



Ameliorative effects of *Trichoderma harzianum* on monocot crops under hydroponic saline environment

Roomana Yasmeen¹ · Zamin Shaheed Siddiqui¹

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Abstract

Ameliorative effects of *Trichoderma harzianum* (*Th-6*) on monocot crops under saline environment using hydroponic system were examined. Both rice and maize seeds were coated with *T. harzianum* (*Th-6*) and used for the saline and non-saline treatment. Germination and seedling growth performance were studied. *T. harzianum* (*Th-6*)-treated seeds showed constantly faster and more uniform germination as compared to untreated seeds. Moreover, seeds treated with *Trichoderma* improved plants' growth and physiological performance under hydroponic saline environment compared to control. The treatments showed higher relative water content (RWC), dark-adapted quantum yield (F_v/F_m ratio), performance index (PI_{ABS}), photochemical quenching (q_p), stomatal conductance (g_s), pigments concentrations and antioxidant enzymes as compared to untreated saline environment. Application of endophyte inhibited the Na⁺ and Cl⁻ ion uptake in leaves when plants were exposed to saline environment. However, H₂O₂ contents of both treated crops declined under hydroponic salt stress environment. Physiological mechanism of *T. harzianum* (*Th-6*) application in mitigating the salt-related consequences of both monocot crops was discussed.

Keywords Antioxidant enzyme activity · Hydroponic system · Maize · Physiological performance · Rice · Salinity · *Trichoderma harzianum* (*Th-6*)

Introduction

The agricultural land is declining day by day due to abiotic stresses that cause substantial reduction in crop growth and yield (Mahajan and Tuteja 2005; Reynolds and Tuberosa 2008; Oliveira et al. 2013). Among the crops monocots are more salt sensitive (Flowers et al. 2010; Ji et al. 2013; Jaarsma et al. 2013). Crops like maize and rice are more demanding for rapidly growing population, particularly, in the developing countries. In this regard, demand for agricultural supplies becomes intense. Due to climatic severity and low water availability it is difficult to get better growth and productivity of crops in arid region of the world (Zhu 2002; Ahmad and Prasad 2012; Siddiqui and Khan 2013; Siddiqui et al. 2014). Salinity stress induces osmotic and

ionic stress that leads to retarded growth in plants (Neumann 2008; Rasool et al. 2013; Alqarawi et al. 2014).

Trichoderma harzianum is an endophytic fungus which has symbiotic relationship with plant roots under arid region of the world (Shukla et al. 2012; Hashem et al. 2014). Previously, the role of *T. harzianum* in mitigating the salinity consequences on crop plants in arid environment was presented (Rawat et al. 2011; Ahmad et al. 2015). However, studies on physiological mechanism and the ameliorative role of *T. harzianum* in germination and early seedling level are rather scarce.

Hydroponic is a vital tool for plant research to grow plants using nutrient solution under axenic without soil condition (Shavrukov et al. 2012). Many studies have been carried out to determine salinity under hydroponic environment (Colla et al. 2006; Mahmood 2009). Only few such studies have shown relating germination enhancements of crop seeds by endophytes under hydroponic saline condition (Bjorkman et al. 1995; Yedidia et al. 2001; Mastouri et al. 2010). However, the method to improve plants' nutritional levels under axenic hydroponic environment through beneficial bio-control endophytic fungi like *T. harzianum* is

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✉ Zamin Shaheed Siddiqui
zaminss@uok.edu.pk

¹ Department of Botany, Stress Physiology Phenomics Centre, University of Karachi, Karachi 75270, Pakistan

still unknown. Therefore, in the current study, physiological mechanism of ameliorating role of the *T. harzianum* (*Th-6*) on germination and early seedling growth using hydroponic growing medium was observed. This study further enlightens the ability of *T. harzianum* (*Th-6*) under soilless or hydroponic saline condition to identify salt tolerance differences in rice and maize by *T. harzianum*.

Materials and methods

Seed selection

Maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel were collected from Plant Protection Department at Karachi (PPD), Pakistan. Later, both varietal seeds were surface sterilized with 10% sodium hypochlorite solution for 3 min and washed thoroughly with distilled water before the experiment.

Culture collection and preparation

The pure strain of *T. harzianum* (*Th-6*) was obtained from Plant Pathology Lab, Department of Botany, and University of Karachi. Later, pure *T. harzianum* culture was prepared in potato dextrose agar (PDA) containing 100 mM NaCl (Fig. 1).

Treatment of seeds by culture

Maize and rice seeds were subject to *T. harzianum* using 2% gum arabic as sticker and the colony forming unit (cfu) was about $64.9 \text{ conidia}/10^{-3}$ of *Trichoderma*.



Fig. 1 *Trichoderma harzianum* (*Th-6*) culture was prepared in potato dextrose agar containing 100 mM NaCl

In vitro seed germination test

Fifteen seeds of both crops treated with and without *T. harzianum* (*Th-6*) were placed in 9-cm-diameter sterilized petri plates. Ten ml of each NaCl concentration (0, 50, 100, and 150 mM) was prepared in half-strength Hoagland solution and were used in each plate. The germination was recorded daily up to 9 days. Germinated seeds were counted as when the radicle protruded through the seed coat shown in Fig. 2. The entire setup was incubated at 30 ± 10 and 22 ± 8 °C D/N periods. The control and treated solutions were changed within 24 h to avoid contamination. Following attributes were calculated.

Germination indices

Two germination indices were calculated: 50% germination (T_{50}) and coefficient of the rate of germination (CRG). However, the time to acquire 50% germination (T_{50}) was measured using the following formula reported by Coolbear et al. (1984) and modified by Farooq et al. (2005):

$$T_{50} = \frac{t_i + \{(N/2) - n_i\}(t_i - t_j)}{n_i - n_j}$$

where N is the final number of germination and n_i, n_j cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Coefficient of the rate of germination (CRG) was calculated according to the following formula of Bewley and Black (1985):

$$\text{CRG} = 100 \times \left(\frac{[N_1 + N_2 + N_3 + \dots + N_n]}{[(N_1 \times T_1) + (N_2 \times T_2) + (N_3 \times T_3) + \dots + (N_n \times T_n)]} \right)$$

where N_1 is the number of germinated seeds at time T_1 , N_2 number of germinated seeds at time T_2 and N_n number of germinated seeds at time T_n .

Seedling growth and transfer

Nine-day-old germinated seedlings were transferred on plastic rectangular tray having 70 (area 4 cm^2) small square to establish proper roots. The soil was autoclaved for 1 h at 80 °C containing 7.1 clay, 8.1 organic carbon, 80.5% silt and 0.20% nitrogen. Soil EC 1.8 ds/m and pH 7.5 were recorded using conductivity meter Linton Cambrige, UK (Dahnke and Whitney 1988). Seedlings were allowed to grow up to 15 days.

Later, seedlings were transferred in hydroponic tank (size 35 cm^2 with depth 15.5 cm) having 13 L half-strength Hoagland solution (Hoagland and Arnon 1950). NaCl

