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Effect of endophytic fungus, *Piriformospora indica*, on growth and activity of antioxidant enzymes of rice (*Oryza sativa* L.) under salinity stress

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ABSTRACT

Abiotic stresses including salinity are the major limiting factors of growth and crop production worldwide. Microbial endophytes as the most important soil microorganisms, by modifying plants at genetical, physiological and ecological levels increase their yield per area unit and provide the possibility of crop production in saline and arid soils or climates with biotic and abiotic stresses. The endophytic fungus, *Piriformospora indica* has a pronounced growth-promoting activity and also increases plant resistance to environmental stresses including salinity, drought and plant pathogens. We compared some growth parameters, physiological and biochemical responses such as total soluble proteins, Relative Water Content(RWC), lipid peroxidation, free proline content, and enzyme antioxidants(catalase, glutathione reductase, superoxide dismutase, ascorbate peroxidase) activity of *p.indica*–inoculated and non-*p.indica*-inoculated(controls) rice(*Oryza sativa*) under salt stress. The obtained results show that *P. indica* increase the biomass of aerial parts and root, total soluble proteins, Relative Water Content (RWC), free proline content and enzyme antioxidants activity of inoculated rice in compared to the controls. In contrast, Lipid peroxidation decreased in inoculated rice in compared to the controls. The obtained results of this research indicated that the effective role of this fungus to improve growth of rice under salt stress conditions.

Key words: Antioxidant enzymes, *Piriformospora indica*, Rice (*Oryza sativa*), Salt stress

INTRODUCTION

Salinity is one of the major abiotic stress limiting plant growth and productivity in many areas of the world (Shannon, 1997). Salt stress has been found to disrupt several morphological, physiological and biochemical processes, many of which are seen at plant cellular levels (Maslenkova *et al*., 1999). Many of important salinity effects in plants are including: Hyperionic and hyperosmotic effects, inhibition of

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enzyme activities in metabolic pathways, decreased carbon-use efficiency, decreased germination percentage, reduction in photosynthesis and the decomposition of protein and membrane structures. Rice (*Oryza sativa*) is the main stable food in Iran. *O.sativa* adapted to flood condition. According to recent estimates 25 million of 165 million hectares of Iran's total arable land area is salt affected (Abtahi, 1992). Main adaptation of plants to salt stress are including: salt exclusion (exclude the salt in aerial portion of the plant (stem and leaves), salt avoidance (production of structures like salt glands), salt compartmentalization (compartmentalize Na^+ and Cl^- in vacuole of the cell) and mutualistic symbiosis with mycorrhizal and endophytic fungi. *Piriformospora indica* (Basidiomycota, Sebacinales) is a root-endophytic fungus which is able to improve growth and overall biomass of both monocot and eudicot plants and increase tolerance to biotic and abiotic stresses. *P.indica* was isolated from the rhizophere of *Prosopis juliflora* and *Zizyphus nummulaia* in the Thar Desert in Rajasthan, India. *p.indica* has at least six chromosomes, genome size of about 15.4-24 Mb and pear-shaped chlamydospores in the plants root cortex(Verma *et al*., 1998; Varma *et al*., 1999). This fungus never finded endodermis roots and shoots portion. It is similar to arbuscular mycorrhizal fungi (AMF) in terms of plant growth promotional effect, root colonization and phosphorus acquisition but, in contrast, *p.indica* can be cultivated easily on synthetic media and colonized cabbage and spinach of *Brasicaceae* family. The main aim of the present study was to investigation of *p.indica* protective effect in *O.sativa* under four levels of NaCl (0, 100, 200 and 300 mM). In order to purpose, We compared some growth parameters, physiological and biochemical responses such as total soluble proteins,H_2O_2 concentration, lipid peroxidation, free proline content, and enzyme antioxidants(catalase(CAT: EC1.11.1.6), glutathione reductase(GR: EC1.6.4.2), superoxide dismutase(SOD: EC 1.15.1.1), ascorbate peroxidase(APX: EC 1.11.1.11)) activity of *p.indica*–inoculated and non-*p.indica*-inoculated(controls) rice under salt stress(Blilou *et al*., 2000; Porcel *et al*., 2003). Many of research exhibited, Inoculation with AMF and root endophytic fungi increased biomass, photosynthetic pigment, Relative Water Content(RWC), hydrogen peroxide, proline content as an osmoprotectant, protein content and antioxidant enzyme activity, instead of reduced MDA(when polyunsaturated fatty acids in the membrane undergo peroxidation) in inoculated plants than non-inoculated plants under salt stress(Hua *et al*., 1997; Hernández *et al*., 1995; Yang *et al*., 2004; Sudhakar *et al*., 1993; Lutts *et al*., 1999). Last study exhibited that *P. indica* protects barley plants from abiotic stresses such as high salt concentrations. In other study showed antioxidant enzyme activity in roots and shoots of maize plants colonized with *P.indica* increase than non-cololnized plants. Reactive oxygen species (ROS) produce in normal condition and under biotic and abiotic stress in plants. ROS are ordinarily generated in plant from leak of electrons from the photosynthetic and mitochondrial electron transport chains to oxygen (photosynthesis and respiration) and oxygenase activity of Rubisco in photorespiration (Dat *et al*., 2000; Imlay, 2003; Alscher *et al*., 2002). The important of ROS are singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet -}$) and hydroxyl radicals (OH•) (Ozkur *et al*., 2009; De Gara *et al*., 2003; Mittler, 2002). ROS are highly reactive and capable of changing normal cellular metabolism through oxidative stress to membranes, nucleic acids, proteins (denaturing) and lipid peroxidation. Generation of ROS increased in several plant species under salt stress than normal condition, that increase symbolized ROS can act as signaling molecules for stress responses. Plants are protected by number of enzymatic and non- enzymatic antioxidants in relation to the scavenge free radicals. The present study focuses on the interaction of P. indica with rice plants, its role in bioprotection against the salt stress; with emphasize activities of various antioxidant enzymes and metabolic compounds.

