significantly detained NaCl induced changes in leaf greenness and chlorophyll fluorescence. Regarding chlorophyll fluorescence in soybean leaves, negligible but significant percent of reduction was observed in seedlings from the untreated seed lot. Under saline conditions, the highest photosynthesis rate was detected for *Trichoderma*-treated seed lot. Net assimilation rate was reduced due to salinity stress by 50% at 9 dS/m. Recorded values for stomatal conductance were reduced due to salinity by over 60% at 9 dS/m. Untreated seedlings averaged about 14% reduction in stomatal conductance across salt stress levels compared with the pre-treated seeds (Table 2).

MSI decreased significantly when salinity level increased from 0 to 9 dS/m (Figure 2a). Maximum MSI value was recorded by the bioprimed seeds (88%) followed by the unprimed seeds (82%) under non-saline condition. The value of membrane stability index was reduced almost 2.5 fold under 9 dS/m (32.5%) from 0 dS/m (85%). Under mild salt stress (3 dS/m), the highest MSI was observed in the *Trichoderma*-treated soybean plants.

The MDA content was higher in the unprimed seeds (control) at all salinity levels (Figure 2b) from 0 dS/m (1.7 μmol/g FW) to 9 dS/m (7.4 μmol/g FW) indicating higher rate of lipid peroxidation caused by the NaCl stress. The accumulation of MDA was lowest in the seed biopriming (mean value at non-saline

condition=1.5 µmol/g FW), which was followed by the same treatment (mean at mild salinity= 2.6 µmol/g FW) revealing reduced accumulation of lipid peroxides in the seedlings from *Trichoderma*-treated seeds under NaCl stress. On the contrary, the ameliorative effect of *T. harzianum* was eliminated under severe salinity stress (9 dS/m).

**Table 2. Effect of salinity and seed biopriming with *Trichoderma* on leaf chlorophyll content, chlorophyll fluorescence, net photosynthesis (*A*) and stomatal conductance ($g_s$)[a]**

| Treatments | Leaf chlorophyll content (mg/g FW) | Chlorophyll fluorescence (Fv/Fm) | Net photosynthesis (*A*) µmol $CO_2/m^2/s$ | Stomatal conductance ($g_s$) mmol $H_2O/m^2/s$ |
|---|---|---|---|---|
| **Salinity effect** | | | | |
| No stress | 2.24±0.052 a | 0.830±0.010 a | 16.32±0.195 a | 106.5±2.70 a |
| 3 dS/m | 1.90±0.050 b | 0.750±0.012 b | 13.67±0.175 b | 91.5±3.02 b |
| 6 dS/m | 1.26±0.048 c | 0.600±0.012 c | 10.62±0.124 c | 69.0±4.21 c |
| 9 dS/m | 0.83±0.043 d | 0.430±0.015 d | 8.68±0.204 d | 40.5±2.82 d |
| LSD (5%) | 0.096 | 0.028 | 0.399 | 7.72 |
| **Biopriming effect** | | | | |
| Primed | 1.67±0.130 a | 0.670±0.037 a | 12.66±0.689 a | 82.5±6.09 a |
| Unprimed | 1.45±0.126 b | 0.635±0.035 b | 11.98±0.662 b | 71.2±5.89 b |
| LSD (5%) | 0.068 | 0.020 | 0.282 | 5.46 |

[a]The interactions between experimental factors were not significant, so means were presented for the main effects of salinity and seed biopriming with salt-tolerant isolate of *Trichodermaharzianum*.
Data represent the mean ± SE (n= 10).
In each column, means having different letters are significantly different at the 0.05 probability level.
LSD (5%), *F*-protected least significant difference, at 5% probability level.