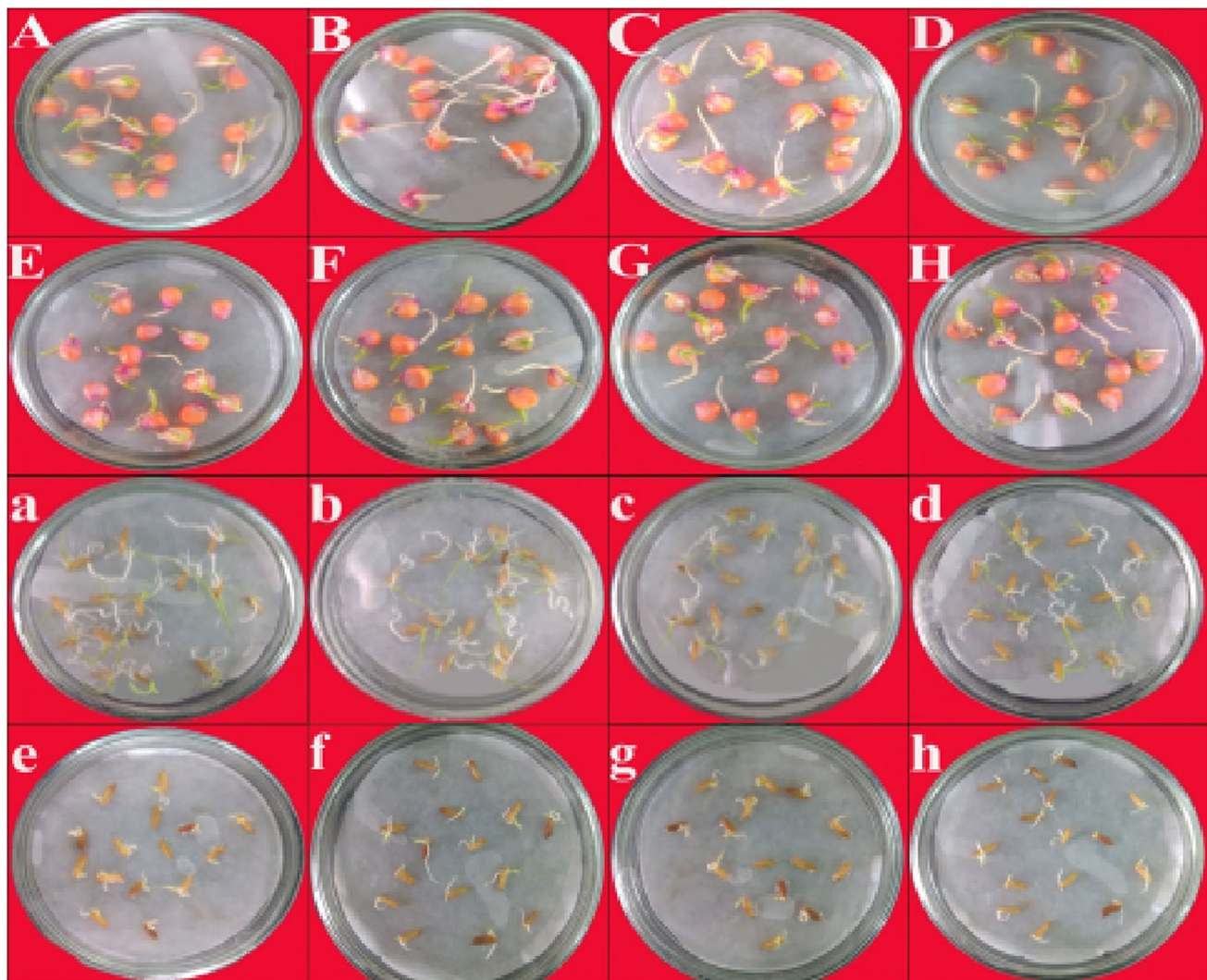


Fig. 2 Nine-day germination data in petri plates of both crops, maize and rice. Capital letters represent maize (*Zea mays* L) and small letters represent rice (*Oryza sativa* L) seedlings. **A, a** = 0 mM, **B,**

b = 0 mM + *Th*, **C, c** = 50 mM, **D, d** = 50 mM + *Th*, **E, e** = 100 mM, **F, f** = 100 mM + *Th*, **G, g** = 150 mM, **H, h** = 150 mM + *Th* NaCl concentrations of both maize and rice crops

concentrations (50, 100 and 150 mM NaCl) were applied along with half-strength Hoagland solution. pH and EC were maintained and are given in Table 1. Seedlings were subjected to average day–night temperatures of 30 ± 10 and 22 ± 8 °C for the growth. Plants treated with only half-strength Hoagland solution served as control. Each treatment and control was replicated four times. Seedling growth (length), biomass and physiological parameters were examined on the 28th day.

Relative water content

Four randomly selected leaves of each control and treated sample of an area 4×2 cm² rice and 1.2 cm² maize were excised from the mid-veins and the edge section and fresh

Table 1 pH and EC of the treatment solution with and without *T. harzianum*

Plants	NaCl concentration (mM)	pH	EC (ds/m)
Maize (<i>Zea mays</i> L.)	0	6.43	0.10
	50	6.45	4.54
	100	6.48	10.93
	150	6.45	15.23
Rice (<i>Oryza sativa</i> L.)	0	5.86	0.91
	50	5.83	4.01
	100	5.83	9.61
	150	5.56	13.15

weights (FW) were determined. Later, leaves were left in an airtight 90-mm plastic Petri-plate having distilled water for the next 12 h to measured turgid weight (TW). Leaf samples were then dried at 80 °C for 48 h in an oven and the dry weight (DW) was determined. Relative water content was calculated using the method described by Barrs and Weatherley (1962) with some modifications as:

$$\text{Relative water content} = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

where FW is the fresh weight, TW is the turgid weight, and DW is the dry weight.

Na and Cl ion determination

For sodium and chloride ion determination in leaf tissue, 0.5 mg dry mass of leaves of each randomly collected treated and control sample was homogenized in 10 mL double-distilled water at 25 °C for 10 min. The homogenate is centrifuged at 3000×g for 15 min, and the supernatant is filtered through Whatman #3 filter paper. Later an aliquot of filtrate was used for Cl⁻ by titration with silver nitrate and Na⁺ by flame photometry.

PSII quantum yield and stomatal conductance (g_s)

Chlorophyll fluorescence emission from the 20 randomly chosen leaves was monitored with a chlorophyll fluorescence meter, model OS-30P (Opti-Science, USA). For the dark adaptation, a leaf was subjected to dark conditions for 30 min using black leaf clips, later it was initially exposed to the modulated beam of far-red light with typical peak at wavelength 735 nm. The original (F_0) and maximum fluorescence (F_m) yields were recorded under 0.5 μmol m⁻² s⁻¹-modulated red light with 1.6 s pulses of saturating light having 6.8 μmol m⁻² s⁻¹ PAR. The variable fluorescence yield (F_v) was calculated by the equation $F_m - F_0$. The proportion of the variable to maximum fluorescence (F_v/F_m) was calculated as the dark-adapted quantum yield of PSII photochemistry and performance index and photochemical quenching as described by Maxwell and Johnson (2000). Similarly stomatal conductance of 20 randomly selected leaves of each treated and control plant was examined in the middle portion on the lower surface of the leaves using a leaf porometer (Model SC-1, Decagon). Four measurements were taken for each plantlet. Unit for stomatal conductance is mmol m⁻² s⁻¹.

Pigment analysis

0.5 g weighed leaf samples was plunged in 10 mL of 96% AR-grade methanol and then centrifuged at 4000 rpm for 10 min. The supernatants were then separated and the optical

density (OD) was observed at 653, 666, and 470 nm. Chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoid contents were calculated according to formulas given by Lichtenthaler (1987):

$$C_a = 15.65 A_{666} - 7.340 A_{653}$$

$$C_b = 27.05 A_{653} - 11.21 A_{666}$$

$$C = C_a + C_b$$

$$C_{x+c} = 1000 A_{470} - 2.860 C_a - 129.2 C_b / 245$$

where C_a is Chlorophyll 'a', C_b is Chlorophyll 'b', C_{x+c} is total carotenoid, and C is total chlorophyll.

Free proline content

Total proline contents were assessed using the method of Bates et al. (1973). According to the methods, leaf samples (0.5 g) of each treated and control samples were crushed in 5 mL sulphosalicylic acid (3% w/v) and then filtered through Whatman no. 1 filter paper. Take 2 mL of filtrate in test tube, afterward, 2 mL each of glacial acetic acid and acid ninhydrin was added. Mixture was heated in boiling water bath at 100 °C for an hour. After cooling the reaction mixture, 4 mL toluene is added and shaken vigorously for at least 15–20 s. Chromophore containing toluene is separated from the aqueous phase. Later absorbance is measured at 520 nm using toluene blank. Proline contents were calculated using the following formula:

$$\text{Proline}(\mu\text{mol g}^{-1}\text{FW}) = \frac{(\mu\text{g proline/mL} \times \text{mL toluene})}{[115.5 \mu\text{g}/\mu\text{mol}]/[\text{g sample}/5]}$$

Total H₂O₂ content

Total hydrogen peroxide content was quantified using the method of Velikova et al. (2000). 100 mg of freshly harvested leaf samples was homogenized in 3 mL of 0.1% (w/v) trichloroacetic acid (TCA). Later the homogenate was centrifuged at 12,000g for 15 min. Subsequently, 0.5 mL of 10 mM phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide (KI) were mixed with 0.5 mL of the supernatant. The optical density (OD) of the supernatant was read at 390 nm. The H₂O₂ contents were estimated using a standard curve.