MATERIALS AND METHODS

Fungal and Plant Material, Symbiotic and NaCl treatment

Piriformospora indica was provided by Vierheilig *et al*(1998) and was propagated on a rotary shaker at 18–22°C in liquid Aspergillus complex medium(CM) (Peškan-Berghöfer *et al*., 2004)(table 1). Fungal mycelium was prepared for root inoculation as described by Druege *et al*. (2007).

Table1. *Aspergillus* complex medium for produce of *P.indica*

Amount	Materials	Amount	Materials	Amount	Materials
120g $NaNO_3$, 10.4g KC1, 10.4g $MgSO_4.7H_2O$, 30.4g KH_2PO_4	20×salt Solution	15g	Agar	20g	Glucose
6g $MnCl_2.4H_2O$, 1.5g H_3BO_3 2.65g $ZnSO_4.7H_2O$, 2.4mg $NaMO_4.2H_2O$, 750mg KI, 130mg $CuSO_4.5H_2o$	Microelement	1mL	Water	2g	Peptone
		1g	Casamin acids	1g	Yeast extract

Seeds of *Oryza sativa* L., cultivars Tarrom were obtained from the University of Agricultural and Natural Resources (Iran, Mazandaran). Seeds were surface-sterilized for 10 min in 25% sodium hypochlorite and finally washed six times with sterile water .Seeds germinated at 28^0C for 4days in the dark on sheets of Whatman No. 1 filter paper in Petri dishes. After 4 d, one part of the germinating seeds (four seeds per pot) was transferred to pots (15 cm height by 12cm diameter) and grown in a mixture of sand/ vermiculite (1:1) in green house at 25: 18 ^{0}C at day: night cycle, 70% relative humidity and a photoperiod of 16 h light: 8 h dark and fertilized daily with Yoshida solution. The other part of the seeds was inoculated with *P. indica*: developing roots of 4-d-old germinating seeds were immersed in *P. indica* homogenate (The spore concentration was adjusted to 500.000 spores/ml) for 12 h before transferring to pots and grown under the same conditions. Three weeks after incubation, Colonization was observed under a light microscope after staining root fragments described by Vierheilig *et al* (1998). After 4 wk, *P. indica*-infected and controls plants were exposed to different concentrations of NaCl (0, 100, 200and 300mM) for one week. In during one wk, plants were grown in Yoshida solution treated with different of NaCl concentration. After NaCl treatment, plants for each group were harvested and divided into separate root and shoot fractions and then stored at −70 ◦C for biochemical and physiological analyses.

Growth parameters and water relation parameters

The fresh weights of shoots and roots were weighed and the lengths of shoots and roots were measured. The samples were then dried in a forced draft oven at 70 ◦C for 48 h, and the dry weights were determined. Relative water content (RWC) estimation was performed by adding 1 g of roots and shoots fresh to 50 ml of distilled water for 4 h. After this period, the turgid samples were oven-dried for 48 h at 70°C before determination of dry weight. The value of RWC was determined by the equation: RWC (%)

= [(FW−DW)/ (TW−DW)]×100, where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight (Sairam and Srivastava, 2002).

