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RESEARCH ARTICLE

Potential of seed biopriming with *Trichoderma* in ameliorating salinity stress and providing resistance against leaf blast disease in finger millet (*Eleusine coracana* L.)

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Abstract

Finger millet (*Eleusine coracana* L.) is an important nutri-cereal crop which is sensitive to salinity and vulnerable to blast disease caused by *Magnaporthe grisea.* The present study was therefore aimed to explore the potential of seed biopriming with four salinity tolerant *Trichoderma* isolates (TRU-21, TRU-14, TRU-33 and TRU-176) on the planting value parameters, physiological and biochemical responses in finger millet grown at various salt stress levels (0, 4, 6 and 8 dSm^{-1}) and to examine their bio-efficacy towards *M. grisea*. The potential of the *Trichoderma* isolates in inhibiting *M. grisea* was assessed by recording the incidence of leaf blast disease using 0–5 SES scale. The *Trichoderma* treatments were found effective in enhancement of germination percentage and minimizing reduction percent germination. Seedlings raised from *Trichoderma* treated seeds exhibited significant enhancement on plant biomass, total chlorophyll content and chlorophyll fluorescence in comparison to untreated plants at all salt stress levels. *Trichoderma* treatments showed lower accumulation of malondialdehyde and H_2O_2 content revealing lower oxidative damage whereas total phenolics, proline content and superoxide dismutase content were higher in plants previously treated with *Trichoderma*. Current study reported resistant to moderately resistant response towards leaf blast disease in *Trichoderma* treated plants whereas susceptible response in untreated plants. Use of *Trichoderma* isolates thus, has been found to provide a sustainable approach to alleviate salt stress and leaf blast disease in finger millet by modulating growth attributes, physiological and biochemical responses, with TRU-14 (*Trichoderma asperellum,* ITCC-7903) showing most consistent effect for most of the traits studied.

Keywords Finger millet · Salt stress · *Trichoderma* · Seed biopriming · Leaf blast disease · *Magnaporthe grisea*

Introduction

Finger millet [*Eleusine coracana* (L). Gaertn.], commonly known as "Ragi", is a nutritious cereal and good source of seed protein, fibre, minerals such as iron, phosphorous, calcium and amino acids viz., cysteine, tyrosine, methionine and tryptophan (Ceasar and Ignacimuthu 2008). Compared to other commonly consumed cereals, finger millet is a crop with a potentially enormous source of nutraceutical properties with potential health benefits. India is Asia's leading producer in finger millet and finger millet serves as staple food in many states viz., Karnataka, Tamil Nadu, Andhra Pradesh, Orrisa, Maharashtra, Bihar and Uttarakhand for millions of people (Gupta et al. 2017). Since time immemorial, it has been cultivated mostly in hilly/tribal areas, but finger millet grains have been relatively neglected as a food resource, but the crop has gained worldwide popularity for its health benefits over the past few decades and has also been receiving growing attention among farmers due to its low cultivation costs, short duration and adaptability to varied agro-climatic conditions (Rawat et al. 2020).

Though finger millet is considered a hardy crop, more than twenty diseases affect the crop, of which the blast

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disease caused by the fungus *Magnaporthe grisea* (Hebert) Barr. is the most devastating disease (Prajapati et al. 2013). The fungus can damage the finger millet plant in a number of ways since it can infect at different growth stages causing leaf, neck and finger blast. Blast disease in finger millet is the most serious disease primarily in eastern Africa and India. It degrades finger millet grain quality and causes yield losses of up to 80% in Kenya and Uganda (Holt 2000; Obilana and Manyasa 2002) and more than 50% in India (Sastri 1989). Nagaraja et al. (2007) reported that the blast disease may cause complete harvest loss if it occurs prior to grain formation.

The crop is also highly affected by abiotic stresses, which reduce its average productivity, and is considered as a crop sensitive to salinity as compared to other millets and cereals viz., barley, oats, wheat and sorghum (Bray et al. 2000). Millets are not resistant to high salt concentrations and thus are grouped into glycophytes which are cruelly repressed or destroyed by an application of 100–200 mmol/L NaCl (Krishnamurthy et al. 2014). Specific electrical conductivity (EC) above 0.8 dSm^{-1} at 25 °C in 1:2 soil water suspension is considered to be high for salt sensitive plants and injurious to germinating seeds or seedlings of most crops while a value above 1.5 dSm^{-1} is considered as excessive salinity, injurious to most plants at all growth stages (Bear 1965). Soil salinization is a major production constraint in Africa and South Asia where finger millet is widely cultivated (Krishnamurthy et al. 2014). Salinity, one of the most important abiotic stresses, restricts plant growth and productivity and is considered to affect almost every aspect of plant physiology and biochemistry (Rawat et al. 2012). Krishnamurthy et al. (2014) have also reported 23–27% reduction in finger millet mean grain yield due to salinity.

Soil conditions and losses due to plant diseases directly affect the yield and nutritive quality of the finger millet and will remain act as major threats to its cultivation in poor and degraded soil. Though crop production and protection can be made by the application of chemical fertilizers and pesticides, but their application in long run may result in poor soil fertility, disrupted soil habitats and may increase environmental and groundwater pollution (Kibblewhite et al. 2008). The reluctant, steadily expanding utilization of chemicals and fertilizers is one of the key reasons causing soil contamination by enhancing its salinity, making it inconvenient for crop bearing and adversely influencing the soil microorganisms. Salinity negatively influences growth and net photosynthetic activity and a reduction in photosynthesis may also be due to a decrease in chlorophyll content (Delfine et al. 1999). As a result of primary salinity stress effects (hyper osmotic stress and ion imbalance), secondary stresses such as oxidative stress often occurs due to reactive oxygen species (ROS) overproduction, causing peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death (PCD) pathway and ultimately leading to death of the cells. As salinity stress increases in plants, the development of malondialdehyde (MDA), which is indicative of oxidative stress, increases and serves as an index of lipid peroxidation. Plasma membrane peroxidation damage contributes to material leakage, rapid desiccation, and cell death (Rawat et al. 2012). Maintenance of leaf turgor can be achieved under salinity stress by osmotic modification in response to proline, sucrose, soluble carbohydrates and other solutes accumulation in cytoplasm, thereby improving water uptake under osmotic stress conditions (Shukla et al. 2014). The process of aggregation of such solutes under osmotic stress is known as osmotic adjustment and is observed to enhance tolerance in plants to water stress (Nayyar and Gupta 2006).