Enzyme assays

Five hundred mg of each treated and control leaf samples were crushed in liquid nitrogen at 4 °C and homogenized in

10 mL protein extraction buffer containing 0.05 mM ethylenediaminetetraacetic acid (EDTA), 50 mg polyvinylpyrrolidone (PVP), 100 mM Tris-HCl, pH 6.8. The contents were centrifuged at 12,000 rpm for 15 min using refrigerated ultracentrifuge (Smart R-17, Hanil) machine. However, total protein content of each sample was recorded by the method of Bradford (1976).

Antioxidant activity of enzyme catalase (CAT; EC 1.11.1.6) was quantified by Patterson et al. (1984). The disintegration of H_2O_2 was measured at 240 nm taking $\Delta\epsilon$ as 43.6 mM cm^{-1} . A 3.0 mL reaction mixture was made up of 10.5 mM H_2O_2 in 0.05 M potassium phosphate buffer (pH 7.0). Reaction was started when 0.1 mL enzyme extract was added at 25 °C. The decline in optical density at 240 nm was used to calculate the activity. One unit of CAT activity is defined as the amount of enzyme that catalyzes the conversion of 1 mM of $\text{H}_2\text{O}_2 \text{ min}^{-1}$ at 25 °C.

Enzymes ascorbate peroxidase (APX; EC 1.11.1.11) activity was performed according to the method of Nakano and Asada (1981). 2.0 mL reaction mixture contained 0.2 mM EDTA, 0.5 mM ascorbic acid, 50 mM potassium phosphate buffer (pH 7.0) and 0.25 mM H_2O_2 . Later the reaction was started at 25 °C soon after the mixing with 0.1 mL enzyme extract. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated from the extinction coefficient 2.8 mM cm^{-1} . The activity unit is expressed as micromole of ascorbic acid oxidized min^{-1} at 25 °C.

Enzyme superoxide dismutase (SOD; EC 1.15.1.1) activity was quantified by the method of Beyer and Fridovich (1987). The reaction mixture was prepared using 27.0 mL of 0.05 M potassium phosphate buffer (pH 7.8), 11.0 mL of nitroblue tetrazolium salt (14.4 mg per 10 mL), and 0.75 mL of Triton X-100 and 0.5 mL of L-methionine (300 mg per 2.7 mL). Subsequently, 1.0-mL aliquots of this mixture were delivered into small glass tubes, followed by the addition of 10 mL of riboflavin (4.4 mg per 100 mL) and 20 mL enzyme extract. The combination was mixed and then subjected to illumination for 15 min in an aluminium foil-lined box containing 25 W fluorescent tubes. Control sample was substituted by 20 mL of extraction buffer and the optical density was measured at 560 nm. The reaction was stopped by switching off the light and placing the tubes in the dark. Increase in absorbance due to the formation of formazan was measured at 560 nm. Under the described conditions, the increase in absorbance in the control was taken as 100% and the enzyme activity in the samples was calculated by determining the percentage inhibition per minute. One unit of SOD is the amount of enzyme that causes a 50% inhibition of the rate for reduction of nitroblue tetrazolium salt under the conditions of the assay.

Guaiacol peroxidase (GPX; EC 1.11.17) activity was measured spectrophotometrically at 25 °C by the method

of Tatiana et al. (1999). The reaction mixture (2.0 mL) consisted of 0.05 M potassium phosphate buffer (pH 7.0), 2 mM H_2O_2 , and 2.7 mM guaiacol. The reaction was started by the addition of 0.1 mL enzyme extract. The initial rate of guaiacol oxidation was measured by the rate of formation of tetra guaiacol and was measured at 470 nm ($\Delta\epsilon = 26.6 \text{ mM cm}^{-1}$). One unit is defined as the amount of enzyme required to catalyze the conversion of one micromole of hydrogen peroxide, with guaiacol as hydrogen donor, per minute under specified conditions.

Statistical analysis

All data from treated and control were subjected to statistical analysis using software SPSS version 20 (IBM, USA). A two-way ANOVA was used to determine significant differences among means within and among each treatment and corresponding controls for each plant separately. The significance of Bonferroni test was represented as (*) and different alphabets on each bar graph.

Results

The results pertaining that seed germination decreases with increasing concentration of NaCl stress in maize and rice and the data are presented in Figs. 2 and 3. The substantial decrease in seed germination was observed at 150 mM NaCl without *Th* treatment. Application of *Th* in plants enhanced germination under NaCl condition showing minimum decrease in maize than rice. However, substantial increase was observed at 50 and 100 mM NaCl in both crops as compared to control.

The results related to the growth and biomass showing significant decline in NaCl stress in the absence of *Th* under hydroponic environment are presented in Table 2. Seed treated with *Th* mitigates the deleterious effect of NaCl stress in both maize and rice plants and restored the growth and biomass substantially at all salinity concentrations (50, 100, 150 mM) over control. However, in comparison, *Th* improved growth and biomass was significantly higher in maize rather than rice.

In saline environment, total Na and Cl ion were examined. Result showed that in both plants substantial amount of both Na and Cl ions were accumulated (Table 3). However, application of endophyte (*Th*) reduced the accumulation of Na and Cl ion in plants when exposed to salt stress. *Th* showed greater reduction of ions in maize as compared to rice.

Salinity significantly decreased fresh weight (FW), turgid weight (TW), dry weight (DW) as well as relative water contents (RWC) in maize and rice plants in the present study (Fig. 4). The maximum reduction was observed at 150 mM NaCl concentration compared to control. However,

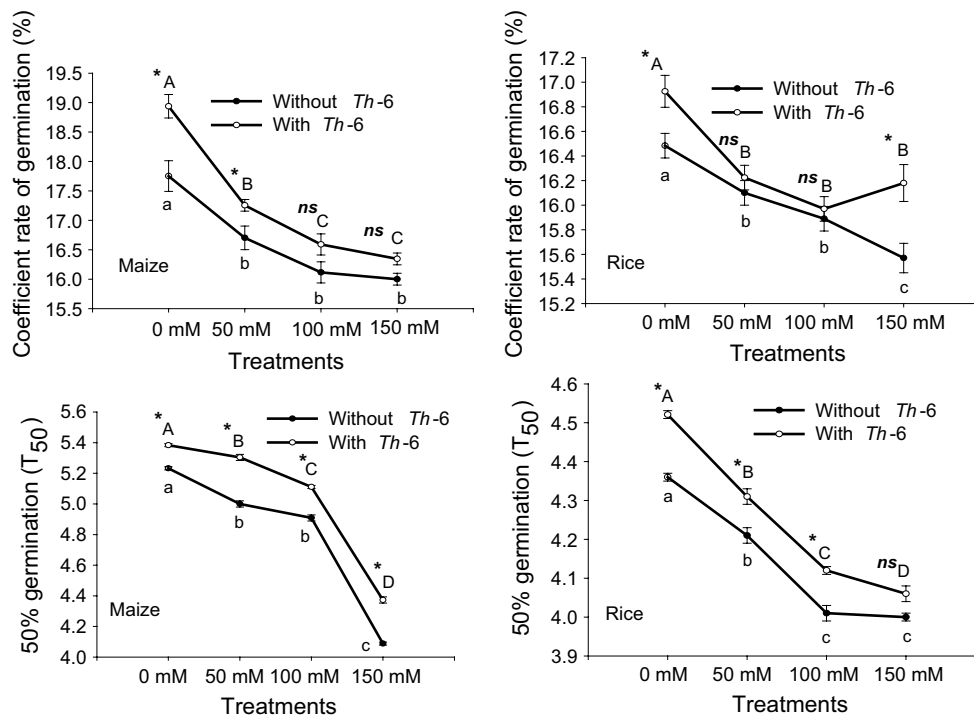


Fig. 3 Effect of *T. harzianum* (*Th*-6) seed treatments on germination indices (T_{50}) and rate of germination (CRG) of two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition. Vertical lines on the graph represent mean \pm stand-

ard error. Same alphabets on the line graphs showed non-significant difference within each treatment. Asterisk stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

Table 2 Effect of *T. harzianum* (*Th*-6) seed treatments on growth and biomass accumulation of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition

Plants	Without <i>Th</i> -6				With <i>Th</i> -6				
	Treatments (mM)	Shoot		Root		Shoot		Root	
		Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)	Length (cm)	Dry wt (g)
Maize (<i>Zea mays</i> L.)	0	34.4667 \pm 0.2333a*	0.2120 \pm 0.0111a*	17.9000 \pm 0.5132a*	0.1793 \pm 8.2529e-3a*	37.4667 \pm 0.2603a*	0.2560 \pm 0.0119a*	20.9667 \pm 0.2667a*	0.2270 \pm 4.2529e-3a*
	50	31.4667 \pm 0.2603b*	0.1513 \pm 0.0110b*	16.6000 \pm 0.5132a*	0.1190 \pm 7.2529e-3b*	34.4667 \pm 0.233b*	0.2083 \pm 0.0110b*	19.4667 \pm 0.2667a*	0.1917 \pm 5.2529e-3b*
	100	24.1333 \pm 1.2414c*	0.1100 \pm 0.0261b*	12.1667 \pm 0.5457b*	0.1090 \pm 8.2529e-3b*	29.8333 \pm 0.7513c*	0.1883 \pm 0.0165b*	15.9333 \pm 0.4256b*	0.1670 \pm 7.2529e-3c*
	150	20.7333 \pm 1.2414c*	0.0700 \pm 0.0115b*	11.1667 \pm 0.5457b*	0.0693 \pm 6.2529e-3c*	24.8333 \pm 0.7513d*	0.1513 \pm 0.0120c*	14.4333 \pm 0.4256b*	0.1070 \pm 8.2529e-3d*
Rice (<i>Oryza sativa</i> L.)	0	15.1333 \pm 1.0414a*	0.0310 \pm 1.283e-3a*	12.1667 \pm 0.5508a*	0.0170 \pm 1.0221e-3a*	19.9333 \pm 0.6119a*	0.0383 \pm 1.0000e-3a*	15.1333 \pm 0.7398a*	0.0219 \pm 1.0075e-3a*
	50	13.9000 \pm 0.8021a*	0.0267 \pm 1.362e-3a*	10.4000 \pm 0.5508b*	0.0154 \pm 1.0019e-3a*	17.2667 \pm 0.5783b*	0.0340 \pm 1.3533e-3a*	13.6667 \pm 0.8398a*	0.0199 \pm 1.0141e-3a*
	100	10.4333 \pm 0.6119b*	0.0213 \pm 1.000e-3b*	9.4000 \pm 0.4096b ns	0.0128 \pm 1.0275e-3b*	13.6333 \pm 1.0414c*	0.0273 \pm 1.2830e-3b*	11.3000 \pm 0.5015b ns	0.0167 \pm 1.0259e-3b*
	150	8.9000 \pm 0.8021b ns	0.0207 \pm 1.462e-3b ns	6.9800 \pm 0.5015c*	0.0121 \pm 1.0119e-3b*	10.966 \pm 0.5783d ns	0.0243 \pm 1.3533e-3b ns	9.9933 \pm 0.4096b*	0.0159 \pm 1.0241e-3b*

Data presented are the mean \pm standard error. Same alphabets on the data showed non-significant difference within each treatment. * stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

Table 3 Na⁺ and Cl⁻ ion concentration (mM) in the leaves of each treated and control samples

Treatments NaCl (mM)	Rice				Maize			
	Without <i>Th-6</i>		With <i>Th-6</i>		Without <i>Th-6</i>		With <i>Th-6</i>	
	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻
0	2.2	1.9	1.5	1.85	2.2	1.9	1.5	1.85
50	83.1	111.5	40.5	33.4	85.2	109.2	35.5	25.6
100	95.2	124.5	68.5	41.2	97.5	116	49.6	41.2
150	123.5	133.5	69.4	45.6	115.6	125.7	55.2	45.6

Values were the means of three replicates

the seeds treated with *T. harzianum* alleviated the antagonistic effect of NaCl stress showing better relative water contents as compared to those treated with NaCl alone. Moreover, the application of *T. harzianum* (*Th-6*) caused significant increase in FW, TW, and DW in maize plant as compared to rice under stress environment.

The results related to the effect of NaCl and *T. harzianum* on total chlorophyll content is depicted in (Table 4). Maximum decline in pigments was seen at 150 mM NaCl in both crops. The application of *T. harzianum* lessens the deleterious effect of salt stress and enhanced the total chlorophyll contents significantly in maize as compared to rice over control.

The result pertaining to the effect of salt stress and *T. harzianum* on photosynthetic attributes such as dark-adapted quantum yield (F_v/F_m ratio), performance index (PI_{abs}), photochemical quenching (q_p) and stomatal conductance (g_s) is illustrated in Fig. 5. Substantial decrease was recorded in all photosynthetic attributes at all stress levels in both crops in the absence of *Th*. However, data clearly indicate that inoculation of *T. harzianum* helped in maize plants as compared to rice in restoring photosynthetic performance including F_v/F_m ratio, PI_{abs} , q_p and g_s under saline hydroponic condition.

Free proline and H₂O₂ contents of maize and rice plants at all NaCl concentrations (50, 100, and 150 mM) with and without *T. harzianum* (*Th-6*) are depicted in Fig. 6. *T. harzianum* (*Th-6*) increases the free proline content but decreases H₂O₂ contents substantially in hydroponic saline environment. Results showed that *T. harzianum* reduces H₂O₂ production and increased proline accumulation in both the crops. However, maximum proline and minimum H₂O₂ contents were recorded at 150 mM NaCl in maize plant as compared to rice.

Activities of antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and guaiacol peroxidase (GPX) in both maize and rice were measured and the results are depicted in Fig. 7. Observations showed that as concentrations of NaCl increased, the activity

of antioxidant enzymes in both plants increased. However, application of *T. harzianum* (*Th-6*) elevated the CAT, SOD, APX, and GPX activities at all NaCl concentrations (50, 100, and 150 mM) relative to control (Fig. 7). Among both crops maize showed significant increase in all antioxidant enzyme activity compared to rice.

Discussion

Application of *T. harzianum* (*Th-6*) in both maize and rice seed before subjecting to salt stress showing substantial improvement in seed germination rate and coefficient. It was observed that seed germination and rate of the germination is greatly reduced by salt stress (Siddiqui and Khan 2011; Rajakumar 2013; Panuccio et al. 2014). Both crops such as rice and maize (Poaceae) are sensitive or moderately sensitive and unable to tolerate higher amount of salt against salinity. It was known that *T. harzianum* have the ability to enhance systemic resistance in plants and enhance tolerance against high concentration of NaCl (Benitez et al. 2004; Harman et al. 2004; Shores et al. 2010).

Delayed seed germination under saline hydroponic conditions indicates decreased seed metabolism which results in decreases in germination (Azam et al. 2005). However, there are almost no reports of the abilities of *T. harzianum* to improve seed germination in salt-stressed hydroponic condition except Bjorkman et al. (1994), Mastouri et al. (2010), that is, it showed increased germination in petri plate experiment.

Seed coated with *T. harzianum* improved seedling length and biomass allocation in both maize and rice crop subjected to salt stress. Maize showed better improvement as compared to rice. There might be one possibility which is that salinity reduces photosynthesis mechanism, resulting in minimized supply of carbohydrates which is needed for better plant growth (Azooz et al. 2011; Iqbal and Ashraf 2013; Hameed et al. 2014). The present study indicated that the application of *T. harzianum* (*Th-6*) in combination