Proline content

Free proline content was extracted and measured according to the method described by Bates *et al* (1973). Fresh of roots and shoots (0.5 g) were homogenized in 10 ml of 3% (w/v) aqueous sulfosalicyclic acid. This aqueous solution was filtered through Whatman's No. 2 filter paper and 2 ml of filtrated solution mixed with 2 ml acid-ninhydrin and 2 ml of glacial acetic acid in a test tube. The mixture was heated at 100°C for 1 h in water bath, and the reaction was stopped by using ice bath. The reaction mixture was extracted with 4 ml toluene, after which the chromophore-containing toluene was aspirated and subjected to absorbance measurement at 520 nm. Proline concentration was determined using a calibration curve and expressed as µmol proline g^{-1} FW.

Lipid peroxidation

Lipid peroxidation was estimated by measuring malondialdehyde (MDA) content according to the method of Heath and Packer (1968). Fresh roots and shoots (1 g) were homogenized in 10 ml of 0.25% thiobarbituric acid (TBA) in 10% trichloracetic acid (TCA). The mixture was heated at 95°C for 30 min and then cooled quickly on an ice bath. Afterwards, the mixture was centrifuged for 10 min at 10,000 × *g* and the absorbance of the supernatant was measured at 532 nm and at 600 nm for correction of nonspecific turbidity. The MDA content was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol g^{-1} FW.

Protein and enzyme extraction

For estimation of total protein content and enzyme activity, homogenate of shoots and roots was extracted with ice-cold 0.5 M Tris-HCl (pH 6.8) buffer. The extracts were centrifuged at 16 000× *g* for 30 min at 4 °C and resulting supernatants were frozen in liquid nitrogen, kept in -70 °C and used for protein determination and enzyme assays. A high-speed centrifuge (*J2-21M, Beckman*, Palo Alto, USA) and UV-visible spectrophotometer (*UV-160, Shimadzu*, Tokyo, Japan) with 10 mm matched quartz cells were used for centrifugation of the extracts and determination of the absorbance, respectively.

Protein determination

Total soluble protein contents were measured according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

Antioxidant enzyme activity

Superoxide dismutase activity was assayed according to the protocol of Beauchamp and Fridovich (1971), measuring inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8) with 0.1 mM ethylenediaminetetraacetic acid (EDTA), 75 µM NBT, 13 mM methionine, 2 µM riboflavin and 20 µl of protein extract. Reactions were carried out for 10 min at a light intensity of 300 μmol^{-1} $m^{-2}s^{-1}$. The non-irradiated reaction mixture served as control and was deducted from absorption at

560 nm. One unit of SOD activity was defined as the amount of enzyme, which causes 50% inhibition of NBT reduction under the assay condition, and the results were expressed as U mg−1 protein. Catalase activity was measured according to the method of Aebi (1974), which measures the decline of the extinction of H_2O_2 at the maximum absorption at 240 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 15 mM H_2O_2 and 20 µl protein extract. The decrease of H_2O_2 level was monitored and quantified by its molar extinction coefficient (36 M^{-1} cm^{-1}), and the results were expressed as µmol $H_2O_2 min^{-1}$ mg^{-1} protein. Ascorbate peroxidase activity was determined according to the method of Jebara *et al* (2005). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM ascorbate, and 15 µl protein extract. The reaction was started by adding of H_2O_2, and ascorbate oxidation was measured at 290 nm for 1 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (ε = 2.8 mM^{-1} cm^{-1}), and the results were expressed as unite of ascorbate oxidized $min^{-1}mg^{-1}$ protein per minute at 25±2°C. Glutathione reductase activity was assayed by the oxidation of NADPH using its extinction coefficient (6.2 mM−1 cm−1) at 340 nm as described by Rao *et al*. (1996). The reaction mixture was composed of 100 mM potassium phosphate buffer (pH 7.8), 2 mM EDTA, 0.2 mM NADPH, 0.5 mM oxidized glutathione and 20 µl protein extract (total reaction mixture 1 ml). The reaction was initiated by the addition of NADPH at 25°C. The results were expressed as micromoles of NADPH oxidized min^{-1} mg^{-1} protein at 25±2°C.

Statistical analysis

At least two independent experiments were carried out in each case. Each data was pointed the mean of three replicates except of root lenghts and shoot heigths of all plants. Mean values and standard deviations (S.D.) were calculated for P. indica-inoculated and controls cuttings of all plants. Analysis of variance was conducted using one-way ANOVA test using SPSS 14 for Microsoft Windows. Means were compared by Duncan's test at the 0.05 level of confidence.

