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Trichoderma Harzianum Enhancing Plant Growth Parameters and Reducing Deleterious Effects of Natural Saline-Sodic Soil in Rice

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Abstract: *As salinity is one of the major abiotic constraints limiting plant productivity and productivity, a pot experiment was conducted in natural saline- sodic soil collected from Jagdishpur (U.P.) to assess the effects of seed biopriming with selected salinity tolerant (ST) isolates of Trichoderma harzianum to reduce the deleterious effects of salt stress on physiological, biochemical and agronomical parameters in rice. The results indicated that the untreated plants were more sensitive to salt stress. Photosynthetic rate, chlorophyll fluorescence (F_v/F_m ratio), stomatal conductance, chlorophyll content, Leaf greenness and Membrane Stability Index reduced in untreated plants in comparison to Trichoderma treated plants under salt stress. Trichoderma treated plants exhibited lower accumulation of malondealdehyde content as compared to control (untreated). The higher proline content, membrane stability index and lower malondealdehyde accumulation might have imparted salt tolerance in Trichoderma treated plants. Agronomical parameters studied in case of rice grown under natural saline-sodic soil indicated that salt stress significantly reduced number of tillers, number of panicles, panicle length and 1000 grain weight. However, per cent decrease was significantly low in Trichoderma treated plants.*

Keywords: *Rice; Trichoderma; Salt stress; Seed biopriming.*

INTRODUCTION

Among cereal crops, rice (*Oryza sativa* L.) is a major source of food after wheat for more than 2.7 billion people on a daily basis. Rice is considered as a salt sensitive monocot (Mass and Hoffman, 1997). Qayyum and Malik (1998) have reported 64 per cent reduction in yield of rice even on moderately salt affected soils. Rice is relatively salt–tolerant at germination but becomes very sensitive at the young seedling stage, which impacts the stand density in salt-affected fields (Lutts *et al*., 1995).

Salinity causes decrease both in growth and net photosynthesis of higher plants. The decline in productivity observed for many plant species subjected to excess salinity is often associated with the reduction in photosynthesis capacity (Long and Baker, 1986). The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content (Delfine *et al*., 1999) and the reduction in growth at higher salinity could be attributed to a reduction in leaf area expansion (Marcelis and Hooijdonk, 1999). Chlorophyll fluorescence, a tool that monitors the function of the photosynthetic apparatus, has also been shown to change in response to water stress and salinity (Jamil *et al*., 2007). Characters like leaf damage, plant height and survival are the most commonly used criteria for identifying salinity tolerance (Gamma *et al*., 2007). Other indices of tolerance have also been proposed that are based on specific physiological characteristics- accumulation of compatible solutes in shoots or leaves and that accumulation may play a role in combating salinity stress (Ashraf and Harris, 2004). Salt stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids (Mittler, 2002). Lipid peroxidation, induced by free radicals, is also important in membrane deterioration (Khan and Panda, 2008).

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Soil salinity causes an imbalance of the cellular ions resulting in osmotic stress, which makes water uptake by roots difficult. However, incorporation of *Trichoderma* enhances deep root growth, which helps in more water acquisition and nutrient uptake (Harman, 2006; Azarmi *et al*., 2011). *Trichoderma* application through seed biopriming helps seeds to germinate even under adverse soil conditions (Singh *et al*., 2003). Seed biopriming is a process of biological seed treatment that refers to a combination of seed hydration and seed inoculation with beneficial organisms to protect seed. Beneficial activities attributed to *Trichoderma*-plant interactions include induced disease resistance, plant growth promotion, and tolerance to abiotic stresses (Shoresh and Harman, 2008; Mastouri *et al*., 2010).

As a consequence of primary effects (hyper osmotic stress, ion imbalance) of high salt concentrations, secondary stresses, such as oxidative stresses due to overproduction of reactive oxygen species (ROS) often occur. However, root colonization by *Trichoderma harzianum* results in increased level of plant enzymes, including various peroxidases, chitinases, β-1,3-glucanases, lipoxygenase-pathway hydroperoxide lyase and such changes in plant metabolism can lead to the accumulation of compounds like phytoalexins and phenols to provide durable resistance against any biotic and abiotic stress (Mohiddin *et al*., 2010).

The present research was, therefore, carried out to elucidate in detail the usefulness of seed biopriming technique with salinity tolerant (ST) isolates of *Trichoderma harzianum* in alleviation of the adverse effects of salt stress on physiological, biochemical and agronomical parameters in rice and also to find the correlation between physiological and biochemical parameters affected by saline-sodic soil in rice plants (var. PD-11) pretreated with selected *Trichoderma* isolates under salt stress. In this study, we have examined and compared the proline, malondealdehyde and phenol accumulation in *Trichoderma* treated and untreated rice seedlings grown under natural saline-sodic soil in order to exploit the salinity tolerant *Trichoderma* strains in relation to salt stress tolerance in rice. This research is important as it reveals the role of *Trichoderma* that imparts stress resistance and provide insight in to the potential for rice plants to adapt to saline conditions.

MATERIALS AND METHODS

Materials and Methods

Experimental Site

The investigation was carried out at Biocontrol Laboratory of Plant Pathology Discipline, College of Forestry, Ranichauri, V.C.S.G UUHF. The experiment was carried out in 5 Kg capacity pots with soil collected from Jagdishpur UP (saline-sodic soil).