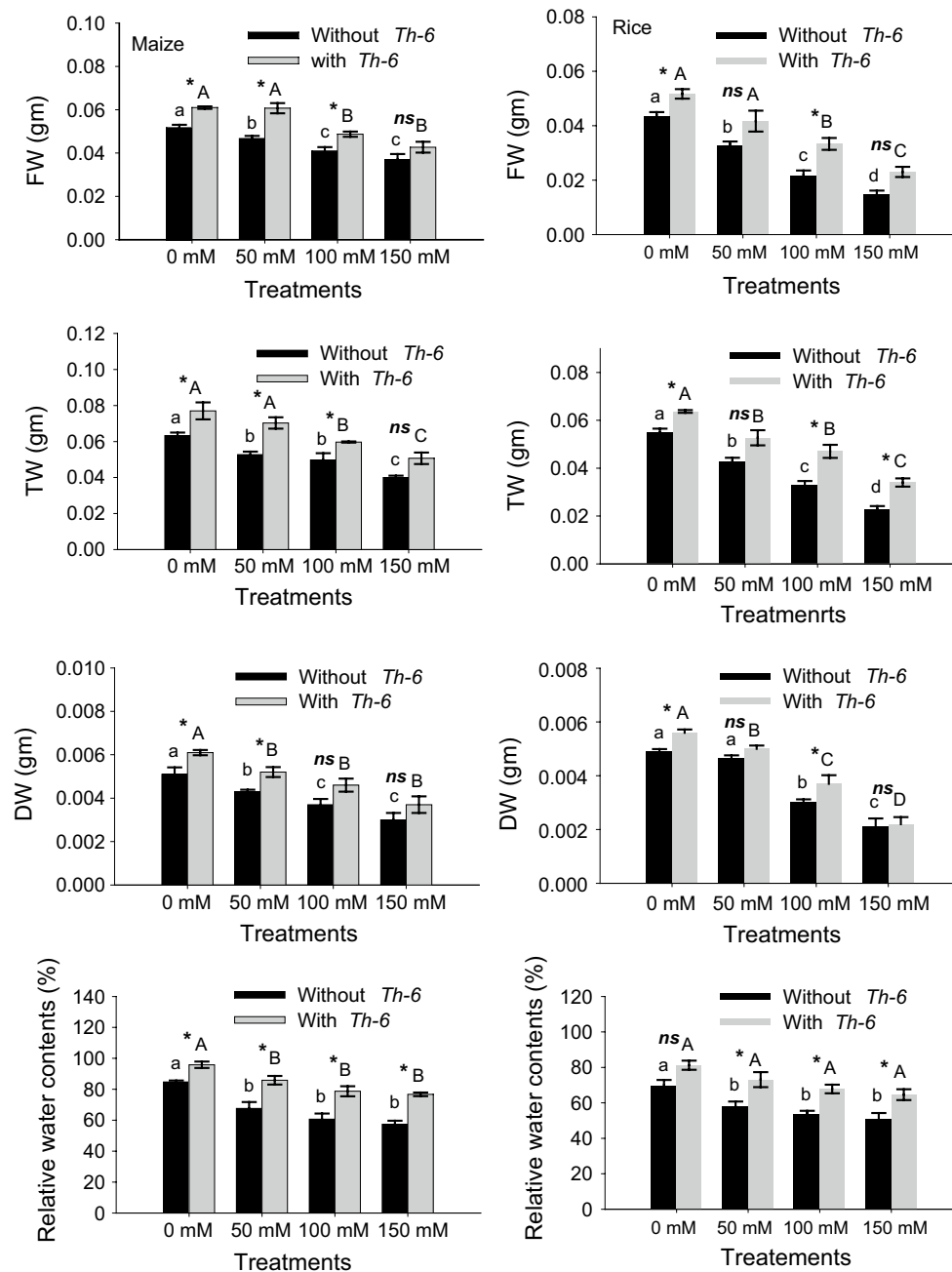


Fig. 4 Effect of *T. harzianum* (*Th-6*) seed treatments on relative water content and biomass accumulation of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition. Vertical lines on the bar represent mean \pm standard

error. Same alphabets on the bar graphs showed non-significant difference within each treatment. Asterisk stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

with NaCl under hydroponic condition showed considerable increase in growth parameters (Rasool et al. 2013; Ahmad et al. 2014) and present results corroborated with the findings of Mastouri et al. (2010) in tomato, who reported that the availability of *Trichoderma* enhanced mineral and hormonal activities under hydroponic habitat and may be

attributed to several growth-promoting mechanisms such as growth hormones such as cytokines (Zhang et al. 2013).

The present study showed that accumulation of toxic Na^+ and Cl^- ions was greatly reduced by the endophytes in both plants. It was observed that prolonged exposure to high salinity can aggravate damage once Na^+ and Cl^- ions

Table 4 Effect of *T. harzianum* (*Th*-6) seed treatments on chlorophyll contents of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition

Plants	Without <i>Th</i> -6					With <i>Th</i> -6			
	Treat-ments (mM)	Chlorophyll <i>a</i> ($\mu\text{g mg}^{-1}$ fresh weight)	Chlorophyll <i>b</i> ($\mu\text{g mg}^{-1}$ fresh weight)	Total chlorophyll ($\mu\text{g mg}^{-1}$ fresh weight)	Carotenoids ($\mu\text{g mg}^{-1}$ fresh weight)	Chlorophyll <i>a</i> ($\mu\text{g mg}^{-1}$ fresh weight)	Chlorophyll <i>b</i> ($\mu\text{g mg}^{-1}$ fresh weight)	Total chlorophyll ($\mu\text{g mg}^{-1}$ fresh weight)	Carotenoids ($\mu\text{g mg}^{-1}$ fresh weight)
Maize (<i>Zea mays</i> L.)	0	4.9767 \pm 0.0961a*	5.7503 \pm 0.2055a*	10.7270 \pm 0.2901a*	0.7272 \pm 0.0415a*	5.7912 \pm 0.0960a*	7.4594 \pm 0.20551a*	3.2506 \pm 0.2901a*	0.9105 \pm 0.0215a*
	50	2.2682 \pm 0.0840b*	3.9542 \pm 0.2055b ns	6.2224 \pm 0.2901b*	0.3754 \pm 0.0715b*	4.3830 \pm 0.0320b*	4.5559 \pm 0.2055b ns	8.9389 \pm 0.2901b*	0.8059 \pm 0.0315a*
	100	1.8310 \pm 0.0540c*	3.1198 \pm 0.2055c*	4.9508 \pm 0.2901b*	0.3646 \pm 0.0615b ns	4.1287 \pm 0.0980b*	4.4516 \pm 0.2055b*	8.5803 \pm 0.2901b*	0.5105 \pm 0.0715B ns
	150	1.4860 \pm 0.0650d ns	1.8484 \pm 0.2055d*	3.3344 \pm 0.2901c ns	0.2410 \pm 0.0715b ns	1.8185 \pm 0.0650c ns	2.8168 \pm 0.2055c*	4.6353 \pm 0.2901c ns	0.4101 \pm 0.0715B ns
Rice (<i>Oryza sativa</i> L.)	0	3.2852 \pm 0.0970a*	1.1579 \pm 0.2055a*	4.4431 \pm 0.2901a*	0.6164 \pm 0.0200a*	4.0427 \pm 0.0980a*	2.2110 \pm 0.2055a*	6.2537 \pm 0.2901a*	0.7183 \pm 0.0200a*
	50	0.8458 \pm 0.0960b*	0.8325 \pm 0.2055a*	1.6783 \pm 0.2901b*	0.3575 \pm 0.0300b*	2.6025 \pm 0.0960b*	2.0358 \pm 0.2055a*	4.6383 \pm 0.2901b*	0.5335 \pm 0.0200b*
	100	0.7896 \pm 0.0690b*	0.8074 \pm 0.2055a*	1.5970 \pm 0.2901b*	0.2777 \pm 0.0200c*	2.5170 \pm 0.0890b*	1.5967 \pm 0.2055a*	4.1138 \pm 0.2901b*	0.4592 \pm 0.0100c*
	150	0.7349 \pm 0.0990b*	0.4978 \pm 0.2055a ns	1.2327 \pm 0.2901b ns	0.2671 \pm 0.0100c ns	2.6611 \pm 0.0790b*	0.7508 \pm 0.2055b ns	3.4119 \pm 0.2901b*	0.2926 \pm 0.0200d*

Data presented are the mean \pm standard error. Same alphabets on the data showed non-significant difference within each treatment. * stands for significant and ns for non-significant difference among the treatments (with and without *Th*)

build up to high concentrations. Consequently, homeostasis of Na^+ and Cl^- becomes an important mechanism to reduce NaCl stress in higher plants. Excessive Na^+ accumulation as compared to Cl^- ions is frequently assumed to be largely responsible for the reductions in growth and yield under salinity (Chi Lin and Huei Kao 2001; Tsai et al. 2004; Hong et al. 2009).

The results on relative water content (RWC) showed decreasing trend with increase in salt stress under soilless or hydroponic condition, later it was improved by *T. harzianum* (*Th*-6). The continuous presence of NaCl concentration in the rooting solution not only caused a disturbance in nutrient uptake but also disrupted substantially intracellular water relations of leaves under hydroponic condition. Relative water content is a sensitive indicator for measuring plant growth against salinity (Siddiqui et al. 2014). It was observed that *T. harzianum* (*Th*-6) inoculation maintains better relative water contents under hydroponic saline condition. Similar to our finding, the increase in RWC due to *T. harzianum* (*Th*-6) under saline soil have also been reported by many scientists (Rawat et al. 2011; Hashem et al. 2014).