RESULT AND DISCUSSION

Piriformospora indica enhances plant biomass under salt stress

Cytological analysis of rice roots in contact with *P.indica* revealed the fungal mycelium penetrate in epidermis and cortical cells although never reaches the endodermis layer and stelar tissue. Pear-shaped chlamydospores were localized interacellularly in root cortical tissue (fig 1). Effect of inoculation *P.indica* on growth parameters of rice on salt stress have been presented in table 2.We observed difference morphological changes in rice colonized with *P.indica* as compared with controls in several levels of salt stress. To understand the effect of *P. indica* on rice morphology, several growth parameters including plant height, root length, fresh and dry weight (shoot and root) were analyzed. Our study exhibited Increasing NaCl concentration decreased growth parameters in controls than inoculated rice plants(table 2). The old leaves of Rice plants grown in saline condition showed typical symptoms as leaf yellowing, stunted growth, wilting, and chlorosis and subsequent necrosis(fig 2). However, these symptoms are increased controls than rice colonized with *P.indica*.

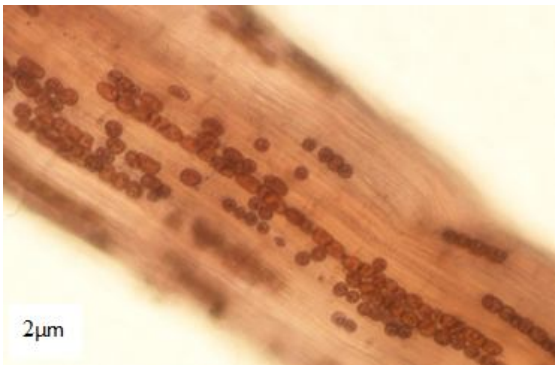

Fig1.Intracellular chlamydospores of *P. indica* on rice root

Table 2. Effects of *P.indica* on growth parameters of rice plant in salt stress.

Root			Shoot				NaCl (mM)
Dry weight(g)	Fresh weight (g)	length(cm)	Dry weight(g)	Fresh weight (g)	length(cm)		
1.8±0.21a	4±0.66a	13±0.24a	4.3±0.9a	13.5±1.6a	72±1.63a	-*P. indica*	0
2±0.4a	4.3±0.16a	13.2±0.4a	4.8±0.16a	15.5±0.4a	76±0.81a	+*P.indica*	
1±0.14b	3±0.08a	12±0.24b	a3.2±0.14a	10±0.81a	69.3±1.2a	- *P. indica*	100
1.8±0.35a	3.8±0.69ab	13±0.08a	4.3±0.97ab	13.3±1.2ab	75±0.81a	+*P.indica*	
0.6±0.04c	1.5±.37b	8.73±0.1c	1.7±0.16bc	6±0.57b	54±2.16b	-*P. indica*	200
1.3±0.29ab	3.1±0.21b	10±0.13b	2.9±0.47ab	11±0.81bc	69±0.81b	+*P.indica*	
0.4±0.21cd	0.9±.24bc	6.3±0.1d	1.2±0.43bc	3.5±0.24c	41±0.8c	- *P. indica*	300
0.8±0.16ab	2±0.32c	9.1±0.1c	2.5±0.4bc	7.6±0.24c	54.3±1.2c	+*P.indica*	

P.indica increase Protein, proline and Relative water content in shoots and roots of salt-treated rice

NaCl increased significantly the protein content of colonized rice with *P.indica* in respect to that of control. Our result showed that shoot and root protein content in colonized rice was higher than that of control plants in all of NaCl concentration (table 3). Proline (Pro) is an osmoprotectant that has been shown to accumulate in plants in response to salinity stress [A.J. Delauney, D.P.S. Verma, Proline biosynthesis and osmoregulation in plants, Plant J. 4 (1993) 215–223.]. In concurrence with this, our results (table. 3) show that rice colonized with *P.indica* was more controls significantly higher levels of Pro under stress. For instance, when exposed to 300mM NaCl, Shoot prolines of rice colonized increased 3-fold but in uncolonized rice were 2-fold relatively to the 0mM NaCl.The relative water growth (RWC) in rice colonized with *P.indica* and control subjected to salinity stress, based on leaf dry weight, is showed in table3. Our results showed that NaCl salinity significantly affected the RWC in both group plants. For instance, when exposed to 300mM NaCl, root RWC of rice colonized decreased 27% but in uncolonized rice was 55% relatively to the 0mM NaCl.