Soil Analysis

The characteristics of normal soil (collected from Crop Improvement Department, Ranichauri Campus, V.C.S.G. UUHF) and saline soil (collected from Jagdishpur, UP) used in the present study are given in the Table 1.

Trichoderma isolates

Five isolates of *Trichoderma* harzianum (*Rani Th-10, Rani Th -14, Rani Th -21, Rani Th-30 and Rani Th-39*) obtained from the repository of biocontrol

Table 1
Characteristics of normal and saline-sodic soils used in the present study.

	Values	
Characteristics	*Normal soil*	*Saline-sodic soil*
1. Electrical conductivity of saturation extract (dS/m)	0.39	6.6
2. pH of soil saturated extract	7.6	9.5

Table 2
List of different treatments used at recommended dose of 10g/kg seeds in evaluation against salinity stress in rice (var. PD-11)

Symbol	*Treatments*
T1	Control (untreated)
T2	*T. harzianum* (*Rani Th-10*)
T3	*T. harzianum* (*Rani Th-14*)
T4	*T. harzianum* (*Rani Th-21*
T5	*T. harzianum* (*Rani Th-30*)
T6	*T. harzianum* (*Rani Th-39*)

*T1, T2, T3, T4, T5 and T6 are symbols of different treatments

laboratory of Discipline of Plant Pathology, College of Forestry, Ranichauri, V.C.S.G Uttarakhand University of Horticulture and Forestry, were used in the experiment.

Preparation of talc-based formulation of Trichoderma

Mass culture of each selected *Trichoderma harzianum* isolate was prepared separately on barnyard millet (*Echinocloa frumentacae*) grains. Grains were soaked in water for 12 h and filled in 250 ml Erlenmeyer flasks (@ 50 g/ flask). Those flasks were autoclaved at 15 *lbs psi* for 30 minutes. After cooling to room temperature, the flasks were inoculated with mycelial discs cut from three-day old culture of *Trichoderma* spp. and incubated at 28°C for 12 days. *Trichoderma* colonized barnyard millet grains were air dried in open shade and ground with Willy Mill to a fine powder. The powder was passed through 50 and 80 mesh size sieves, simultaneously to obtain a pure spore powder. The talc formulation was prepared by diluting this powder with talcum powder (350 mesh, 95% whiteness) and 1 % carboxy methyl cellulose (CMC), used as a sticker, to get desired concentration of biocontrol agents in the formulation. The final colony forming units of *Trichoderma* were adjusted to 5×10^6 Cfu/g of the formulation.

Seeds

Seeds of Rice (var. PD- 11) were obtained from Crop Improvement Department, Ranichauri Campus, V.C.S.G. UUHF.

Seed biopriming

Prior to their use, seeds were surface sterilized with 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and dried. Seeds were bioprimed separately with each drought tolerant isolates of *Trichoderma* spp @ 10g/kg of seeds. After presoaking of seeds in sterile distilled water, seeds were coated with powder formulation of *Trichoderma* spp. and mixed thoroughly to provide uniform coating. Seeds were then kept under warm and moist conditions at 25 ± 2°C for 24 hrs until prior to radical emergence as this technique helps *Trichoderma* to increase in number by almost hundred times on spermosphere during incubation period as compared to normal seed treatment (Singh *et al*., 2004).

Observations

Physiological and biochemical parameters observed were:

Total chlorophyll content (CC)

The total CC of fresh leaves was estimated following the method suggested by Barnes *et al*. (1992) according to the following formula:

$$\text{Total CC (mg/g)} = (2.02 \times A_{645}) + (8.02 \times A_{663}) / (\text{weight in gm} \times 1000)$$

SPAD Value

A chlorophyll meter was used to estimate the nitrogen status of crops. The instrument measures transmission of red light at 650 nm, at which chlorophyll absorbs light and transmission of infrared light at 940 nm, at which no absorption occurs. On the basis of these two transmission values the instrument calculates a SPAD (Soil Plant Analysis Development) value that is quite well correlated with chlorophyll content. SPAD readings were recorded by a portable SPAD meter (Opti Science, CMM-200, USA). At each evaluation, the content was measured 6 times from leaf to base and the average was used for analysis.

Chlorophyll fluorescence (Fv/Fm ratio)

Chlorophyll '*a*' fluorescence emitted by green plants reflects photosynthetic ability of PS-II. A handy plant efficiency analyzer (Handy PEA, Hansatech, UK) was used to monitor chlorophyll fluorescence (F_v/F_m). Measurements were recorded in the forenoon (9-10 am) to avoid photo inhibition.

Photosynthetic rate and stomatal conductance

Photosynthesis (μ mol CO_2 m^{-2} s^{-1}) was determined with the help of portable CO_2 gas analyzer (CID inc. USA). It was measured in an open system in which instrument takes reference CO_2 from atmosphere. The air flow rate, response time and added interval time were 0.4 LPM, 15 S and 20 s, respectively. The area of window of leaf chamber (broad rectangular) was 11 cm^2. Other system set up values was those which are already present in the memory of instruments. The time of measurement was kept

constant. Photosynthetic rate and stomatal conductance were assessed on intact leaves. All readings were taken in full sun light and data were taken in triplicates for each plant to get the mean value.