Application of *T. harzianum* in maize and rice showed high chlorophyll content, subsequently better photosynthetic performance including F_v/F_m ratio, PI_{abs} , q_p and g_s under saline hydroponic condition. Photosynthetic pigments like

Chl *a*, *b* significantly reduced under NaCl stress due to the production of chlorophyll-degrading enzyme activities such as chlorophyllase enzyme (Sultana et al. 1999; Sairam et al. 2002; Parida and Das 2005). It is assumed that *T. harzianum* may reduce the salt stress consequences on enzymes like chlorophyllase which not only improved chlorophyll content but also improved photosynthetic attributes such as dark-adapted quantum yield (F_v/F_m ratio), performance index (PI_{abs}), photochemical quenching (q_p) and stomatal conductance (g_s) reduced under NaCl stress. It was observed that maximum reactive oxygen species production in salt stress environment caused substantial damage at molecular levels such as DNA, RNA, and proteins decreasing carotenoids contents in plants (Siddiqui et al. 2014). However, mitigation of negative effects of NaCl stress as well as improvement in the photosynthetic pigments by *Trichoderma* has been demonstrated by many scientists under saline soil condition (Hashem et al. 2014; Ahmad et al. 2015). In the present study application of *T. harzianum* (*Th*-6) restored all pigments in hydroponic condition which might be due to exclusive production of hormones such as gibberellins, auxin, and cytokinins to appreciable level to stimulate of chlorophyll production.

Results demonstrated that *T. harzianum* (*Th*-6)-treated plant sample showed substantial increase in proline

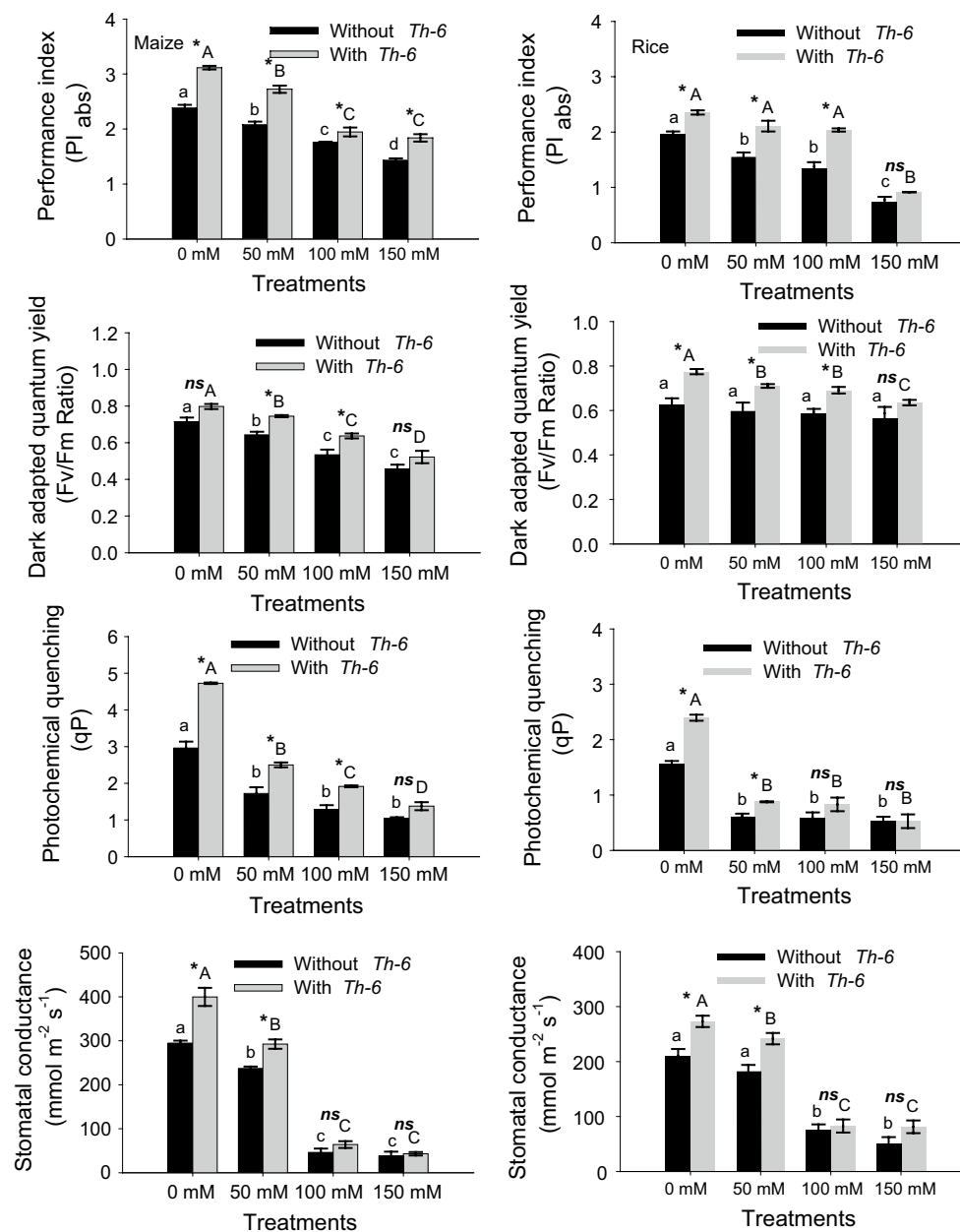


Fig. 5 Effect of *T. harzianum* (*Th-6*) seed treatments on photosynthetic attributes of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition. Vertical lines on the bar represent mean \pm standard error. Same alphabets on

the bar graphs showed non-significant difference within each treatment. Asterisk stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

contents and decreased H_2O_2 production in saline hydroponic condition. H_2O_2 production plays a pivotal role in metabolic processes to facilitate plant growth. H_2O_2 is produced through the unbalance of superoxide ($O_2^{\cdot-}$), in response to salinity stresses (Bienert et al. 2007; Siddiqui

et al. 2014). Proline is a known compatible osmolyte that not only maintain osmoregulation under salt stress in crop plant but also protect plant against stress by balancing the osmotic or solute potential of cytoplasm (Ahmad et al. 2010) with that of vacuole and outer environment

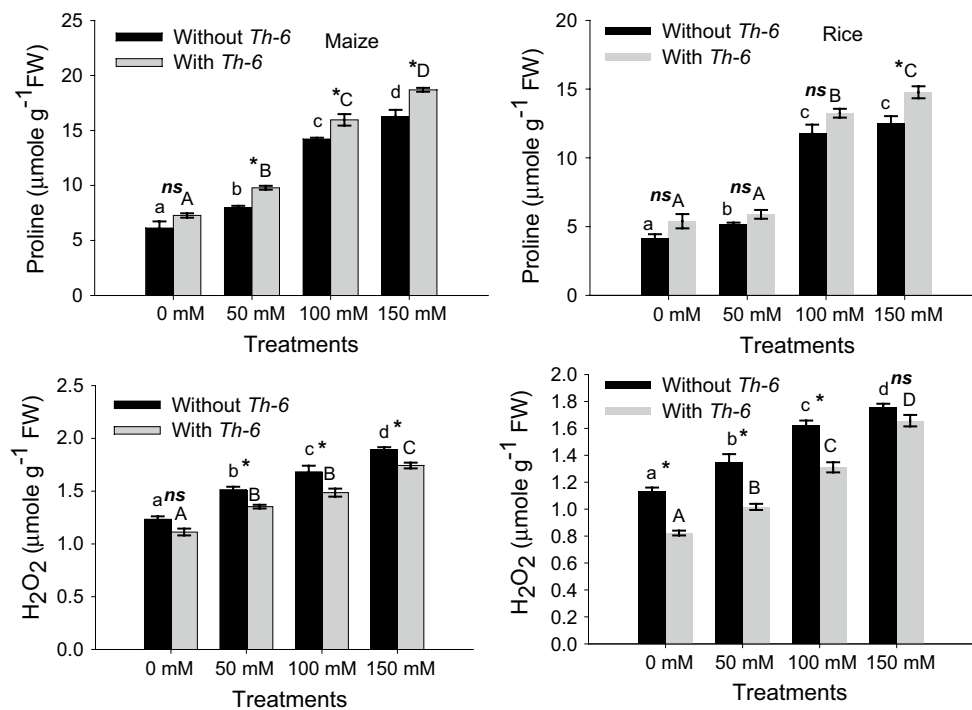


Fig. 6 Effect of *T. harzianum* (*Th-6*) seed treatments on free proline and H_2O_2 contents of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition. Vertical lines on the bar represent mean \pm standard error. Same alphabets

on the bar graphs showed non-significant difference within each treatment. Asterisk stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

(Siddiqui et al. 2014). Free proline accumulation generally has antioxidant property which lessens the severity of environmental stresses in plants, particularly under saline condition (Jogaiah et al. 2013) excessive amount of free proline takes part also in nitrogen fixation in plants (Greenway and Munns 1980; Abeer et al. 2015). It has been presumed that inoculation of *T. harzianum* minimizes the H_2O_2 production under saline environment due to more proline and carotenoid accumulation. It was reported that photosynthetic rate and maximum quantum yield are the main physiological attributes responsible for controlling production of ROS generation (Siddiqui and Khan 2013; Siddiqui et al. 2014).