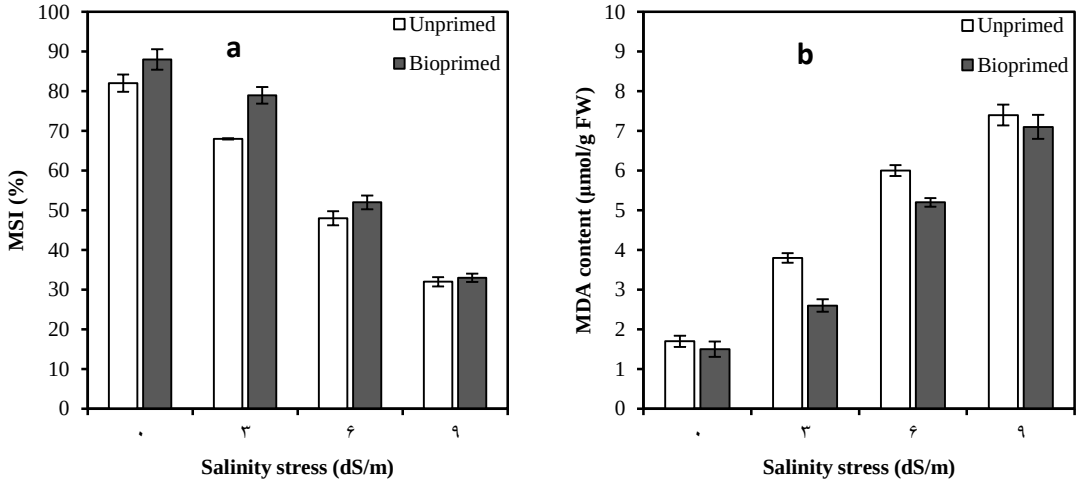


**Figure 2. Effect of seed biopriming with *Trichoderma harzianum* on a) membrane stability index (MSI) and b) malondealdehyde (MDA) content of soybean seedlings grown under different saline conditions. The relevant *F*-protected LSDs at 5% probability level are 4.83 and 0.526, respectively. Data represent the mean ± SE of five replicates.**

In general, increase in the activity of catalase was observed under salt stress (Figure 3). The greater increase was recorded in the *Trichoderma* treated seeds especially under mild salt stress (3 dS/m). Maximum increase at 9 dS/m over non-stress condition (relevant control) was recorded in the bioprimed seed lot (over three-fold) followed by the unprimed seedlings at the same salinity level, and lowest significant increase was noticed in the untreated seeds at the mild salt stress condition (Figure 3). The interactions between salinity and biopriming for the activity of superoxide dismutase and ascorbate peroxidase were not statistically significant (Table 1). The SOD activity drastically increased with increasing NaCl concentrations in the medium (Table 3), although the difference between two high levels of salt stress (6 and 9 dS/m) was not significant. Averaged over all salinity levels, *Trichoderma* isolate promoted the activity of SOD in soybean seedlings. The activity of ascorbate peroxidase from soybean seedling increased substantially with an increase in the salt stress level (Table 3). Maximum increase of the enzyme activity at 9 dS/m over non-stress condition was obtained (about 65%) followed by salinity levels of 3 and 6 dS/m. On the whole, the highest ascorbate peroxidase activity in soybean leaves was observed in the bioprimed seeds.

**Table 3. Effect of salinity and seed biopriming with *Trichoderma* on superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity[a]**

| Treatments | SOD activity (Unit) | APX activity (Unit) |
|---|---|---|
| **Salinity effect** | | |
| No stress | 0.340±0.015c | 0.046±0.0034 c |
| 3 dS/m | 0.540±0.022 b | 0.106±0.0046 b |
| 6 dS/m | 0.695±0.019a | 0.114±0.0043 b |
| 9 dS/m | 0.740±0.019a | 0.130±0.0046 a |
| LSD (5%) | 0.048 | 0.008 |
| **Biopriming effect** | | |
| Primed | 0.608±0.039 a | 0.109±0.0079 a |
| Unprimed | 0.550±0.036 b | 0.089±0.0073 b |
| LSD (5%) | 0.034 | 0.005 |

aThe interactions between experimental factors were not significant, so means were presented for the main effects of salinity and seed biopriming with Trichodermaharzianum.
Data represent the mean ± SE (n=10).
In each column, means having different letters are significantly different at the 0.05 probability level.
LSD (5%), F-protected least significant difference, at 5% probability level.