Membrane Stability Index (MSI)

Membrane stability index was examined according to Sairam *et al*., 1997. Four to five leaves were taken in test tube containing 10 ml of double distilled water in two sets. One set was kept at 40°C for 30 min. another set at 100°C in boiling water bath at for 15 min. and there respective electrical conductivity, C_1 and C_2 was measured by conductivity meter using the formula,

$$MSI = [1 - (C_1/C_2)] \times 100$$

Where, C_1= conductivity at 40°C; C_2= conductivity at 100°C

Proline Content

Proline content was determined following the method of Bates *et al*. (1973). A pre-weighed (0.2g) leaf sample was homogenized in 4 ml of sulfosalicylic acid (3%) and centrifuged at 10000 g for 30 min. About 2ml of extract supernatant was taken in a test tube and to it 2ml of glacial acetic acid and 2 ml of ninhydrin reagent was added. The reaction mixture was boiled in water bath at 100°C for 30 min. After cooling the reaction mixture, 4 ml of toluene was added and vortexed for 30 sec. The upper phase containing proline was measured with spectrophotometer at 520 nm using toluene as a blank. Proline content (µ mol/g fr.wt.) was quantified using the ninhydrin acid reagent method by using L-proline as a standard.

Malondialdehyde (MDA) content

Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Heath and Packer, 1968 with some modifications. A pre-weighed (0.2g) fresh leaf sample was ground homogenate in 3 ml of 0.1% TCA (Trichloro acetic acid). Homogenate was centrifuged at 1000g for 10 min. 1.2 ml of (0.5% thio butyric acid in 20% TCA) was added to 0.3 mL supernatant and incubated in water bath at 95°C for 30 min. Reaction was terminated in ice, centrifuged at 1000g for 10 min. Absorbance of supernatant was determined at 532 and 600 nm .After subtracting the non specific absorbance at 600 nm, the MDA concentration (µ mol/g fr.wt.) was determined using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Agronomical parameters

Plant height, number of tillers, number of panicles, panicle length and 1000 grain weight were recorded manually.

RESULTS

Evaluation of Selected Trichoderma Isolates to Alleviate Salt Stress in Rice Under Natural Saline-sodic Soil

Results of analysis of variance of data for different parameters are presented in Tables (3 to 7). While, correlation between physiological and biochemical parameters is depicted in Table 8. Effect of salt injury on rice seedlings at early stage in natural saline-sodic soil is shown in Figure 1.

Physiological and Biochemical Parameters

Total chlorophyll content

Total chlorophyll content in all treatments was found to decrease under stress condition (saline soil) in comparison to non-stress condition (normal soil). Under stress, maximum chlorophyll content was recorded in *Rani Th-14* (1.65 mg/g fr.wt.) followed by *Rani Th-10* (1.69 mg/g fr.wt.) and minimum was calculated in control (1.34 mg/g fr.wt) followed by *Rani Th-39*(1.59 mg/g fr.wt). Per cent decrease in chlorophyll content was found to range from 35.98% to 46.61% (Table 3).

All *Trichoderma* treated plants showed relatively least per cent decrease in chlorophyll content (Table 3) than control (untreated). Correlation study (Table 8) showed that chlorophyll content was significantly positively correlated with photosynthetic rate (r = 0.950*), chlorophyll fluorescence (r = 0.875**) and stomatal conductance (r = 0.910**) whereas negatively correlated with MDA (r = –0.867**).