In traditional and organic farming systems throughout the world, the use of bioinoculants and the utilization of novel beneficial plant microbes deliver sustainable, promising, and environment friendly strategies of managing plant stresses/plant diseases (Zhou et al. 2016). Among various fungal biocontrol agents, *Trichoderma* spp. have been reported to have tolerance towards different biotic and abiotic stresses (Singh et al. 2003). *Trichoderma* inoculation in various vegetable and cereal crops through seed biopriming technique has previously shown enhancement in plant growth hormone levels and improvement in seed output (Howell 2003; Harman et al. 2004a). Seed biopriming is a technique that combines biological (seed inoculation with beneficial organism) and physiological (hydration of seed) aspects to safeguard seeds. It is a pre-sowing treatment that leads to a physiological state which enables seed to germinate evenly and more efficiently even under adverse soil conditions (Singh et al. 2003; Rawat et al. 2016). *Trichoderma* releases a number of compounds and the resulting plant-mediated process strengthens natural defenses and induces resistance response to different biotic and abiotic stresses in plants. Previous studies have shown that *Trichoderma*- host interaction results in increased levels of plant proteins and compounds such as phytoalexins and phenols that induce defense mechanisms in plants to various pathogens as well as environmental stresses (Shoresh et al. 2010; Rawat et al. 2013).

The aim behind the present investigation was to explore the potential of selected potent salinity tolerant *Trichoderma* isolates in alleviating salinity stress and enhancing resistance against leaf blast disease in finger millet. Therefore, the current study was attempted to determine whether exogenous application of selected promising four salinity tolerant *Trichoderma* isolates through seed biopriming technique could modulate the plant water relations, chlorophyll content, chlorophyll fluorescence, stress induced metabolites, enzyme activity and suppress leaf blast disease in finger millet under salt stress conditions.

Materials and methods

Experimental site

The experiments were carried out during 2018 & 2019 at Biocontrol Laboratory of Plant Pathology Department, College of Forestry, Ranichauri, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar. The pot assay of both the years was conducted in the greenhouse of the department under the following conditions: temperature of 25 °C during the night and 28 °C during the day, automatic venting at 33 ± 3 °C with supplemental light for 12 hd^{-1}.

Seed material

Finger millet seeds of variety PRM 2, procured from a core set of finger millet germplasm maintained under the project entitled "All India Coordinated Research Project on Small Millets" funded by ICAR- IIMR, Hyderabad, running at College of Forestry, Ranichauri, Tehri Garhwal, Uttarakhand, were used in the present study. The finger millet variety PRM 2 is having semi compact ear head, 90–95 cm plant height and light copper colored seed. Surface sterilization of seeds was made using 1% sodium hypochlorite solution for 3 min, and thereafter the seeds were washed with sterilized distilled water and air dried before application.

Trichoderma strains and their morphological characterization

Four isolates of salinity tolerant (ST) *Trichoderma* viz. TRU-21, TRU-14, TRU-33 and TRU-176 were obtained from the well characterized repository of Biocontrol Lab, Plant Pathology Department, College of Forestry, Ranichauri, Tehri Garhwal (Uttarakhand), India (Fig. 1).

All the four isolates (previously isolated from rhizospheric soil samples of finger millet crop grown at Ranichauri, Tehri Garhwal, Uttarakhand), used during the study, were investigated for morphological characterization to identify up to species level. For morphological studies of *Trichoderma*, Cornmeal dextrose (CMD) agar medium was used. A block of the fungal mycelium from each isolate

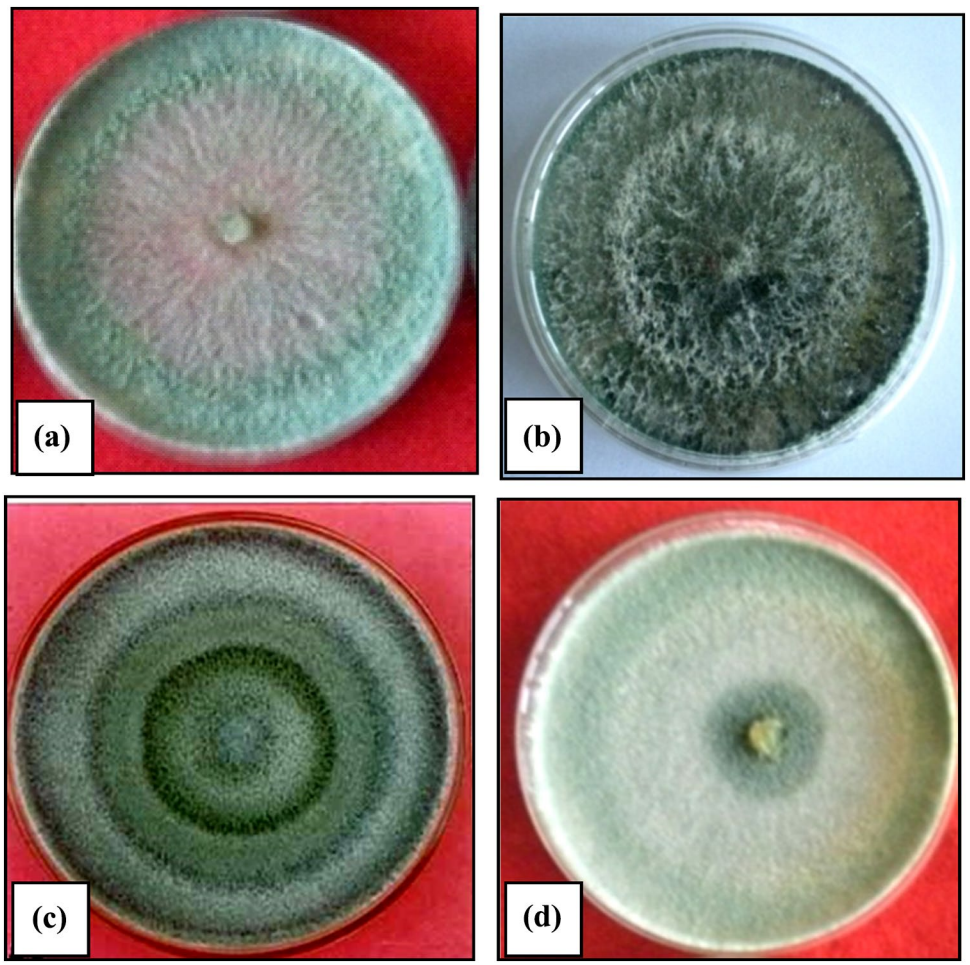

Fig. 1 Four salinity tolerant isolates of *Trichoderma* (**a** = TRU-21; *Trichoderma harzianum*, **b** = TRU-14; *Trichoderma asperellum,* **c** = TRU-33; *Trichoderma asperellum*, **d** = TRU-176; *Trichoderma harzianum*) were used in the present study