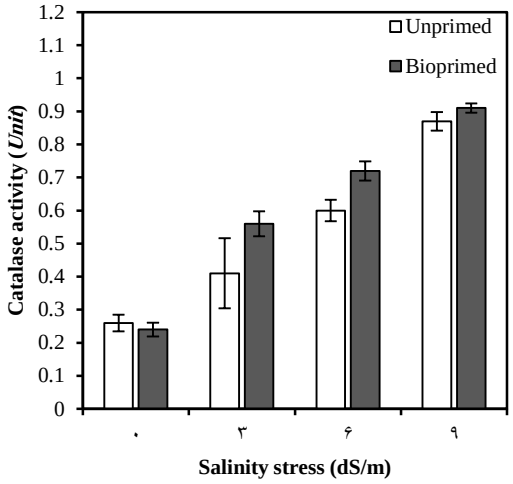


**Figure 3. Effect of salinity stress on catalase activity of soybean seedlings obtained from bioprimed seed lot. *F*-protected LSD at 5% probability level is 0.078. Data represent the mean ± SE of five replicates Discussion**

Salinity is a major environmental stress limiting plant productivity. It is an elaborate event involving osmotic stress, ion toxicity, nutrient imbalance, etc. thereby influencing different physiological and biochemical processes related to plant growth and development. Among several strategies used to improve plant growth under salt stress, use of salt-tolerant isolate of *Trichoderma* through seed biopriming could be the efficient and convenient approach. To enhance the capability of such technique and to develop newer options, a better understanding of the physiological and molecular bases of salt tolerance in plants is required. Among several mechanisms employed by *Trichoderma* to improve salt tolerance, the most studied mechanism relates to *Trichoderma*-induced changes in the plant metabolism, which in most cases, favours the plant (Brotman *et al.* 2013). The results of this work provided straightforward experimental validation that seed biopriming with salt-tolerant isolate of *Trichoderma harzianum* improved plant growth under salinity in *Glycine max*.

It is possible that decline in the seedling growth and root volume in NaCl affected seedlings could be due to several reasons including down-regulated photosynthesis, long distance signalling and perturbation in mineral acquisition. However, seedlings produced from the bioprimed seeds showed greater seedling dry weight and root volume. These results were in agreement with those of other researchers (Howell 2003; Benitez *et al.* 2004), who reported that *Trichoderma* produce plant growth hormones (e.g. gibberellins). The application of *Trichoderma* in plants increases root extension, which helps in more water uptake and thereby increasing the plants capability to tolerate abiotic stresses (Schuster and Schmoll 2010). It is well understood that *Trichoderma* increases plant biomass. On the other hand, seedling vigour i.e. higher seedling biomass, has been regarded to have dilution effects on ion building up in leaves (Fan *et al.* 2013).

Abiotic stress factors have detrimental impact on the photosynthesis, especially by disrupting it's all major determinants including the thylakoidal electron transport, the carbon reduction cycle and the stomatal control of the $CO_2$ supply (Gururani *et al.* 2015) together with lipid peroxidation and degradation of leaf chlorophyll (Munns and Tester 2008). In the present experiment, maximum leaf chlorophyll content, chlorophyll fluorescence, net photosynthesis and stomatal conductance were observed in the *Trichoderma* treated seedlings while minimum in the control (untreated) seed lot under both normal and salt stress conditions. However, seedlings exposed to varying concentrations of NaCl generally had low leaf greenness, chlorophyll fluorescence, net photosynthesis and stomatal conductance. Shukla *et al.* (2015) reported that chlorophyll content, net photosynthesis and stomatal conductance reduction were delayed in wheat seedlings pre-treated with *Trichoderma harzianum*. Salinity stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme i.e. chlorophyllase (Jamil *et al.* 2007), inducing the destruction of the chloroplast structure and the loss of pigment complexes (Sudhir and Murthy 2004). It has been indicated that chlorophyll content and net assimilation rate declines in the salinity susceptible plants such as common bean (Yasar *et al.* 2008) and soybean (Mannan *et al.* 2009). In the present analysis, the absence of any salinity×biopriming interaction on all measured leaf photosynthesis characteristics implies that *Trichoderma*-mediated tolerance presumably encompasses attitudes other than improving leaf photosynthetic performance to attenuate the impacts of salt stress.