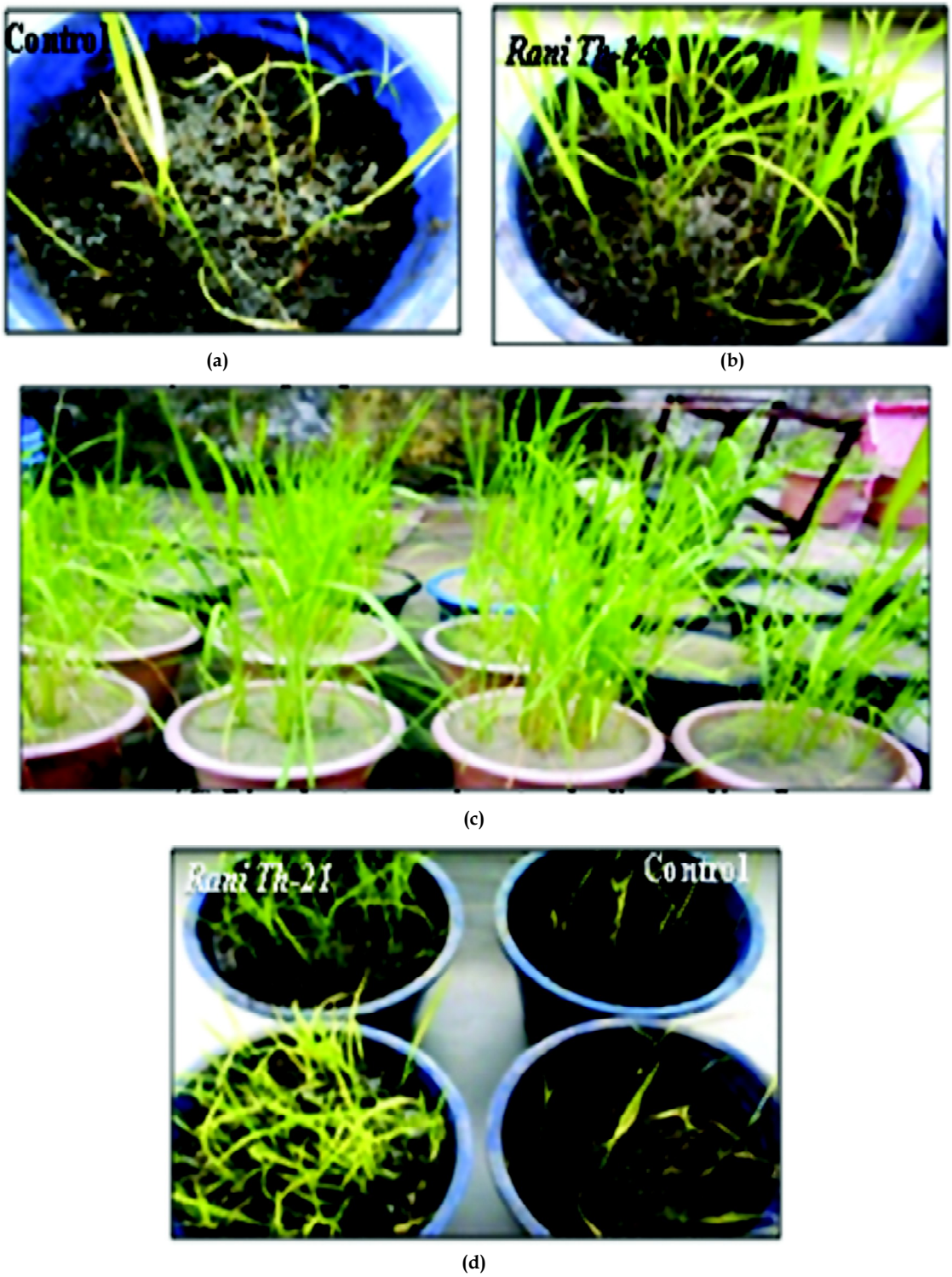

Figure 1. Effect of salt injury on rice seedlings at early stage in natural saline–sodic soil. (A) Mortality of rice seedlings due to salt stress at early seedling stage. (B) Seedlings obtained from *Rani Th–14* treated rice seeds. (C) Rice seedlings grown in natural saline–sodic soil. (D) Comparative difference between treated (*Rani Th–21*) and untreated seeds (control).

SPAD value

SPAD value showed a positive significant correlation with total chlorophyll content ($r = 0.85^{**}$). Per cent decrease in greenness among the treatments varied from 35.98% to 46.61% (Table 3) under stress condition (*i.e.*, saline soil). Under stress, treatments *Rani* Th-10, *Rani Th-14*, *Rani Th-21*, *Rani Th-30* and *Rani Th-39* showed comparatively minimal effect on

Table 3
Effect of saline-sodic soil on total chlorophyll content, SPAD value, and chlorophyll fluorescence in rice (var. PD-11) plants obtained from seeds bioprimed with selected *Trichoderma* isolates

	Total Chlorophyll(mg/g fr.wt)			*SPAD value*			*Chlorophyll fluorescence*		
Treatments	*NS*	*SS*	*% decrease*	*NS*	*SS*	*% decrease*	*NS*	*SS*	*% decrease*
Control	2.51	1.34	46.61	35.41	23.79	32.81	0.66	0.35	46.96
Rani Th-10	2.64	1.69	35.98	42.27	36.46	13.74	0.73	0.43	41.09
Rani Th-14	2.85	1.75	38.59	43.22	37.99	12.10	0.75	0.47	37.33
Rani Th-21	2.65	1.61	39.24	41.89	36.23	13.51	0.71	0.43	39.43
Rani Th-30	2.60	1.66	36.15	39.97	30.40	23.94	0.70	0.40	42.85
Rani Th-39	2.58	1.59	38.37	39.01	29.32	24.83	0.67	0.38	43.28
CD at 5%	0.165	0.103		2.28	3.597		0.043	0.406	

NS = Normal soil; SS = Saline-sodic soil

Table 4
Effect of saline-sodic soil on photosynthetic rate, stomatal conductance, and membrane stability index in rice (var. PD-11) plants obtained from seeds bioprimed with selected *Trichoderma* isolates

	Photosynthetic rate (μ *mol* CO_2 m^{-2} s^{-1})			*Stomatal conductance* (*mol* H_2O m^{-2} s^{-1})			*Membrane stability Index (%)*		
Treatments	*NS*	*SS*	*% Decrease*	*NS*	*SS*	*% Decrease*	*NS*	*SS*	*% Decrease*
Control	13.42	11.39	15.12	0.72	0.42	41.66	80.72	67.88	15.91
Rani Th-10	14.06	12.09	14.01	0.83	0.67	19.27	84.68	74.75	11.72
Rani Th-14	14.43	12.76	11.57	0.90	0.77	14.44	86.25	75.71	12.22
Rani Th-21	14.22	12.46	12.37	0.89	0.75	15.73	84.83	73.66	13.16
Rani Th-30	14.00	12.31	12.07	0.82	0.71	13.41	84.08	74.52	11.37
Rani Th-39	13.75	11.79	13.11	0.81	0.67	17.28	85.43	73.16	14.36
CD at 5%	0.481	0.457		0.374	0.066		2.807	2.448	

NS = Normal soil; SS = Saline-sodic soil

greenness with a per cent decrease of 35.98% to 39.24% while it was maximal (46.61%) for T1 (control).

Chlorophyll fluorescence

A significant decrease was observed in chlorophyll fluorescence for all treatments under stress with percent decrease ranging from 37.33% to 46.96% (Table 3). Maximal decrease in chlorophyll fluorescence was recorded in control (46.96%) followed by *Rani Th-39* (43.28%) and *Rani Th-30* (42.35%) while it was minimal in *Rani Th-14* (37.33%) followed by Th-21 (39.43%). Chlorophyll fluorescence showed a significantly positive correlation with photosynthetic rate (r = 0.937**), SPAD value (r = 0.915**), stomatal conductance (r = 0.925) and MSI (r = 0.902**).