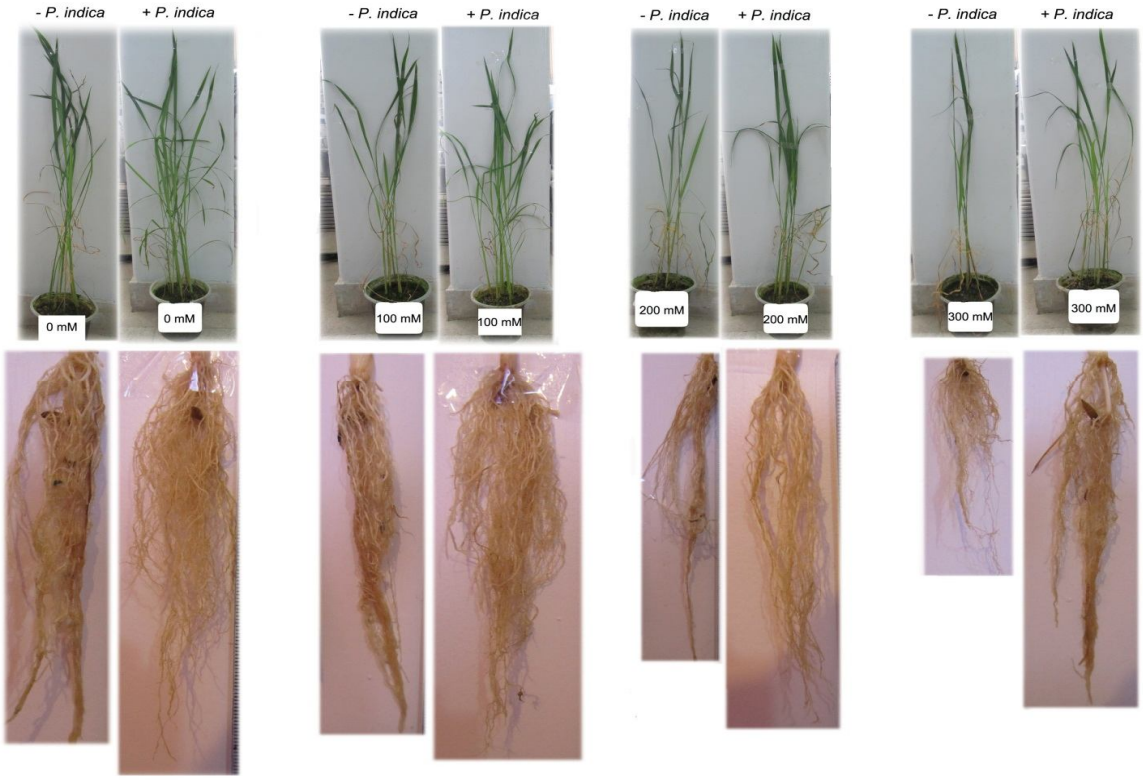

Fig 2. Effects of *P.indica* on root and shoot morphological of rice plant under salt stress.

Piriformospora indica reduces lipid peroxidation in salt-treated rice

Changes in lipid peroxidation serves as an indicator of the extent of oxidative damage under stress (Borsani, *et al.*, 2001) To determine if NaCl causes oxidative damage in rice, we monitored changes in lipid peroxidation by measuring thiobarbituric acid reactive substances in shoots and roots of inoculated rice and control plants. Lipid peroxidation levels in shoots and roots rice inoculated with *P.indica* and controls, measured as the content of MDA, are given in table3. Increased lipid peroxidation was observed with increasing NaCl concentrations in both groups. This increase was significantly higher in control as compared to colonized rice with *P.indica* at all NaCl concentrations. For instance, when exposed to 300mM NaCl, root MDA of rice colonized increased 5.2-fold but in uncolonized rice were 9.8 relatively to the 0mM NaCl.

Table 3. The effect of *P.indica* on proteins, proline, RWC and lipid peroxidation under NaCl concentration