was inoculated onto 90 mm petri dishes seeded with CMD and the cultures were incubated separately at 20 °C. Microscopic preparations for morphological studies were made from pustules where there were still white conidia, generally within a week of incubation. For preparing slides, a very small amount of the material was placed in drop of 3% KOH on a slide. After preparing the mount, KOH was replaced with lactophenol cotton blue. Before placing the cover slip, the hyphae and conidiophores were separated using needles and thereafter the slide was observed under the microscope. The branching pattern of conidiophores, their angle to main axis, conidia, and phialides arrangement were recorded for species specific characterization using the key provided by Bisset (1991a, 1991b) and Prameela (2018).

Preparation of *Trichoderma* formulation and seed biopriming

Mass culture of each *Trichoderma* isolate was prepared separately on barnyard millet (*Echinochloa frumentaceae*) grains (locally known as "Jhangora/Sawan") as described by Rawat et al (2012). To obtain the optimal concentration of biocontrol agents in the talc formulation, spore powder was mixed with 350 mesh talcum powder (95 percent whiteness) and 1 percent carboxy methyl cellulose (CMC), which was used as a sticker. The final *Trichoderma* inoculum was adjusted to 5 X 10^6 CFU/g in the prepared formulation. For seed biopriming (integration of biological and physiological aspects), after pre-soaking of finger millet seeds in sterilized distilled water, seeds were separately treated with powder (talc) formulation of each strain of *Trichoderma* @ 10 g/kg of seeds and mixed thoroughly to provide uniform coating and thereafter treated seeds were kept under warm and humid conditions in an incubator at 25 ± 2 °C until prior to emergence of radical to facilitate *Trichoderma* colonization on spermosphere during incubation period. Seeds without *Trichoderma* treatment were used as control (Rawat et al. 2016).

Pot assay

The selected isolates were then evaluated at each salt stress level for their ability to enhance salt stress tolerance in finger millet plants in a factorial completely randomized design with four salt stress levels (0, 4, 6 and 8 dSm^{-1}) and five treatments (T1 = TRU-21, T2 = TRU-14, T3 = TRU-33, T4 = TRU-176 and T5 = Control). The experiment was performed with three replications repeated twice at different time intervals. Bulk surface soil (0–15 cm), collected from the B- Block Plant Pathology Field, Ranichauri, was air dried, mixed thoroughly and then passed through 2 mm sieve. Plastic pots of 5 kg capacity were filled with 4.5 kg autoclaved sandy loam soil having pH 7.7 with four levels of salt stresses separately. The soil was made saline following the method of Rawat et al. (2011). Twenty healthy seeds (treated and untreated as per the treatments) of similar shape and size were sown in each pot. Pots were irrigated daily with saline solution (2800 ppm of NaCl) to maintain salinity levels in the pot, while the control pots received only normal irrigation water. Specific electrical conductivity (dSm^{-1} at 25 °C) of 1: 2 soil water suspension was taken as a criterion of salinity stress. The EC of soil in plastic pots was tested at regular intervals and final salt stress level was recorded to be 0.45, 3.76, 5.76 and 7.43 dSm^{-1} in pots with 0, 4, 6 and 8 dSm^{-1}, respectively at the end of the experiment.

Observations

Subsequent to salt stress levels application and sowing of bioprimed and untreated seeds in their respective pots, observations on germination, growth, physiological and biochemical responses of finger millet were analyzed. The Data presented is the average of six replicates obtained from two independent experiments (three replicates in each) conducted over two different time periods under identical conditions.

Germination test

Germination was recorded at every 24 h after first seed germinated in any of the treatments and continued till germination process is over.

Reduction in percent germination (RPG) or emergence (RPE) was estimated as defined by Madidi et al. (2004).

$$\text{RPG (or RPE)} = 1 - \text{Nx}/\text{N}_c \times 100$$

"Nx" is the number of seedlings germinated under salt stress and "Nc" is the number of seedlings germinated under no stress conditions (0 dSm^{-1}).

Growth parameters

Plants were carefully uprooted and rinsed using distilled water. The lengths, fresh weights of root and shoot of the plants were observed and measured manually after 21 days of salinity treatment. Leaves and roots were then oven- dried for 48 h at 80 ^{0}C to acquire their respective dry weights.

Physiological and biochemical parameters

Both triplicate experiments were performed using plant tissues obtained after 3 weeks from the individual pots from each treatment using fully expanded leaves.

Relative water content

In accordance with Barrs (1968), the relative water content (RCW) of leaves was quantified using fresh weight (FW), dry weight (DW) and turgor weight (TW) as follows:

$$RCW = [(FW - DW)/(TW - DW) \times 100].$$

The turgor weight was obtained by leaving the leaf in distilled water at 5 °C in darkness overnight and taking dry weight after 24 h at 80 °C.

Leaf electrolyte leakage

The electrolyte leakage (E_L) was measured as percentage of leaked electrolytes from cut cells ($E_{I=}$ Initial electrical conductivity) compared to the total electrolyte pool (E_T) in the sample as suggested by Tabot and Adams (2012).

$$E_L(\%) = \left(E_I/E_T\right) \times 100$$

Total chlorophyll content (TCC)

Chlorophyll a, b and total chlorophylls (a + b) were determined according to Lichtenthaler (1987). Approximately 100 mg of fresh leaves were taken, finely ground and extracted in 5 mL of absolute ethanol (99%). Extracts were filtered through Whatman no.1 filter paper and the absorbance was recorded with a UV–Visible spectrophotometer (UV-2450, Shimadzu Analytical, Japan) at 664.5 and 647 nm, respectively.