Photosynthetic rate

Photosynthetic rate seems to be significantly reduced when plants are grown under stress. However, maximum photosynthetic rate was shown by plants pretreated with Th-14 (12.76 μ mol$CO_2m^{-2}s^{-1}$) followed by *Rani Th-21* (12.46 μ mol $CO_2m^{-2}s^{-1}$) and minimum by control (11.39 μ mol $CO_2m^{-2}s^{-1}$) under stress condition (Table 4). Per cent decrease in photosynthetic rate was recorded in a range of 11.57% to 15.12% with maximum in control (15.12%) followed by *Rani Th-10* and *Rani Th-39* with 14.01%, and 13.11% decrease, respectively. Per cent decrease was relatively lower in *Rani Th-14* (11.57%) followed by *Rani Th-30* (12.07%). Positive significant correlation was found between photosynthetic rate and total chlorophyll content (r = 0.950**).

Stomatal conductance

A positive significant correlation was found between stomatal conductance and photosynthetic rate ($r = 0.939^{**}$). Under stress, stomatal conductance was found to be significantly decreased in all treatments with per cent decrease ranging from 13.41% to 41.66%. *Rani Th-14* showed maximal stomatal conductance both under non-stress (0.90 mol H_2O $m^{-2}s^{-1}$) and stress condition (0.77 mol H_2O $m^{-2}s^{-1}$) followed by *Rani Th-21* (0.89 mol H_2O $m^{-2}s^{-1}$ and 0.75 mol H_2O $m^{-2}s^{-1}$ under non-stress and stress conditions, respectively). However, *Rani Th-30* followed by *Rani Th-14* found to have minimum per cent decrease in stomatal conductance recording 13.41% and 14.44% respectively. Minimal stomatal conductance both under non-stress (0.72 mol H_2O $m^{-2}s^{-1}$) and stress condition (0.42 mol H_2O $m^{-2}s^{-1}$) and maximal per cent decrease (41.66%) was measured in control (Table 4).

Membrane stability index (MSI)

MSI was also severely affected under stress condition (saline soil) with a percent decrease ranging from 11.37% to 15.91% (Table 4). Maximal per cent decrease was measured in control (11.37%) while it was minimal in Th-33 (15.91%) followed by *Rani Th-10* and *Rani Th-14* (11.72% and 12.22%, respectively). Under stress, highest membrane stability index was recorded in *Rani Th-14* (75.71) followed by *Rani Th-10* (74.75) and *Rani Th-30* (74.52) and lowest in control (67.88). MSI showed a significantly positive correlation with proline content ($r = 0.855^{**}$).

Proline content

Among all the parameters observed under stress condition, highest fluctuations in values were observed in proline content. The proline content increased significantly under stress condition in all the treatments with per cent increase ranging from 77.12% to 82.01% (Table 5). In T1 (control), proline content was found to increase from 2.50 µmol/g fr.wt (normal soil) to 10.93 µmol/g fr.wt (saline soil) with a percent increase of 77.12%. *Rani Th-14* showed maximum proline content (18.38µmol/g fr.wt) under stress. However, per cent increase in proline content was maximal for *Rani* Th-39 (82.01%) followed by *Rani Th-21* (81.72% and *Rani Th-30* (81.61%).

Malondealdehyde content (MDA content)

Lipid peroxidation in leaves was measured as MDA content. It was observed that MDA content increased in all treatments under stress condition with a per cent increase ranging from 55.78% to 69.4% (Table 5). Per cent increase in MDA content was found maximum 69.4% for T1 (control) while it was quiet lower in all *Trichoderma* treated plants (Table 4.38) recording lowest per cent increase in *Rani Th-14* (55.78%) followed by *Rani Th-10* (58.88%). MDA content showed a significantly negative correlation with membrane stability index ($r = -0.823^{**}$) and proline content ($r = -0.894^{**}$).

Agronomical Parameters

Plant height

Different treatments showed significant differences in plant height under both non-stress and stress conditions. Plant height seems to be significantly decreased under stress condition in all treatments (Table 6).Under stress, maximum plant height was observed in *Rani Th-14* (108.30 cm) followed by *Rani Th-21* (107.93 cm) and minimum was recorded in control (93.30cm). Per cent decrease in plant height recorded ranging from 29.85% with minimal in Th-21 to 35.84% with maximal in control.

Table 5
Effect of saline-sodic soil on proline content and malondealdehyde content in rice (var. PD-11) plants obtained from seeds bioprimed with selected *Trichoderma* isolates

	Proline content (µ mol/g fr.wt)			*MDA (µ mol/g fr.wt)*		
Treatments	*NS*	*SS*	*% increase*	*NS*	*SS*	*% increase*
Control	2.50	10.93	77.12	2.38	7.78	69.40
Rani Th-10	3.59	18.25	80.32	1.55	3.77	58.88
Rani Th-14	3.62	18.38	80.30	1.26	2.85	55.78
Rani Th-21	3.19	17.46	81.72	1.57	5.36	63.99
Rani Th-30	3.14	17.08	81.61	1.81	4.95	63.43
Rani Th-39	2.96	16.46	82.01	1.77	5.27	62.09
CD at 5%	0.518	1.066		0.448	0.779	