Root				Shoot					
RWC (%)	MDA (µmol g^{-1} FW)	Proline (µmol proline g^{-1} FW)	Proteins (mg/g FW)	RWC (%)	MDA (µmol g^{-1} FW)	Proline (µmol proline g^{-1} FW)	Proteins (mg/g FW)		NaCl (mM)
71±3 a	10.75±2.8c	43±2.27c	9.9±2.08a	86.3±1.3a	53±7.11c	150±9.09c	40±1.2a	*-P. indica*	0
75±3.5 a	10±2.85c	46±7.3c	10±0.94a	95±2.1a	50±9.6cd	163±916d	47±3.8a	*+P.indica*	
66±2.5 a	27±1.63c	50±8.16c	7.4±0.32b	81.7±2.05a	77±15.76c	180±37bc	43.6±3.09a	*-P. indica*	100
69±3.1a	14.2±2.68c	80±29.4c	12.2±0.48a	86.6±4.02ab	55±7.8bc	271±38c	58.6±1.24b	*+P.indica*	
37±2.62bc	7.13±2.05b	88±14.9b	3.1±0.29c	54±1.63b	140±8.6b	220±8.1abc	24.4±3b	*-P. indica*	200
67±3.9a	24±0.81b	217±14b	7.2±0.16b	80.6±3.1bc	70±7.11bc	349±3.3b	64±0.8c	*+P.indica*	
32±1.3bc	105.6±3.3a	143±2.9a	1.58±0.13d	40.3±1.24c	190±4.08a	300±57ab	12.3±1.2c	*-P. indica*	300
54±2.5b	52±9.79a	266±16a	4.2±0.94c	75.33±3.7bc	90±8.16a	490±45a	39.6±1.2d	*+P.indica*	

***Piriformospora indica* further increases antioxidant enzyme activities induced by salt treatment in rice**

Statistical analysis revealed significant ($P < 0.05$) effects of *P.indica* on the activities of antioxidant enzymes in several of NaCl concentration (fig 3). Enzyme activities were affected in roots and shoots of rice by NaCl. We assay SOD, APX, CAT and GR activity. In both roots and shoots, ascorbate peroxidase (APX) activity increased with increasing NaCl levels, but superoxide dismutase (SOD) activity incresead with increasing NaCl to 200mM and then decreased at 300mM NaCl in both controls and *p.indica* – inoculated rice. Shoots and roots of rice inoculated with *P.indica* catalase (CAT) activity increased with increasing NaCl levels, similar to shoot catalase activity of controls but, root catalase activity of control increased with increasing NaCl to 200mM and then decreased at 300mM NaCl. Roots and shoots of inoculated rice glutathione reductase (GR) activity increased with increasing NaCl levels, But in shoots of control increased with increasing NaCl to 200mM and then decreased at 300mM NaCl. GR activity in roots of controls increased with increasing NaCl to 100mM and then decreased at 200 to 300mM NaCl(Fig 3).