Our results indicated a decreasing trend in MSI with the increase in NaCl concentration in both treated and untreated seedlings but the leakage was higher in the untreated seedlings than *Trichoderma* treated seedlings. Presence of salt in the medium caused a disturbance in membrane stability expressed by an increase in solute leakage (Xue *et al.* 2014). Enhanced hydrogen peroxide accumulation and subsequent lipid peroxidation was probably the major reason of electrolyte leakage under NaCl stress (Ashraf and Harris 2004), an important consequence of salt stress which is the ROS attack. These free radicals, formed under salinity, cause membrane disorganization and metabolic toxicity, resulting in higher leakage of solutes. Harman (2006) also concluded that enhanced activity of antioxidant enzymes such as peroxidases caused by *Trichoderma harzianum*, detoxify the ROS and thus bring about the membrane stability.

Measurement of malondialdehyde serves as a reliable index of oxidative hazard during abiotic stresses. MDA increased in the salt-stressed plants. Lipid peroxidation is the main indicator of the increase in free radicals and MDA is the major output of the lipid peroxidation process (Ashraf 2009; Gill and Tuteja 2010). Our records showed that the degree of accumulation of MDA was higher in the untreated seedlings as compared to the pre-treated seedlings at all salinity levels. Lowest MDA content was observed in the *Trichoderma* treated seeds which might be due to an elevation in biosynthesis of stress induced enzymes such as glutathione S-transferase,

glutathione-dependent formaldehyde dehydrogenase and peroxidase (Parida and Das 2005). Similar effect was obtained by the inoculation of *Trichoderma* in wheat plants (Rawat *et al.* 2011). Under abiotic stresses, when ROS are generated, the scavenging enzymes triggered by *Trichoderma* application play a pivotal role in protecting the cell from oxidative stress.

Increase in the activity of catalase in the soybean seedlings, crucial in the detoxification of ROS in confronting with different levels of salinity, was the highest activity recorded from the *Trichoderma* treated seeds. To mitigate and repair the damage initiated by free radicals, plants have developed a complex antioxidant system (Bailly 2004; Ashraf 2009). The primary components of this system include enzymes such as superoxide dismutase, catalase and peroxidases (Gill and Tuteja 2010; Walters *et al.* 2010). Kibinza *et al.* (2011) reported the key role of antioxidative enzymes mediating oxidation protection during seed ageing and repair followed by priming treatment of sunflower seeds. As reported by Brotman *et al.* (2013) salt stress tolerance of cucumber seedlings offered by *Trichoderma* is dependent on activation of the plant antioxidant defence machinery.

The results of the present research suggest that seed biopriming with salt tolerant isolate of *Trichoderma harzianum* increased the capability of soybean to grow successfully under salt stress conditions. *Trichoderma* application was considered best in providing salt tolerance to soybean seedlings. Evidences from the present research indicated that salinity stress resistance is promoted through seedling vigour, amelioration of damage caused by ROS and accumulation of stress induced metabolites. These effects along with increased plant growth performance and antioxidant defence system, all energy requiring, suggest that both photosynthetic efficiency and antioxidant capacity are elevated in the presence of salt tolerant isolate of *Trichoderma harzianum*. This study deserves notice as it further provides new approaches for application of *Trichoderma* through seed biopriming in soybean plants for enhanced salinity tolerance.