Chlorophyll fluorescence (F_v/F_m ratio)

Chlorophyll "a" fluorescence produced by green plants represents the photosynthetic potential of PS II. To monitor chlorophyll fluorescence (F_v/F_m ratio) according to the below given equation, a portable plant efficiency analyzer (Handy PEA, Hansatech, UK) was used.

$$F_v/F_m = \left(F_m - F_0\right)/F_m$$

Proline content

Proline content in the tissue, as defined by Bates et al. (1973), was determined by colorimetric method. Using L-proline as normal, the material (μ mol/g fr. wt.) was quantified by the ninhydrin acid reagent process.

Malondialdehyde (MDA) content

The measure of MDA produced by thiobarbituric acid reaction was taken as a criterion of lipid peroxidation as proposed by Heath and Packer (1968).

Total phenolics content

The total content of phenolics was calculated by the method suggested by Thimmaiah (1999) using Folin-Ciocalteu reagent and the absorbance against each blank was measured at 650 nm. The phenol content was derived from different catechol concentrations and was expressed as mg/100 g.

Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide was measured spectrophotometrically after reaction with potassium iodide (KI). The reaction mixture consisted of 0.5 ml of 0.1 percent trichloroacetic acid (TCA) leaf extract supernatant, 0.5 ml 100 mM K-phosphate buffer and 2 ml reagent (1 M KI w/v in fresh double-distilled water H_2O). In the absence of leaf extract, the blank probe was composed of 0.1 percent TCA. The reaction was developed for 1 h in darkness and absorbance measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2 and then expressed as mg g^{-1} fresh weight (Alexieva et al. 2001).

SOD activity

SOD activity was determined by estimating the capacity to retard the photochemical decrease of nitroblue tetrazolium chloride (NBT) as portrayed by Giannopolitis and Ries (1977).

Salt tolerance index

A salt sensitive treatment (control) was selected as a susceptible standard to allow comparison between treatments, and the performance of other treatments was compared by measuring a salt tolerance index (Zeng et al. 2003). As a susceptible standard, we selected treatment T5 (control) as it had highest mean for RPG, MDA and H_2O_2 content and lower mean for other characters.

$$\text{Salt tolerance index} = \frac{\text{Mean of a treatment for a trait over salt stress treatments}}{\text{Mean of susceptible standard for the trait over salt stress treatments}}$$

Highest salt tolerance index for different parameters was used as criterion for selecting more saline tolerant isolate of *Trichoderma* to alleviate salinity stress in finger millet except for RPG, MDA and H_2O_2 content for which reverse order was accepted.

Pathogen challenge/inoculation

After taking growth, physiological and biochemical parameters, plants in each pot were assessed for the effect of various treatments towards leaf blast disease response caused by *Magnaporthe grisea*. The pathogen inoculum was prepared by inoculating 6 mm mycelial discs of isolate cut from 7 days old culture of *Magnaporthe grisea,* obtained from Plant Pathology Laboratory, College of Forestry, Ranichauri, VCSG Uttarakhand University of Horticulture & Forestry, Uttarakhand, India, on oat meal agar (OMA) medium at 26 ± 1 °C. Mass multiplication of spores for inoculation was achieved by growing isolate (5 discs/plate) on OMA medium at 26 ± 1 °C for 15 days. The plates were flooded with 10 ml of distilled water and the fungal growth containing mycelium and conidia was gently extracted by scrapping with a sterile plastic inoculation loop. Approximately 30 ml of a spore suspension of isolate was transferred into 100 ml conical flask, thoroughly mixed for release of conidia into water by vertexing. The harvested spores were filtered through a double-layered muslin cloth, the resulting concentration was adjusted to 1×10^5 conidia ml^{-1} and 0.02 percent (vol/vol) Tween 20 (polyoxyethylene sorbitan monolaurate) was applied to the final suspension just before the inoculation (Jia et al. 2003). Seedlings were artificially inoculated using a hand-operated atomizer by spraying the inoculum on the foliage. To avoid dislodging of the spores, inoculated plants were allowed to partially dry for 30 min and the seedlings sprayed with water were maintained as control. All the inoculated seedlings were incubated at 23 °C with > 95% Relative Humidity (RH) and leaf wetness under 12 h photoperiod for 7 days. Leaf blast disease severity was recorded on the plant's leaves. The symptoms emerged on leaves in the form of spindle shaped spots with yellowish margin and grayish center which later on became ash colored.

Disease screening

Infected plants were analyzed for development of lesions and disease severity was estimated based on 0–5 SES (Standard Evaluation System) Scale (Mackill and Bonman, 1992), where 0- No visual symptoms; Highly Resistant (HR), 1- brown specks smaller than 0.5 mm in diameter (lesion expansion up to 5%); Resistant (R) type lesions without sporulation, 2- brown specks about 0.5–1 mm in diameter (20%); Moderately Resistant (MR), 3- roundish to elliptical lesions about 1–3 mm in diameter (50%) with gray centers and brown margins, occasionally sporulating; Moderately Susceptible (MS), 4-typical spindle- shaped blast lesions, 3 mm or longer (80%) with little or no coalescence of lesions with well-defined brown margins; Susceptible (S), 5- same as 4, but 50% of infected leaves killed by coalescence of lesions (> 80%), brown margin not well defined; Highly Susceptible (HS).

Statistical analysis

The data from the experiments were subjected to two-way ANOVA according to the completely randomized factorial design with six replications (two independent but identical experiments with three replications conducted at different time periods) followed by separation of means at $P \leq 0.05$. Standard error of each mean was calculated to represent the same on tables and graphs. The CD values were determined by multiplying the standard error of difference (SEd) with table t value at error degrees of freedom as described by Gomez and Gomez (1984).