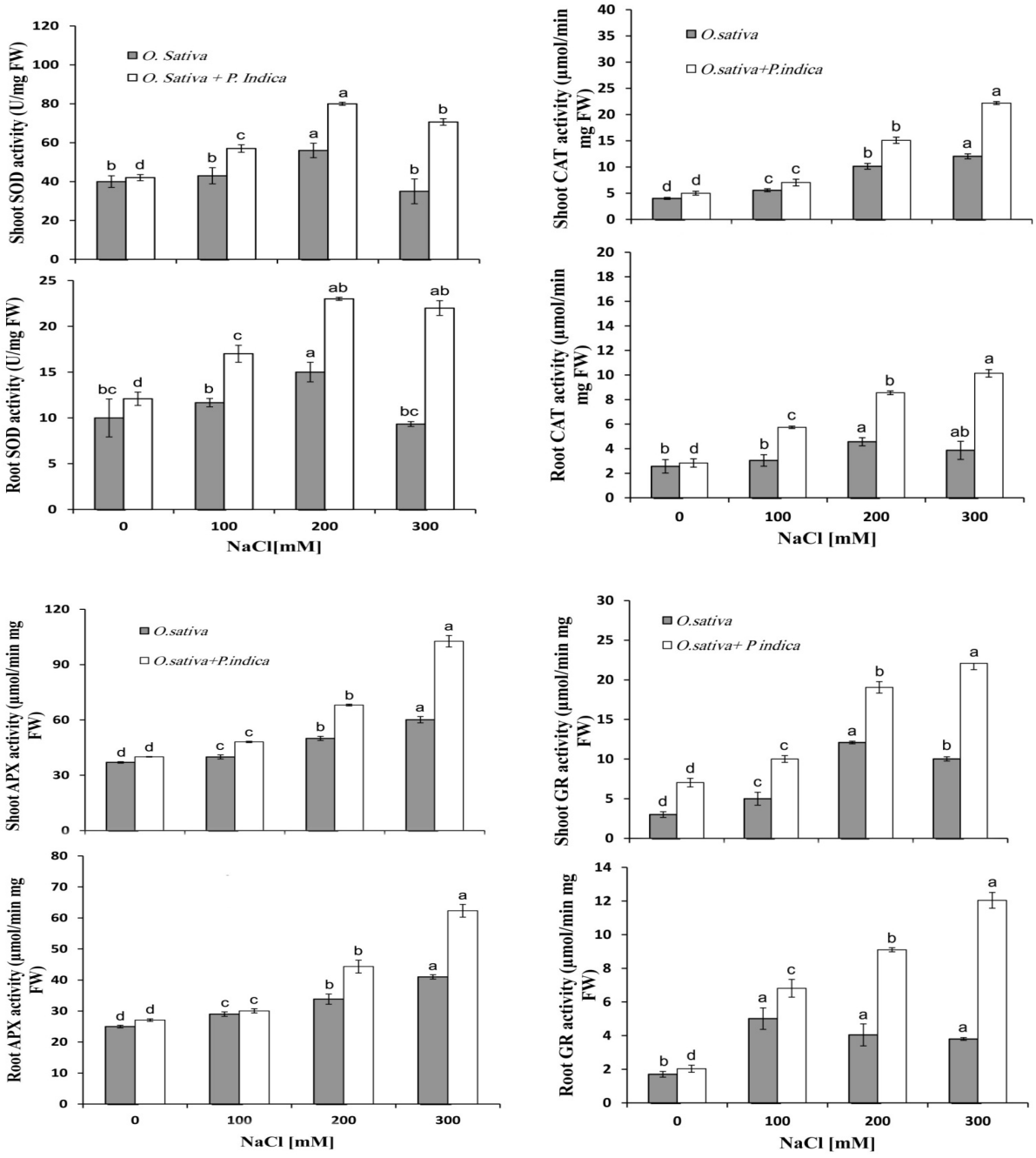

Fig 3. Positive effect of *P.indica* on antioxidant enzyme activity

High salt concentrations in soil and irrigation water are a major threat to agricultural production in arid and semiarid regions. The presence of excess ions in the rhizosphere causes injury to plant roots, followed by their gradual accumulation in the aerial parts with heavy damage to plant metabolism, which leads to stunted growth and reduced yield. Plants have developed complex mechanisms to avoid NaCl toxicity and low water potential in soil caused by salinity as well as drought. Furthermore, mutualistic symbiosis with mycorrhizal and endophytic fungi can confer salt tolerance to plants and decrease yield losses in

cultivated crops grown in saline soils. As a result of the symbiosis of *P. indica*, with rice is very depreciation. Here we could show that *P. indica* protects rice even from high salt stress (300 mm NaCl). However, the mechanism of *P. indica*-induced salt tolerance has not yet been investigated. In order to get a better understanding of the impact of *P. indica* on the establishment of salt tolerance, we assessed biochemical markers for salt stress, such as metabolic activity and antioxidant enzyme activities. In this research we found that *P. indica*-colonized rice showed an increase in biomass production as compared with control plants. This increase in biomass indicates the mycorrhiza-like growth-promoting activity of *P. indica*. Similar results have also been found by Waller *et al* (2005). In the interaction between *P. indica* and barley plants, which supports our data. Although very little is known of the biochemical mechanisms involved in this association, we hypothesize that the increased plant growth-associated activities in the present study due to *P. indica* colonization may be attributed to enhanced nutrient uptake (especially of phosphorus and nitrogen), as observed in the case of mycorrhizal associations (Toro *et al*., 1998; Requena *et al*., 2001; Sherameti *et al*., 2005). However, in the present work, we have not studied this, although it warrants investigation. According to our results incolonized rice with *P.indica* showed a better protection mechanism against salinity induced oxidative damage than control plants. The "disappeared" proteins in response to 300mM NaCl are a result of their denaturation. Depressed protein synthesis and acceleration and its degradation in plants in response to salt stress have been reported by number of workers. The results showed that rice root colonization with *P. indica* increased the proline accumulation. Proline accumulation is a sensitive physiological index of the response of plants to salt and other stresses. For plants to survive under salt stress conditions, adjustment of leaf osmotic potential is very important, and this process requires intracellular osmotic balance. Under salt stress, plants accumulate some organic solutes (proline, soluble sugars, and so on) and inorganic ions to maintain higher osmotic adjustment. Therefore, better growth of *P. indica*-inoculated rice compared to control plants when exposed to NaCl may be a result of increased proline in the leaves of colonized plants. Greater concentrations of proline in leaves may have enabled *P. indica* inoculated plants to maintain higher leaf water potential during stress and kept plants protected against oxidative stress. Salt stress enhanced generation of reactive oxygen species (ROS) in plants (lee *et al.,* 2001). These ROS can seriously cause oxidative damages by disrupting of lipids, proteins and nucleic acids. Our result showed lipid peroxidation decreased in rice colonized than controls plant Thus, lipid desaturation could be an important component of plant tolerance in response to salt stress. Previous studies have demonstrated a salt-induced increase in lipid peroxidation (Hernández *et al*., 1995; Yang *et al*., 2004). Earlier studies have suggested that tolerance of plants to salt stress is associated with the induction of antioxidant enzymes (Hernández *et al*., 2000; Bor *et al*., 2003; Sekmen *et al*., 2007). In this work, we observed antioxidant enzyme activity in roots and shoots of rice plants colonized with *P. indica*. Induction of CAT activity was associated with higher growth rates of the most infective fungus, suggesting that CAT plays an important role in the regulation of intraradical fungal growth. The scavenging of H_2O_2 by CAT may be an efficient mechanism to attenuate the elicitation of plant defense responses (Wu *et al*., 1997), facilitating intraradical fungal growth and differentiation. Significant induction of CAT activity has also been observed in the compatible interaction between Hordeum vulgare and B. graminis Alg-S (Vanacker *et al*., 1998). We observed the same pattern of induction of GR, and SOD activities in plant roots and shoots colonized with *P. indica* as in the case of CAT activity. In an earlier report, induction of GR activity was observed during the *P. indica*–barley plant interaction, and it was suggested that the higher GR activity maintains an enhanced level of reduced glutathione which is involved in maintaining antioxidant capacity (Waller *et al*., 2005).We also observed significant induction of SOD activity throughout the experimental period in roots and shoots. Our data suggest that induction of SOD in this interaction might be associated with recognition of *P. indica* and activation of the plant defense system. It has been reported that unusually strong induction of antioxidative enzymes during the colonization period