NS = Normal soil; SS = Saline-sodic soil; fr.wt = Fresh weight

Table 6
Effect of saline-sodic soil on plant height, number of tillers per plant, and number of panicles in rice (var. PD-11) plants obtained from seeds bioprimed with selected *Trichoderma* isolates

	Plant height (cm)			*Number of tillers*			*Number of panicles*		
Treatments	*NS*	*SS*	*% decrease*	*NS*	*SS*	*% decrease*	*NS*	*SS*	*% decrease*
Control	145.43	93.30	35.84	4.80	3.53	26.45	4.00	3.03	24.25
Rani Th-10	157.36	104.33	33.69	5.00	4.00	20.00	4.30	3.40	20.93
Rani Th-14	159.86	108.30	32.25	5.43	4.16	23.38	4.50	3.53	21.55
Rani Th-21	153.86	107.93	29.85	5.36	4.10	23.50	4.30	3.30	23.25
Rani Th-30	154.76	103.96	32.82	5.13	3.93	23.39	4.40	3.40	22.72
Rani Th-39	155.83	105.40	32.36	5.06	3.93	22.33	4.26	3.30	22.53
CD at 5%	4.331	4.203		0.287	0.393		0.167	0.145	

NS = Normal soil; SS = Saline-sodic soil

Number of tillers

Number of tillers under study showed a significant variation among different treatments under non-stress condition whereas difference was non-significant under stress condition (Table 6). Number of tillers varied from 4.8 to 5.43 with maximal in *Rani Th-14* under non-stress condition and from 3.53 to 4.16 again with maximal in *Rani Th-14* under stress condition. However, per cent decrease in number of tillers varied from 20% with minimum in *Rani Th-10* followed by *Rani Th-39* (22.33%) to 26.45% with maximum in control.

Number of panicles

Number of panicles averaged over treatments was found to be significantly decreased from non-stress (4.26) to stress condition (3.30). Under stress, plants treated with *Rani Th-14* followed by *Rani Th-10* attained maximum number of panicles (3.53 and 3.4, respectively) while minimum number of panicles was occupied by untreated plants (control). Per cent decrease in number of panicles ranged from 20.93% with minimum in *Rani Th-14* to 24.25% with maximum in control (Table 6).

Panicle length

Panicle length also showed a significant difference among different treatments both under non-stress and stress conditions. Panicle length seems to be reduced under stress condition in all treatments with highest length recorded in *Rani Th-14* (24.16 cm) followed by *Rani Th-10* (23.90 cm) and *Rani Th-21* (23.80 cm while lowest panicle length was recorded in control (21.18 cm). Per cent decrease calculated varied from 6.74% with minimal in *Rani Th-10* followed by *Rani Th-21* (7.14%) to 13.76% with maximal in control (Table 7).

1000 grain weight

Stress condition imparted significant effect on 1000 grain weight with lowest grain weight measured in control (11.38 g) and highest grain weight in *Rani Th-14* (12.30 g) followed by *Rani Th-10* (12.2g). Per cent decrease in 1000 grain weight recorded ranging from 9.82% to 15.26% with minimal per cent

Table 7
Effect of saline-sodic soil on panicle length and 1000 grain weight in rice (var. PD- 11) plants obtained from seeds bioprimed with selected *Trichoderma* isolates

	Panicle length (cm)			*1000 grain weight (g)*		
Treatments	*NS*	*SS*	*% Decrease*	*NS*	*SS*	*% Decrease*
Control	24.56	21.18	13.76	13.43	11.38	15.26
Rani Th-10	25.63	23.90	6.74	13.65	12.20	10.62
Rani Th-14	26.16	24.16	7.64	13.66	12.38	9.37
Rani Th-21	25.63	23.80	7.14	13.50	12.17	9.85
Rani Th-30	25.70	23.52	8.48	13.49	12.14	10.00
Rani Th-39	25.86	23.23	10.17	13.92	12.18	9.82
Sem	0.33	0.334		0.892	0.086	
CD at 5%	1.017	1.03		0.275	0.267	

NS = Normal soil; SS = Saline-sodic soil

Table 8
Correlation between physiological and biochemical parameters affected by saline–sodic soil in rice plants (var. PD– 11) pretreated with selected *Trichoderma* isolates

S. No.	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	0.875**	–0.867**	0.903**	0.950**	0.902**	0.850**	0.910**
2		–0.909**	0.902**	0.932**	0.868**	0.915**	0.925**
3			–0.823**	–0.859**	–0.894**	–0.832**	–0.891**
4				0.910**	0.855**	0.833**	0.893**
5					0.843**	0.925**	0.939**
6						0.793**	0.911**
7							0.919**
8							–

1 = Total chlorophyll 2 = Fv/Fm ratio 3 = MDA content
4 = Membrane stability index 5 = Photosynthetic rate 6 = Proline content
7 = SPAD value 8 = Stomatal conductance

decrease in *Rani Th-14* (9.82%) followed by *Rani Th-21* (9.85%) and maximal per cent decrease in control (15.26%).