Results

Two types of arrangement of conidiophores and phialides were recorded among the four *Trichoderma* isolates when slides were observed for various morphological characters under the microscope**.** On the basis of recorded observations related to colony growth, conidiophores branching pattern, phialides, conidia and chlamydospores, two isolates (TRU-21 and TRU-176) were classified as *T. harzianum* (phialides were verticillate and usually 3–4; conidia were smooth, 2.5–3.0 × 2.0–2.5 µm and pale green) and the remaining two isolates (TRU-14 and TRU-33) were classified as *T. asperellum* (phialides were straight and typically in whorls of 2–4; conidia were dark green, 3.5–6.0 × 3.0–5.0 µm and finely spinulose). The specifics of the salinity tolerance of these strains of *Trichoderma* are shown in Table 1.

Data summarized in Tables 2, 3 and Figs. 2, 3, 4 indicated that finger millet response to imposed salt stress conditions was distinctly affected by treatments of salinity tolerant *Trichoderma* isolates. Treatments also significantly influenced the blast disease incidence in finger millet plants under both normal and various levels of salt stress (Table 4).

Germination percentage and reduction percentage of germination

Results revealed that 100% germination was recorded in all the treatments including untreated control (Fig. 2a), but as the salt stress increased, *Trichoderma* isolates exerted significant effect on germination percent in finger millet. Under salt stress levels of 4, 6 and 8 dSm^{-1}, *Trichoderma*

Table 1 Effect of different salinity levels on linear growth, mycelia dry weight and sporulation of *Trichoderma* isolates on potato dextrose agar (PDA) medium supplemented with four levels of salt stress[a]

S. no	*Trichoderma* isolate used	Linear growth (cm)				Mycelial dry weight (mg)				Sporulation			
		0 mM	70 mM	160 mM	250 mM	0 mM	70 mM	160 mM	250 mM	0 mM	70 mM	160 mM	250 mM
1	TRU-21	9.00	8.87	8.86	8.45	195.93	193.42	189.76	175.63	1256.3	1082.0	952.3	764.2
2	TRU-14	9.00	8.91	8.90	8.54	198.23	194.96	192.99	178.56	1469.7	1184.3	984.0	855.4
3	TRU-33	9.00	8.72	8.72	8.37	194.60	192.51	189.13	174.82	1178.3	1032.1	883.0	733.2
4	TRU-176	9.00	8.72	8.69	8.26	193.13	189.47	187.70	172.38	1098.3	997.8	767.7	692.4
5	TRU-56	9.00	5.66	3.53	1.77	194.90	70.24	52.15	36.53	1039.7	455.5	92.7	48.0
C.D			0.312	0.226	0.129	2.744	1.610	2.707	2.507	122.020	102.422	25.092	39.267
SE(m)			0.098	0.071	0.040	0.860	0.504	0.848	0.785	38.229	32.089	7.861	12.302
SE(d)			0.138	0.100	0.057	1.216	0.713	1.200	1.111	54.064	45.381	11.118	17.398
C.V			2.068	1.585	0.991	0.762	0.520	0.905	0.920	5.479	5.849	1.850	3.444

[a]*Trichoderma* isolates were grown on PDA medium supplemented with different concentrations of NaCl viz., 0, 70, 160 and 250 mM salt concentration. Plates (90 mm) were then incubated at 27 ± 2 °C for 4 days. Saline medium was prepared by dissolving calculated or weighed amount of commercially grade NaCl pellets to make 0, 70, 160 and 250 mM salt concentration. The growth of the *Trichoderma* isolates in natural (0 mM) and saline media (70, 160 and 250 mM) was estimated by the measurement of linear growth after four days of incubation. After filtration on muslin of two weeks old *Trichoderma* liquid cultures, the collected mycelium was washed twice and put to dry at 80 °C over the night. Thereafter, dry weight of the mycelium was measured. The level of sporulation was determined in the filtrate using a haemocytometer

Trichoderma isolates at S. no. 1–4 are salinity tolerant as showed no adverse effects on recorded linear growth, mycelial dry weight and sporulation with increasing trend of salinity while isolate at S. no. 5 is salinity sensitive (used for comparative study) which showed drastic reduction in all recorded observations when cultured under salinity stress conditions

bioprimed seeds germinated consistently faster and more uniformly than untreated (control). Under saline conditions, maximum germination was observed in treatment T2 (mean at salt stress = 91.67%) with salt tolerance index of 1.32 followed by T3 (90.56%), T1 (89.45%) and T4 (86.11%) which were statistically at par with salt tolerance indices of 1.30, 1.29 and 1.24 respectively, while T5 (control) recorded minimum germination (mean at salt stress = 69.45) with minimum salt tolerance index of 1.00 (Table 3). With respect to reduction percentage of germination (RPG), maximum (mean at salt stress = 40.44%) was observed in T5 (control) at all stress levels (Fig. 2b) with salt tolerance index of 1.00 whereas minimum RPG (mean at salt stress = 10.67%) with minimum index of 0.26 was observed in treatment T2 followed by T3 (12.11%) with tolerance index of 0.30 (Table 3).

Evaluation of *Trichoderma* isolates on growth response of finger millet grown under salt stress conditions

Shoot length and root length

All the *Trichoderma* treatments showed significant enhancement in planting value parameters as compared to control under both normal and different salt stress levels. The reduction in shoot and root lengths from 5.80 cm and 2.93 cm, respectively at 0 dSm^{-1} to 4.77 cm and 4.63 cm, respectively at 8 dSm^{-1} was recorded in T5 (control). The data summarized in Fig. 3a & b showed that the treatment T2 exhibited maximum enhancement in shoot length and root length (11.18 cm and 6.60 cm, respectively, considering mean at salt stress) with maximum salt tolerance index of 1.46 and 1.42 respectively over plants raised from other treatments (Table 3).