It was observed that NaCl inhibited the antioxidant enzyme activities. However, application of endophyte (*Th-6*) induced substantial increase in all antioxidant enzyme activities. Maize showed greater increase as compared to rice. It was reported that in salt-tolerant plants antioxidant enzyme activities such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX) and glutathione reductase (GR) were elevated (Azooz et al. 2011; Siddiqui 2013; Ahmad et al. 2014). Increase in antioxidant enzyme activities after *T.*

harzianum application showed the mechanism by decreasing ROS production and reducing the damages in the cell organelles of plants subjected to salt (Borde et al. 2012; Gusain et al. 2014). It was depicted from the results that increase in antioxidant enzyme activities caused substantial decline in H_2O_2 production indicating the endophyte induced tolerance mechanism in both plants under saline environment. Many reports have been shown to illustrate that antioxidant played a considerable role to subsidize the damage effects of ROS production (Siddiqui et al. 2014; Jogaiah et al. 2013; Hashem et al. 2014; Ahmad et al. 2015).

In comparison, applications of *T. harzianum* (*Th-6*) in maize perform well against salinity as compared to rice. It is presumed that *T. harzianum* might be more compatible to established symbiotic relation with maize rather than rice which not only improved seed germination rate coefficient, inhibited ions accumulation in leaf but also improved photosynthetic performance in saline environment. It was observed that *T. harzianum* (*Th-6*) inhibited H_2O_2 production due to better enzymatic and non-enzymatic performance.

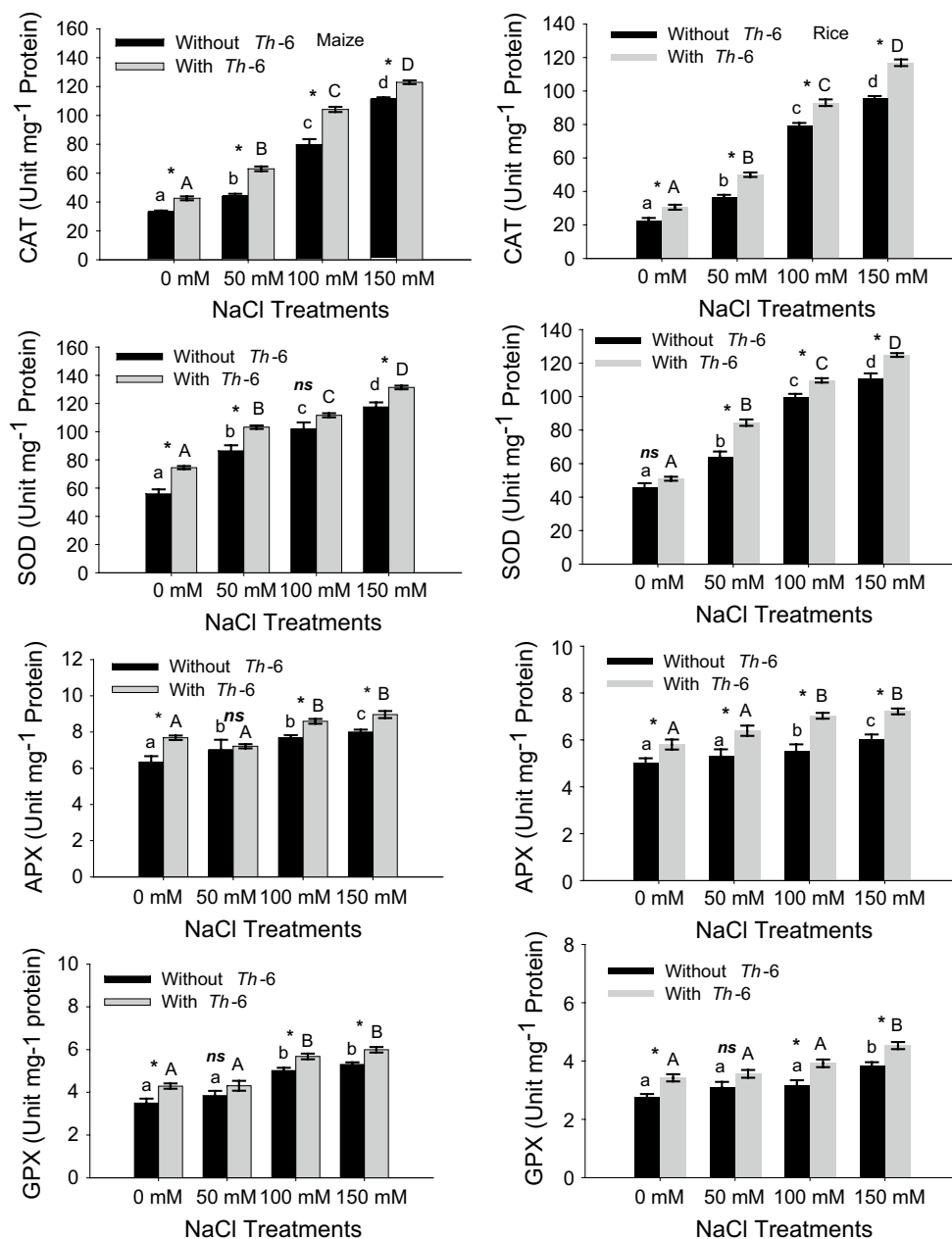


Fig. 7 Effect of *T. harzianum* (*Th-6*) seed treatments on antioxidants enzymes activities of maize (*Zea mays* L.) var. NT6621 and rice (*Oryza sativa* L.) var. kernel under hydroponic saline condition. Vertical lines on the bar represent mean \pm standard error. Same alphabets

on the bar graphs showed non-significant difference within each treatment. Asterisk stands for significant and *ns* for non-significant difference among the treatments (with and without *Th*)

Author contribution statement Miss RY contributed to whole physiological and other experimental works under the supervision of Dr. ZSS. Dr. ZSS not only helped her throughout the experimental work but also gave idea, and supervised article writing and data interpretation.