DISCUSSION

Evaluation Against Salinity Stress

The main objective of this study was to isolate and screen for salinity tolerant *Trichoderma* isolates and their evaluation in reducing the detrimental effects of salinity in rice

Salt stress is an important abiotic factor limiting crop productivity. It is an intricate phenomenon involving osmotic stress, specific ion effect, nutrient deficiency, etc. thereby affecting different physiological and biochemical mechanisms concerned with plant growth and development. Agronomical parameters have also been established for the screening of salinity tolerance (Singh *et al*., 2008). Panicle length, tiller numbers per plant and harvest index are important agronomic characters for the prediction of final yield in rice. These yield components are severely affected by salinity (Zeng *et al*., 2004). However; in the present study, we observed a highly significant effect of seed biopriming with selected isolates of *Trichoderma harzianum* in reducing the negative effects of salt stress in tested crop though the amelioration was better in *Trichoderma harzianum Rani Th-14* in rice among all the tested five isolates. In the present study, Seed biopriming technique has been found beneficial under adverse soil conditions.

Physiological Parameters

Plants exposed to saline environment showed reduced chlorophyll content (CC) and SPAD value with no significant effect on leaf water content. The earlier reports in radish in cabbage and sugarbeet (Jamil *et al,* 2007) supported our results. The present results indicated that, CC and SPAD value significantly reduced by increasing salinity levels and the magnitude of the reduction varied among treatments. NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme; Chlorophyllase (Rao and Rao, 1981), inducing the destruction of the chloroplast structure and the instability of pigment protein complexes (Singh and Dubey, 1995). It has been reported that chlorophyll content decreases in salt susceptible plants (Hamada and El-Enany, 1994). Comparatively, maximum chlorophyll content and SPAD value was recorded in Th-14 and minimum in control both under normal and salt stress condition. However, the decrease in above parameters with increased salinity was observed in all the treatments during investigation.

Chlorophyll fluorescence is a rapid and non intrusive tool used to screen for salt tolerance (Moradi and Ismail, 2007). F_v/F_m ratio which indicates maximum quantum yield of PS II is found

to be decreased under salt stress condition and directly correlated with plant health under stress condition (Chaum *et al*., 2007). In present investigation, maximal quantum yield of PS2 (F_v/F_m) was reduced consistently as the salt level increased especially at higher salt concentrations and the rate of decrease was higher in control among all the treatments. The reduction of chlorophyll fluorescence is associated with the increase of Na accumulation (Dionisio–Sese and Tobita, 2000). Salt decreases efficiency of photosynthesis (Ashraf and Shahbaz, 2003). Our results in this study indicated that *Trichoderma* application through seed bioprimig enhanced chlorophyll fluorescence at all stress levels in comparison to control (untreated), with substantial reduction under salt stress.

It has been reported that stomatal conductance decreases significantly with salinity treatment (Djanaguiraman *et al.,* 2006). Stomatal conductance is related to turgor pressure of the cell and has been suggested to be the most effective screening strategy for salinity tolerance (Singh *et al*., 2007). The turgor pressure is controlled by solute regulation within guard cell protoplast and the RWC of epidermal tissues. External high salt solution enhanced the accumulation of sodium and chloride that altered the osmotic activity causing a reduction in water potential and an influx of water from the surrounding cells. Stomatal conductance significantly decreases even under mild salt stress and often considered as a rapid initial response to salt stress (Walia *et al*., 2005). In the present study *Rani Th-14* was found significantly superior to all with maximal stomatal conductance and minimum % decrease followed by *Rani Th-21* while minimal stomatal conductance and maximum % decrease was in untreated plants, revealing loss of turgor pressure of the cells due to salt injury.

Higher photosynthetic rate is one of the factors for realizing higher grain yield because, it is expected to provide the raw material and the energy required for growth and development (Channappagoudar *et al*., 2007). It is reported earlier that with salt stress, photosynthetic rate decreases and it might be due to

(i) salt stress severely impairs photosynthetic activities as well as photosynthetic apparatus (Kim *et al*., 2007),

(ii) direct effect of stomatal resistance via a higher reduction in guard cell turgor (Moradi and Ismail, 2007). Stomatal closure is assumed to be the main cause of decrease in photosynthetic rate because it cause decrease in CO_2 availability in mesophyll cell (Tezara *et al*., 2003). In our study also, a significant positive correlation between stomatal conductance and photosynthetic rate was found.

(iii) may be due to a cumulative effect of other non stomatal and biochemical components. In the present investigation, under natural saline soil, reduction in photosynthetic rate was highest in control which showed maximum per cent decrease of 15.12% while *Rani Th-14* showed maximum photosynthetic rate and minimum % decrease of 11.57%. Similarly, under artificially created salt stress, maximum photosynthetic rate was observed in *Rani Th-14* followed by *Rani Th-21*. Photosynthesis is directly co-related with number of leaves and leaf area per plant (Reich *et al*., 1999) with the hypothesis that no species can improve photosynthetic capacity without increasing number of leaves and leaf area due to biophysical limitations. It is well documented that *Trichoderma* increases the total biomass of plants which might contribute to higher photosynthesis.

Biochemical Parameters

The results on MSI showed decreasing trend with the increase in salt concentration. The presence of NaCl in the rooting medium caused a disturbance in membrane permeability expressed by an increase in solute leakage (Ghoulam *et al*., 2002). The leakage was higher in untreated plants than *Trichoderma* treated plants, indicating severe membrane damage for the former under salt stress. The higher leakage of solutes was probably due to enhanced H_2O_2 accumulation and lipid per oxidation under salt stress (Dionisio-Sese and Tobita, 1998). An important consequence of salt stress is generation of ROS (reactive oxygen species). These oxidants, formed under salt stress, cause membrane disorganization and metabolic toxicity, resulting in higher leakage of solutes. The leakage was lowest in *Rani Th-14* treated plants revealing reduced

accumulation of lipid peroxides under osmotic stress. Gachomo and Kotchtoni (2008) also concluded that root colonization by *Trichoderma harzianum* T22 results in enhanced concentration of antioxidant enzymes (like peroxidases, chitinases etc). These antioxidant enzymes act as scavengers of ROS and thus cause membrane stability.