Shoot and root fresh and dry weight

Results suggested the higher potential of these *Trichoderma* isolates in improving planting value parameters even under adverse soil conditions. When subjected to four levels of salt stress (0, 4, 6 and 8 dSm^{-1}) both shoot fresh weight and root fresh weight decreased progressively with increase in salt stress levels in all the treatments. Though, the data on average fresh and dry weight of shoot and root showed a strong inhibition with increasing level of salt stress in T5 (control) wherein maximum reduction in fresh and dry weight of shoot and root (40.80 g, 5.27 g and 18.87 g, 3.60 g, respectively) under higher level of salt stress i.e., 8 dSm^{-1} was detected (Fig. 3c, d, e and f). The Treatment T2 (TRU-14) showed maximum average enhancement amongst all the treatments in suppressing the deleterious effects of salt stress with 84.54 g and 37.29 g of fresh weight of shoot and root, respectively and 15.59 g and 7.59 g of dry weight of shoot and root, respectively, considering mean at salt stress followed by T3 (TRU-33) with 80.23 g (mean at salt stress) and 34.52 g (mean at salt stress) of fresh weight of shoot and root, respectively and 14.63 g (mean at salt stress) and

Table 2 Effect of salt stress and salinity tolerant *Trichoderma* isolates on Relative water content, Total chlorophyll content, Chlorophyll fluorescens, Proline content, Malondialdehyde content and Total phenolics content of finger millet plants under different levels of salt stress

Treatment	Relative water content (%)					Total chlorophyll content (mg/g fr. wt.)					Chlorophyll fluorescens (F_v/F_m)				
	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
T1: TRU-21	87.73	82.77	75.93	65.67	78.03	5.28	3.21	2.66	2.62	3.44	0.413	0.387	0.321	0.308	0.357
T2: TRU-14	94.33	91.57	82.63	78.73	86.82	5.83	3.94	3.49	3.10	4.09	0.428	0.391	0.359	0.314	0.373
T3: TRU-33	90.60	85.30	76.07	70.03	80.50	4.90	3.49	3.11	2.83	3.58	0.402	0.348	0.341	0.309	0.350
T4: TRU-176	89.13	85.40	72.57	63.23	77.58	5.25	3.30	2.74	2.07	3.34	0.388	0.330	0.315	0.295	0.332
T5: Control	88.60	82.70	68.27	51.77	72.83	4.95	2.45	1.81	1.48	2.67	0.429	0.301	0.194	0.150	0.269
	SE(d)					SE(d)					SE(d)				
S	0.643					0.047					0.006				
T	0.719					0.053					0.007				
S X T	1.437					0.105					0.014				
Treatment	Proline content (µmol/g fr. wt.)					Malondialdehyde content (µmol/g fr. wt.)					Total phenolics content (µg/g fr. wt.)				
	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean
T1: TRU-21	3.70	5.97	9.50	11.10	7.57	1.82	2.30	4.07	6.10	3.57	67.70	87.60	131.73	193.50	120.13
T2: TRU-14	3.80	7.33	13.47	16.27	10.22	1.25	2.08	2.56	3.68	2.39	56.03	108.97	145.40	247.97	139.59
T3: TRU-33	3.23	6.57	10.93	13.20	8.48	1.89	2.28	2.86	4.10	2.78	63.83	98.40	136.50	217.13	128.97
T4: TRU-176	3.70	5.90	9.20	9.77	7.14	1.15	2.86	4.35	7.54	3.98	63.70	81.23	127.70	172.47	111.28
T5: Control	3.47	4.63	5.20	6.73	5.01	1.15	4.55	7.77	9.12	5.65	56.43	68.90	86.47	116.13	81.98
	SE(d)					SE(d)					SE(d)				
S	0.151					0.037					1.281				
T	0.169					0.041					1.432				
S X T	0.337					0.082					2.864				

fr. wt. fresh weight, *S* Salt stress (0, 4, 6 and 8 dSm^{-1}), *T* Treatment (T1, T2, T3, T4 and T5)

Data are the mean values ($n=6$)

Table 3 Salt tolerance index values amongst treatments at mean value of salt stress

Treatment	Germination (%)	RPG (%)	SFW (g)	SDW (g)	RFW (g)	RDW (g)	SL (cm)	RL (cm)
T1: TRU-21	1.29	0.34	1.19	1.47	1.38	1.35	1.34	1.27
T2: TRU-14	1.32	0.26	1.33	1.67	1.60	1.50	1.42	1.46
T3: TRU-33	1.30	0.30	1.26	1.57	1.48	1.38	1.33	1.27
T4: TRU-176	1.24	0.45	1.13	1.42	1.26	1.26	1.18	1.16
T5: Control	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
CD at 5%	0.293	0.169	0.115	0.394	0.080	0.201	0.154	0.204

RWC (%)	LEL (%)	TCC (mg/g fr. wt.)	CF (F_v/F_m)	PC (µmol/g fr. wt.)	MDA content (µmol/g fr. wt.)	TPC (µg/g fr. wt.)	H_2O_2 content (µmol/g fr. wt.)	SOD content (U/mg protein)
1.07	0.68	1.29	1.33	1.51	0.63	1.47	0.67	3.89
1.19	0.64	1.53	1.39	2.04	0.42	1.70	0.44	5.90
1.11	0.66	1.34	1.30	1.69	0.49	1.57	0.54	3.88
1.07	0.71	1.25	1.24	1.43	0.70	1.36	0.81	3.65
1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
0.121	0.132	0.281	0.349	0.423	*ns*	0.286	0.129	0.995

RPG Reduction percent germination, *SFW* Shoot fresh weight, *SDW* Shoot dry weight, *RFW* Root fresh weight, *RDW* Root dry weight, *SL* Shoot length, *RL* Root length, *RWC* Relative water content, *LEL* Leaf electrolyte leakage, *TCC* Total chlorophyll content, *CF* Chlorophyll fluorescens, *PC* Proline content, *MDA* Malondialdehyde, *TPC* Total phenolics content, H_2O_2 Hydrogen peroxide, *SOD* Superoxide dismutase, *fr. wt.* fresh weight, *ns* non- significant