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References

- Abeer H, Abd-Allah EF, Alqarawi AA, Alwhibi MS, Alenazi MM, Dilfuza E (2015) Arbuscularmycorrhizal fungi mitigates NaCl induced adverse effects on *Solanum lycopersicum* L. Pak J Bot 47:327–340
- Ahmad P, Prasad MNV (2012) Abiotic stress responses in plants metabolism, productivity and sustainability. Springer, New York
- Ahmad P, Jaleel CA, Salem MA, Nabi G, Sharma S (2010) Roles of enzymatic and non-enzymatic antioxidants in plants during abiotic stress. Crit Rev Biotechnol 30:161–175
- Ahmad P, Ozturk M, Sharma S, Gucel S (2014) Effect of sodium carbonate-induced salinity-alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. J Plant Interact 9:460–467
- Ahmad P, Abeer H, Elsayed FAA, Alqarawi AA, Riffat J, Dilfuza E, Salih G (2015) Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. Front Plant Sci 6:868
- Alqarawi AA, Hashem A, Abd Allah EF (2014) Alleviation of salt-induced adverse impact via mycorrhizal fungi in *Ephedra aphylla* Forssk. J Plant Interact 9:802–810
- Azam F, Lodhi A, Farooq S, Harry-Ökuru R, Imam SH (2005) Seed treatment with phytohormones and crop productivity. III. Physiological/biochemical changes in germinating seeds and rooting characteristics of wheat (*Triticum aestivum* L.) following exposure to 2, 4 D. Pak J Bot 37:865–874
- Azooz MM, Youssef AM, Ahmad P (2011) Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater. Int J Plant Physiol Biochem 3:253–264
- Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci 15:413–428
- Bates L, Waldren PP, Teare JD (1973) Rapid determination of proline for water stress studies. Plant Soil 39:205–207
- Benitez T, Rincon AM, Limon MC, Codon AC (2004) Biocontrol mechanisms of *Trichoderma* strains. Int J Microbiol 7:249–260
- Bewley JD, Black M (1985) Seed physiology of development and germination. Plenum Publishing Corporation, New York, p 445
- Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in condition. Ann Biochem 161:559–566
- Bienert GP, Moller AL, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282:1183–1192
- Bjorkman T, Harman GE, Price HC (1994) Effects of the biocontrol fungus *Trichoderma harzianum* on root development in *Zea mays*. Plant Physiol (Rockville) 105:44
- Bjorkman T, Harman GE, Blanchard L (1995) Root development in sweet corn inoculated with the biocontrol fungus *Trichoderma harzianum*. HortScience 30:810
- Borde M, Dudhane M, Jite P (2012) Growth, water use efficiency and antioxidant defense responses of mycorrhizal and non mycorrhizal *Allium sativum* L. under drought stress condition. Ann Plant Sci 1:6–11
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem 72:248–254
- Chi Lin C, Huei Kao C (2001) Relative importance of Na⁺, Cl⁻, and abscisic acid in NaCl induced inhibition of root growth of rice seedlings. Plant Soil 237:165–171
- Colla G, Youssef R, Mariateresa C (2006) Effect of salinity on yield, fruit quality, leaf gas exchange, and mineral composition of grafted watermelon plants. HortScience 41:622–627
- Coolbear P, Francis A, Grierson D (1984) The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. J Exp Bot 35:1609–1617
- Dahnke WC, Whitney DA (1988) Measurement of soil salinity. In: Dahnke WC (ed) Recommended chemical soil test procedures for the North Central Region, pp 32–34
- Farooq M, Basra SMA, Hafeez K, Ahmad N (2005) Thermal hardening: a new seed vigor enhancement tool in rice. J Integr Plant Biol 47:187–193
- Flowers TJ, Gaur PM, Gowda CLL, Krishnamurthy L, Samineni S, Siddique KHM, Turner NC, Vadez V, Varshney RK, Colmer TD (2010) Salt sensitivity in chickpea. Plant Cell Environ 33:490–509
- Greenway H, Munns H (1980) Mechanisms of salt tolerance in non halophytes. Annu Rev Plant Physiol 31:49–190
- Gusain YS, Singh US, Sharma AK (2014) Enhance activity of stress related enzymes in rice (*Oryza sativa* L.) induced by plant growth promoting fungi under drought stress. Afr J Agric Res 9:1430–1434
- Hameed A, Egamberdieva D, Abd-Allah EF, Hashem A, Kumar A, Ahmad P (2014) Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: Use of microbes for the alleviation of soil stresses, vol 1, pp 139–159
- Harman GE, Howell CR, Vitarbo A, Chet I, Lorito M (2004) *Trichoderma* species—opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56
- Hashem A, Abd-Allah EF, Alqarawi AA, Al-Huqail AA, Egamberdieva D (2014) Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. J Plant Interact 9:857–868
- Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Article Calif Agric Exp Stn 347:32
- Hong CY, Chao YY, Yang MY, Cho SC, Huei Kao C (2009) Na⁺ but not Cl⁻ or osmotic stress is involved in NaCl-induced expression of glutathione reductase in roots of rice seedlings. J Plant Physiol 166:1598–1606
- Iqbal M, Ashraf M (2013) Alleviation of salinity-induced perturbations in ionic and hormonal concentrations in spring wheat through seed preconditioning in synthetic auxins. Acta Physiol Plant 35:1093–1112
- Jaarsma R, De Vries RSM, De Boer AH (2013) Effect of salt stress on growth, Na⁺ accumulation and proline metabolism in potato (*Solanum tuberosum*) cultivars. PLoS ONE 8:60183
- Ji H, Pardob JM, Batellic G, Van Oostend MJ, Bressane RA, Li X (2013) The salt overly sensitive (SOS) pathway: established and emerging roles. Mol Plant 6:275–286
- Jogaiah S, Govind SR, Tran LS (2013) Systems biology-based approaches toward understanding drought tolerance in food crops. Crit Rev Biotechnol 33:23–39
- Lichtenthaler HK (1987) Chlorophylls and carotenoids pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382
- Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158
- Mahmood A (2009) A new rapid and simple method of screening wheat plants at early stage of growth for salinity tolerance. Pak J Bot 41:255–262
- Mastouri F, Bjorkman T, Harman GE (2010) Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic and physiological stresses in germinating seed and seedling. Phytopathology 100:1213–1221

- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J Exp Bot* 51:659–668
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- Neumann PM (2008) Coping mechanism for crop plants in drought prone environments. *Ann Bot* 101:901–907
- Oliveira ABD, Alencar NLM, Filho EG (2013) Comparison between the water and salt stress effects on plant growth and development. In: *Agricultural and biological sciences, responses of organism to water stress*, chap 4
- Panuccio MR, Jacobsen SE, Akhtar SS, Muscolo A (2014) Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB Plants* 6:047
- Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Saf* 60:324–334
- Patterson BD, Macrae EA, Ferguson IB (1984) Estimation of hydrogen peroxide in plant extracts using titanium (IV). *Ann Biochem* 139:487–492
- Rajakumar R (2013) A study on effect of salt stress in the seed germination and biochemical parameters of rice (*Oryza sativa* L.) under in vitro condition. *Asian J Plant Sci Res* 3:20–25
- Rasool S, Hameed A, Azooz MM, Rehman M, Siddiqi TO, Ahmad P (2013) Salt stress: causes, types and response of plants. In: Ahmad P, Azooz MM, Prasad MNV (eds) *Ecophysiology and response of plants under salt stress*. Springer LLC, New York, pp 1–24
- Rawat L, Singh Y, Shukla N, 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 Soil* 347:387–400
- Reynolds M, Tuberosa R (2008) Translational research impacting on crop productivity in drought-prone environments. *Curr Opin Plant Biol* 11:171–179
- Sairam RK, Rao KV, Srivastava GC (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Sci* 163:1037–1046
- Shavrukov Y, Genc Y, Hayes J (2012) The use of hydroponics in abiotic stress tolerance research. In: Asao T (ed) *Hydroponics—a standard methodology for plant biological researches*. In: Tech, pp 39–66
- Shoresh M, Harman GE, Mastouri F (2010) Induce systemic resistance and plant response to fungal biocontrol agents. *Annu Rev Phytopathol* 48:21–43
- Shukla N, Awasthi RP, Rawat L, Kumar J (2012) Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. *J Plant Physiol Biochem* 54:78–88
- Siddiqui ZS (2013) Effects of double stress on antioxidant enzyme activity in *Vigna radiata* (L.) Wilczek. *Acta Botan Croat* 72:145–156
- Siddiqui ZS, Khan MA (2011) The role of enzyme amylase in two germinating seed morphs of *Halopyrum mucronatum* (L.) Stapf. in saline and non-saline environment. *Acta Physiol Plant* 33:1185–1197
- Siddiqui ZS, Khan MA (2013) Some physiological attributes of dimorphic seeds of *Halopyrum mucronatum* (L.) Stapf. *Pak J Bot* 45:1975–1979
- Siddiqui ZS, Cho JI, Hanpark S, Kwon TR, Ahn BO, Lee GS, Jeong MJ, Whankim K, Konlee S, Chulpark S (2014) Phenotyping of rice in salt stress environment using high-throughput infrared imaging. *Acta Botan Croat* 73:149–158
- Sultana N, Ikeda T, Itoh R (1999) Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environ Exp Bot* 42:211–220
- Tatiana Z, Yamashita K, Matsumoto H (1999) Iron deficiency Induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber root. *Plant Cell Physiol* 40:273–280
- Tsai YC, Hong C-Y, Liu L-F, Kao H (2004) Relative importance of Na⁺ and Cl⁻ in NaCl-induced antioxidant systems in roots of rice seedlings. *Physiol Plant* 122:86–94
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain treated bean plants: protective role of exogenous polyamines. *Plant Sci* 151:59–66
- Yedia I, Srivastava AK, Kapulnik Y, Chet I (2001) Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant Soil* 235:235–242
- Zhang F, Yuan J, Yang X, Cui Y, Chen L, Ran W (2013) Putative *Trichoderma harzianum* mutant promotes cucumber growth by enhanced production of indoleacetic acid and plant colonization. *Plant Soil* 368:433–444
- Zhu JK (2002) Salt and drought stress signal transduction in plants. *Annu Rev Plant Physiol Plant Mol Biol* 53:247–273