Proline accumulation is an important biochemical index for plant response to salt stress and is supposed to correlate with adaptation to salinity (Ashraf and Harris, 2004). Our results clearly indicate that proline gets accumulated in response to NaCl stress and the level rises with increasing NaCl concentration. The synthesis and accumulation of proline was significantly higher in *Trichoderma* treated plants. Greater the accumulation of proline indicates a greater degree of tolerance; being an organic osmolyte proline helps in the homeostasis establishment. Thus, accumulation of proline is a biological indicator of stress tolerance in plants by balancing the osmotic level of cytosol with vacuole and external environment (Gadallah, 1999) Proline accumulations may also help in non-enzymatic free radical detoxification caused by salinity (Khan *et al*., 2002). In present study, the higher osmolyte concentration in *Rani Th-14* under salt stress helped maintain structure and function of cellular macromolecules.

Lipid peroxidation measured as the amount of thiobarbituric acid reactive substance or malondialdehyde is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals. As lipid peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Khan and Panda, 2008). The results of present study showed that the degree of accumulation of MDA was higher in treatment T1 (control) and least accumulation was in treatment T3 (*Rani Th-14*) followed by T4 (*Rani Th-21*) in case of wheat and rice under artificial salt stress and in treatment T4 (*Rani Th-21*) followed by T6 (*Rani Th-39*) in rice under natural saline soil, indicating reduction in rate of lipid peroxidation as a result of seed biopriming with the above *Trichoderma strains*. Thus, our experimental findings clearly demonstrate that *Trichoderma* treated seedlings can alleviate the damage caused by reactive oxygen species, resulting in better growth of the seedlings. Whereas, an increase in MDA level in untreated seedlings at higher salt concentration could be because of the limited capacity to detoxify ROS at higher salt concentration.

In the present investigation, total chlorophyll content, SPAD value, Fv/Fm ratio, stomatal conductance, photosynthetic rate, proline content, MSI were found to be significantly positively correlated with each other and at the same time significantly negatively correlated with MDA content. A similar kind of relationship was observed by Jamil *et al*. (2007).

Agronomical Parameters

Yield is a very complex character which comprise of many components and these yield components are related to final grain yield which are also severely affected by salinity (Zeng *et al*., 2004). Grain yield is a function of interplay of various yield components such as number of tillers, number of panicles, panicle length and 1000 grain weight (Safdar *et al*., 2006).

Though the plant height is basically a genetically controlled character, it is being influenced by environmental conditions (Channappagoudar *et al*., 2007). In the present investigation, Plant height was significantly affected in saline soil with maximum per cent decrease in untreated plants while on *Rani Th-21* and *Rani Th-14*, the effect was found least showing minimum per cent decrease of 29.85 and 32.25% respectively.

Among yield components, productive tillers are very important because the final yield is mainly a function of the number of panicles bearing tillers per unit area (Motamed *et al*., 2008). In the present investigation, effect of salinity on number of tillers per plant was found significant though it was non-significant among the treatments. The number of tillers of different treatments under salt stress was found to range between 3.53 to 4.16 with minimum in control and maximum for *Rani Th-14*.

Number of panicles and panicle length are also important yield contributing factor which are significantly affected by salinity (Shereen *et al*., 2005). In the present study, number of panicles

seems to reduce under stress condition but the *Trichoderma* treatments effect were found significant with minimum per cent decrease by *Rani Th-14* followed by *Rani Th-21*. Regarding panicle length, the saline effect was found least in *Rani Th-14* followed by *Rani Th-30* with minimum per cent decrease of 6.74 and 7.14 % respectively and again the saline effect was highest in control plants.

1000 grain weight is an important yield determining component. In the present investigation, 1000 grain weight significantly reduced under saline stress. It is previously reported that salt stress may reduce the grain weight as a consequence of a decrease in its size through an inhibition of husk elongation (Ndayiragije and Lutts, 2007). The study indicates a minimum percent decrease in grain weight in plants bioprimed with *Rani Th-14* among all the treatments.

Our experiments indicate that seed biopriming with different salinity tolerant isolates of *Trichoderma* reduced severity of the effects of salinity though the amelioration was better in *Rani Th-14* on most of the parameters studied under present experimental conditions.

CONCLUSION

The present study is mainly confined to explore the potential of *Trichoderma* against salt stress a need in the present scenario of climatic change affecting sea level and cultivable land in adjoining areas. The present research thus offers a new approach to alleviate salt stress conditions using salinity tolerant isolates of *Trichoderma harzianum*.

Reduction in detrimental effects of salinity in plants pretreated with *Trichoderma* might be due to colonizing and penetrating root tissues and thus initiating a series of physiological and biochemical changes in the plant, the resulting plant mediated mechanism enhances natural defences against any abiotic stresses. In future, these seed biopriming treatments (*Rani Th-10*, *Rani Th-14*, *Rani Th-21*, *Rani Th-30* and *Rani Th-39*) may be exploited for improving plant growth and yield in saline areas. It is also concluded that though all tested salinity tolerant isolates of *Trichoderma harzianum* reduced severity of the effects of salinity but the amelioration was better in *Rani Th-14* under present experimental materials and conditions.

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