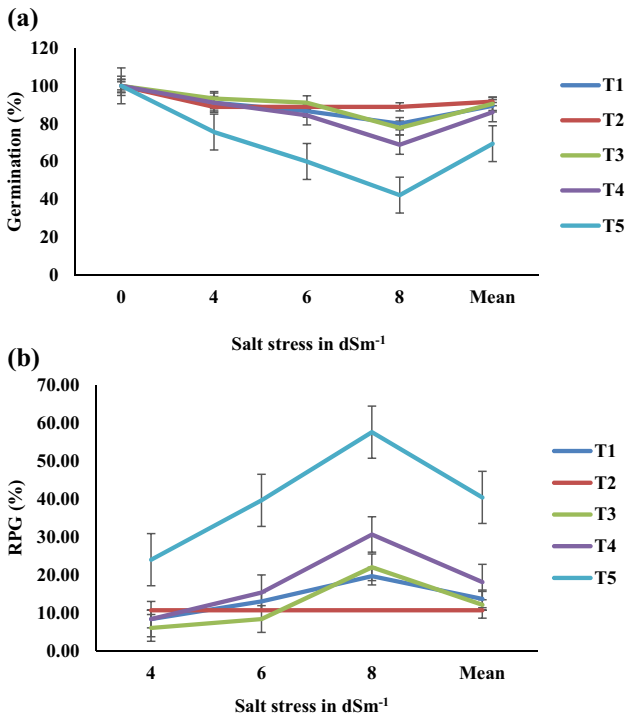

Fig. 2 Effect of seed biopriming by *Trichoderma* strains on percent germination **(a)** and reduction percentage of germination **(b)** in finger millet grown under various salt stress levels. Treatments included four *Trichoderma* strains (TRU-21, TRU-14, TRU-33 and TRU-176) colonized seedlings and one (control) untreated seedlings

6.98 g (mean at salt stress) of dry weight of shoot and root, respectively.

Physiological and biochemical responses

Relative water content (RWC)

A severe loss in the amount of RWC in untreated finger millet plants was detected due to the action of salt stress (Table 2). However, in plants pretreated with various salinity tolerant *Trichoderma* isolates, a comparatively less fluctuation was observed with increasing trend of salt stress. At 8 dSm^{-1} salt stress, marked decrease in the amount of RWC (mean at salt stress = 51.77%) was recorded in untreated plants (control) with a lesser decrease noticed in plants pretreated with different *Trichoderma* isolates (63.23% to 78.73%, considering mean at salt stress). The salt tolerance index revealed highest value for treatment T2 (1.19) followed by T3 (1.11) while lowest value for treatment T5 (control) with 1.00 index followed by T1 and T4 with 1.07 index in each (Table 3).

Total chlorophyll content (TCC)

Total chlorophyll content (TCC) was significantly decreased under salt stress conditions as the stress increased from 0 to 8 dSm^{-1}. The TCC averaged over treatments from 0 dSm^{-1} to 8 dSm^{-1} was in the range of

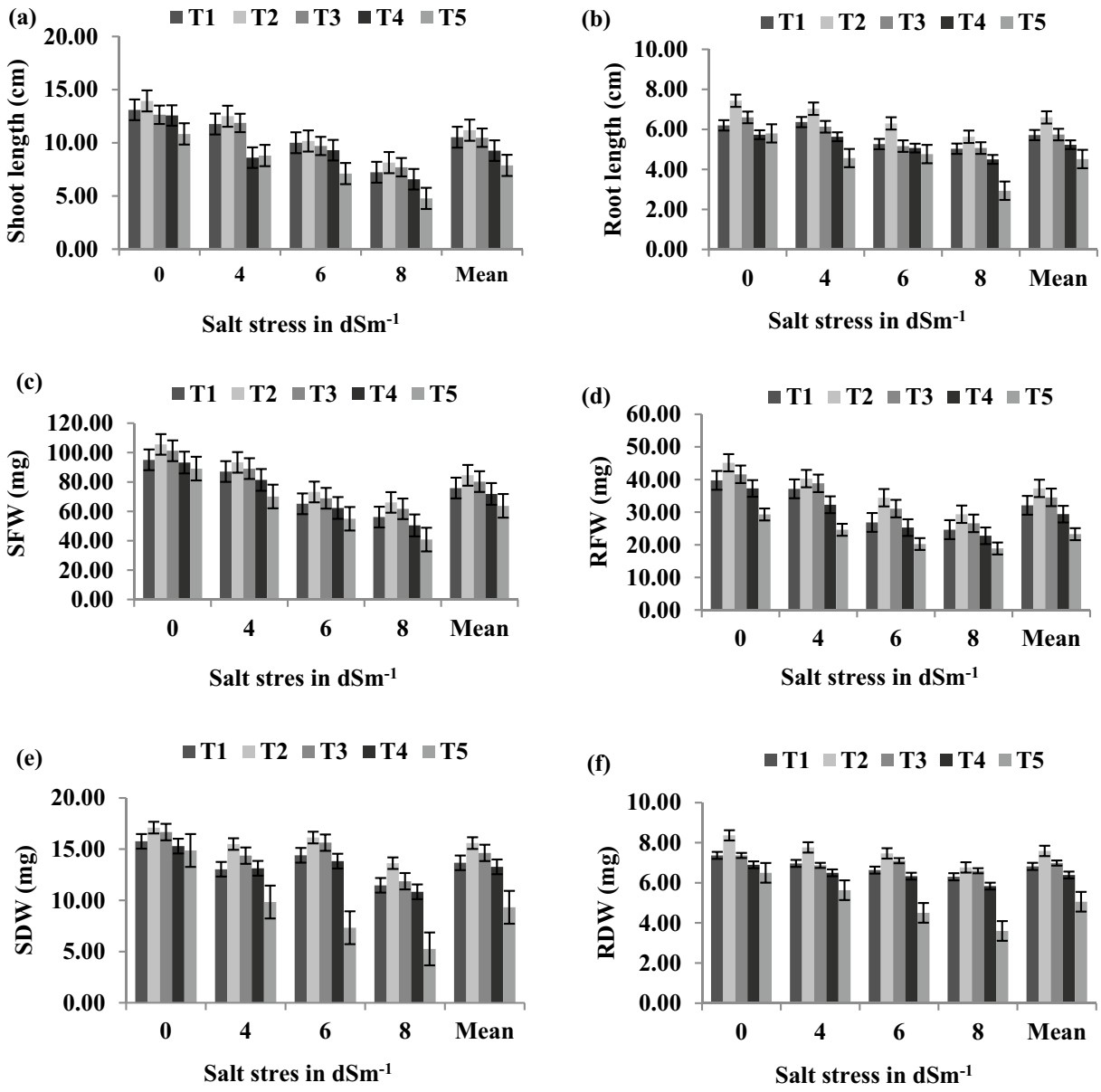

Fig. 3 Effect of *Trichoderma* isolates on shoot length **(a)**, root length **(b)**, shoot fresh weight **(c)**, root fresh weight **(d)**, shoot dry weight **(e)** and root dry weight **(f)** of finger millet plants grown under four levels of salt stress viz., 0, 4, 6 and 8 dSm^{-1}. Seeds were bioprimed with selected *Trichoderma* isolates (TRU-21, TRU-14, TRU-33 and TRU-176) or untreated (control)

5.24 mg/g to 2.42 mg/g. However, *Trichoderma* isolates suppressed the reduction in net chlorophyll content significantly as compared to control. The highest TCC content of 4.09 mg/g was recorded in treatment T2 followed by T3 (3.59) and T1 (3.45), considering mean at salt stress (Table 2). Among *Trichoderma* treatments, minimum TCC was observed in treatment T4 (TRU-179) with 3.34 mg/g which was significantly higher than T5 (control) with 2.67 mg/g TCC. The maximum salt tolerance index was observed again in treatment T2 (Index = 1.53), wherein, minimum index (1.00) was recorded in T5 as presented in Table 3.