## Acknowledgements

We are deeply grateful to the personnel of the Agricultural Research Centre of the University of Mohaghegh Ardabili for their technical support to this research and useful comments that improved the manuscript and statistical analysis.

**References**

Amini S, Ghadiri H, Chen C and Marschner P, 2016. Salt-affected soils, reclamation, carbon dynamics and biochar: a review. Journal of Soils and Sediments 16 (3): 939-953.

Arnon DL, 1949. Copper enzymes in isolated chloroplasts: polyphenol oxidase in *Beta vulgaris* L. Plant Physiology 24: 1-15.

Ashraf M, 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. Biotechnology Advances 27 (1): 84-93.

Ashraf M and Harris PJC, 2004. Potential biochemical indicators of salinity tolerance in plants. Plant Science 166: 3-16.

Bailly C, 2004. Active oxygen species and antioxidants in seed biology. Seed Science Research 14 (2): 93-107.

Bailly C, Benamar A, Corbineau F and Côme D, 1998. Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. Physiologia Plantarum 104 (4): 646-652.

Benitez T, Rincon AM, Limon MC and Codon AC, 2004. Biocontrol mechanisms of *Trichoderma* strains. International Microbiology 7: 249-260.

Brotman Y, Landau U, Cuadros-Inostroza A, Takayuki T, Fernie AR, Chet I, Viterbo A and Willmitzer L, 2013. *Trichoderma*-plant root colonization: escaping early plant defence responses and activation of the antioxidant machinery for saline stress tolerance. PLOS Pathogens 9 (3): 1-15.

Burzynski M and Klobus G, 2004.Changes of photosynthetic parameters in cucumber leaves under Cu, Cd, and Pb stress. Photosynthetica 42 (4): 505-510.

Fan XD, Wang JQ, Yang N, Dong YY, Liu L, Wang FW, Wang N, Chen H, Liu WC, Sun YP, Wu JY and Li HY, 2013. Gene expression profiling of soybean leaves and roots under salt, saline-alkali and drought stress by high-throughput illumina sequencing. Gene 512 (2): 392-402.

Giannopolitis CN and Ries SK, 1977. Superoxide dismutase: I. Occurrence in higher plants. Plant Physiology 59: 309-314.

Gill SS and Tuteja N, 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930.

Gururani MA, Venkatesh J and Tran LSP, 2015. Regulation of photosynthesis during abiotic stress-induced photoinhibition. Molecular Plant 8 (9): 1304-1320.

Hamilton CE, Gundel PE, Helander M and Saikkonen K, 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Diversity 54: 1-10.

Harman GE, 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology 96: 190-194.

Howell CR, 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Disease 87: 4-10.

Jamil M, Rehman S, Lee KJ, Kim JM, Kim HS and Rha ES, 2007. Salinity reduced growth PS2 photochemistry and chlorophyll content in radish. Scientia Agriculturae 64 (2): 111-118.

Javid MG, Sorooshzadeh A, Moradi F, Modarres-Sanavi SAM and Allahdadi I, 2011. The role of phytohormones in alleviating salt stress in crop plants. Australian Journal of Crop Science 5 (6): 726-734.

Kamal AHM and Komatsu S, 2015. Involvement of reactive oxygen species and mitochondrial proteins in bio-photon emission in roots of soybean plants under flooding stress. Journal of Proteome Research 14 (5): 2219-2236.

Khajeh-Hosseini M, Powell AA and Bingham IJ, 2003. The interaction between salinity stress and seed vigor during germination of soybean seeds. Seed Science and Technology 31 (3): 715-725.

Kiani R, Arzani A and Habibi F, 2015. Physiology of salinity tolerance in *Aegilops cylindrica*. Acta Physiologia Plantarum 37 (7): 1-10.

Kibinza S, Bazin J, Bailly C, Farrant JM, Corbineau F and El-Maarouf-Bouteau H, 2011. Catalase is a key enzyme in seed recovery from ageing during priming. Plant Science 181: 309-315.