results in detoxification of ROS (generated during colonization) and plays a protective role in the interaction between plants and fungi (Alguacil *et al*., 2003). Increased activity of antioxidant enzymes minimizes the chances of oxidative burst (excessive ROS production), and therefore *P. indica* might be protected from the oxidative defense system during colonization. Induction of antioxidant enzymes was higher in plants inoculated with *P. indica* than in control plants. These data suggest the systemic induction of antioxidative defenses in the case of colonization by *P. indica*. The activities of antioxidant enzymes did not vary with alternate and simultaneous inoculation of *P. indica*.. Suppression of CAT, GR and GST activities and induction of SOD activity may induce the accumulation of a higher level of H_2O_2. A higher concentration of H_2O_2 has also been observed in *P. indica*-colonized wheat plants after infection with *B. graminis* f. sp. tritici (Serfling *et al*., 2007), which supports our hypothesis of accumulation of H_2O_2 in rice plants interacting with P. indica. In agreement with these data, overexpression of antioxidant enzyme in transgenic plants enhanced tolerance to salt stress (Badawi *et al*., 2004; Ushimaru *et al*., 2006; Nagamiya *et al*.,2007).These observations suggest a slight alteration in the level of antioxidant enzymes, which probably favours the plant–fungal association and increases resistance to the pathogen. Here, we have provided additional information on the mechanism for biotic tolerance and systemic resistance conferred by *P. indica* as well as the antioxidative system, as also proposed by Waller *et al* (2005). The colonization of rice plants by *P. indica* leads to increased growth (due to its growth-promoting abilities), systemic resistance to biotic stress and enhanced antioxidant capacity. We show here that *P. indica*-colonized rice plants are more resistant to abiotic stress and that the reprogrammed metabolic state, which includes an enhanced antioxidant capacity, does not negatively affect biomass yield. Because *P. indica*, unlike AMF, can easily be propagated on a large scale in axenic culture in the absence of a host plant (Varma *et al*., 1999), we suggest the consideration of this endophyte as a tool for sustainable agriculture. Exploitation of *P. indica* may not only complement crop-growing strategies but also serve as a model system to study molecular traits that affect disease resistance and grain yields in plants.

Conclusion

Since *P. indica* has a broad host range and can easily be propagated in axenic culture on a large scale, we emphasize the high potential of the endophyte in protecting crops against salt stress in arid and semiarid agricultural regions. Our results demonstrated that salt stress has been negative effect on all of physiological and biochemical parameters on *P.indica* inoculated rice and controls, but these negative effect was reduced on rice barley when previously inoculated with the mutualistic basidiomycete *P. indica*.This endophyte appears to confer tolerance to salt stress, at least partly, through the activation of antioxidant enzymes. However, several possible symbiotic mechanisms proposed for salt tolerance. For example, root endophytes may act as a biological mediator allowing symbiotic plants to activate stress response systems more rapidly and strongly than nonsymbiotic plants.

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