Maathuis FJM, 2006. The role of monovalent cation transporters in plant responses to salinity. Journal of Experimental Botany 57 (5): 1137-1147.

Mahajan S and Tuteja N, 2005. Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics 444: 139-158.

Manchanda G and Garg N, 2008. Salinity and its effects on the functional biology of legumes. Acta Physiologia Plantarum 30: 595-618.

Mannan MA, Karim MA, Khaliq QA, Haque MM, Mian MAK and Ahmed JU, 2009. Proline accumulation, water status and chlorophyll content in leaf in relation to salt tolerance in soybean. Indian Journal of Plant Physiology 14 (2): 130-134.

Mass EV and Hoffman GJ, 1977. Crop salt tolerance: current assessment. Journal of Irrigation and Drainage 103: 115-134.

Munns R, 2005. Genes and salt tolerance: bringing them together. New Phytologist 167: 645-663.

Munns R and Tester M, 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology 59: 651-681.

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

Parida AK and Das AB, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60 (3): 324-349.

Qadir M, Qureshi AS and Cheraghi SAM, 2008. Extent and characterisation of salt-affected soils in Iran and strategies for their amelioration and management. Land Degradation and Development 19 (2): 214-227.

Rameeh V, 2012. Ions uptake, yield and yield attributes of rapeseed exposed to salinity stress. Journal of Soil Science and Plant Nutrition 12 (4): 851-861.

Rawat L, Singh Y, Shukla N and Kumar J, 2011. Alleviation of the adverse effects of salinity stress in wheat (*Triticum aestivum* L.) by seed biopriming with salinity tolerant isolates of *Trichoderma harzianum*. Plant and Soil 347: 387-400.

Reddy PP, 2013. Recent Advances in Crop Protection. Springer, New Delhi, India.

SAS Institute Inc., 1998. SAS Stat View Reference. Cary, NC, USA.

Schuster A and Schmoll M, 2010. Biology and biotechnology of *Trichoderma*. Applied Microbiology and Biotechnology 87 (3): 787-799.

Shoresh M, Harman GE and Mastouri F, 2010. Induced systemic resistance and plant responses to fungal bio-control agents. Annual Review of Phytopathology 48: 21-43.

Shukla N, Awashti RP, Rawat L and Kumar J, 2015. Seed biopriming with drought tolerant isolates of *Trichoderma harzianum* promote growth and drought tolerance in *Triticum aestivum.* Annals of Applied Biology 166 (2): 171-182.

Sudhir P and Murthy SDS, 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42 (4): 481-486.

Wahid A, Rasul E and Rao AR, 1999. Germination of seeds and propagules under salt stress. In: Pessarakli M (Ed). Handbook of Plant and Crop Stress. Pp. 153-167. Marcel Dekker, New York, USA.

Walters C, Ballesteros D and Vertucci VA, 2010. Structural mechanics of seed deterioration: standing the test of time. Plant Science 179: 565-573.

Wang K, Zhang L, Mei G, Lixia L, Yonggui Z, Linsen Z, Binzhi L, Mingyu H and Ashok KA, 2013. Influence of salt stress on growth and antioxidant responses of two malus species at callus and plantlet stages. Pakistan Journal of Botany 45 (2): 375-381.

Wu GQ, Jiao Q and Shui QZ, 2015. Effect of salinity on seed germination, seedling growth and inorganic and organic solutes accumulation in sunflower (*Helianthus annuus* L.). Plant, Soil and Environment 61 (5): 220-226.

Xue Z, Zhao S, Gao H and Sun S, 2014. The salt resistance of wild soybean (*Glycine soja* Sieb. Et Zucc. ZYD 03262) under NaCl stress is mainly determined by $Na^+$ distribution in the plant. Acta Physiologia Plantarum 36 (1): 61-70.

Yasar F, Ellialtioglu S and Yildiz K, 2008. Effect of salt stress on antioxidant defence systems, lipid peroxidation and chlorophyll content in green bean. Russian Journal of Plant Physiology 55 (6): 782-786.