Chlorophyll fluorescens (CF)

Data presented in Table 2 indicated that CF (F_v/F_m ratio) was almost equivalent in all the treatments under normal condition (0 dSm^{-1}) but treatments exerted significant effect as the salt stress level increased from 4 dSm^{-1} to 6 dSm^{-1}. Treatment T2 (TRU-14) showed relatively higher F_v/F_m ratio (mean at salt stress = 3.7) with highest salt tolerance

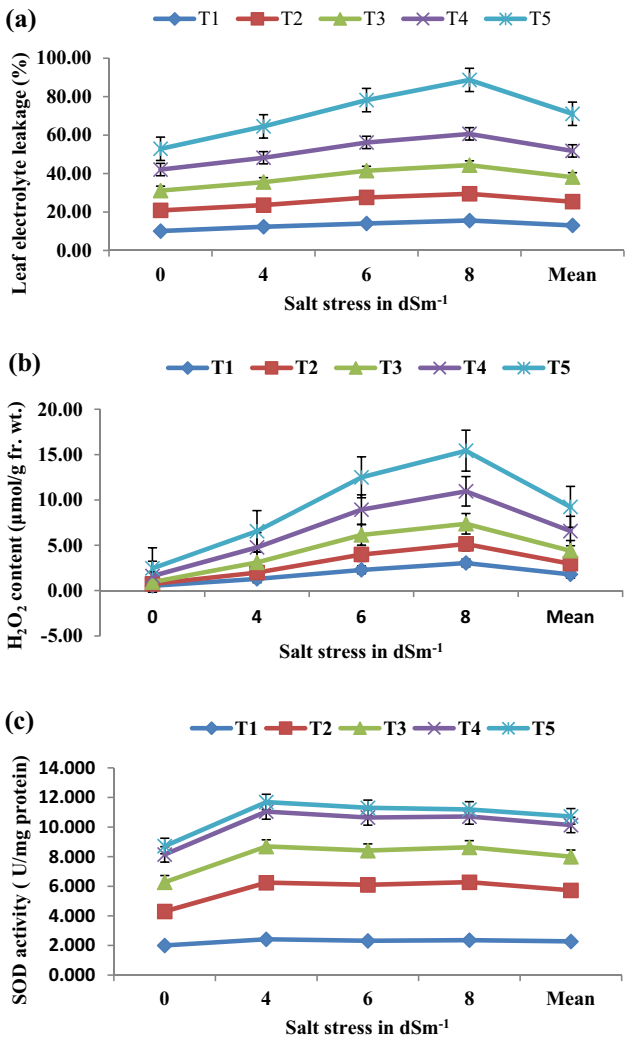

Fig. 4 Effect of salinity tolerant *Trichoderma* isolates on leaf electrolyte leakage **(a)**, hydrogen peroxide (H_2O_2) content **(b)** and superoxide dismutase (SOD) content **(c)** of finger millet plants grown at various salt stress levels viz., 0, 4, 6 and 8 dSm^{-1}. The treatments consisted of four *Trichoderma* primed (TRU-21, TRU-14, TRU-33 and TRU-176) seedlings and one untreated seedling (control)

index of 1.39 followed by T1 (mean at salt stress = 0.357) and T3 (mean at salt stress = 3.50) which were statistically at par with salt tolerance index of 1.33 & 1.30, respectively. The lowest mean F_v/F_m ratio over salt stress treatments was recorded in T5 (control) along with lowest salt tolerance index (Table 3).

Proline content

Proline content was significantly influenced by both *Trichoderma* isolates and salt stress levels. The analysis of data revealed a significant increase in all the treatments with increase in salt stress levels (Table 2). Treatment T2 had shown maximum proline content (mean at salt stress = 10.22 µmol/g) with highest salt tolerance index (index = 2.04) followed by T3 (mean at salt stress = 8.48 µmol/g) and T2 (mean at salt stress = 7.57 µmol/g) with salt tolerance indices of 1.69 and 1.51, respectively under salt stress (Table 2). Minimum proline content was recorded in control at all stress levels with the lowest salt tolerance index (index 1.00). Treatment T2 showed potential impact by exhibiting maximum percent increase in proline content (approximately 2 folds) followed by T3 and T1 (1.69 fold and 1.51 fold, respectively) as compared to unstressed plants considering mean value of salt stress.

Malondialdehyde (MDA) content

The effect of different *Trichoderma* isolates on the concentration of lipid peroxides under salt stress was measured in terms of MDA content in finger millet. A substantial increase in the amount of MDA content was observed due to the action of salt stress. The MDA content was found highest in treatment T5 (control) at all stress levels (Table 2) than the plants raised from *Trichoderma* bio-primed seeds under respective salt stress level. The accumulation of MDA content appeared lowest in treatment T2 (mean at salt stress = 2.39 µmol/g) with lowest index 0.42 followed by treatment T3 (mean at salt stress = 2.78 µmol/g) with index 0.49 (Table 3) revealing reduced accumulation of lipid peroxides in plants raised from TRU-14 and TRU- 33 bio-primed seeds under salt stress. MDA content was found to be 7.9 folds higher in treatment T5 (control) at 8 dSm^{-1} salt stress level (9.12 µmol/g) as compared to 0 dSm^{-1} salt stress level (1.15 µmol/g). However, a less significant change (2.2–6.6 fold) was found among the *Trichoderma* pretreated plants with minimum in treatment T3 (2.9 fold) which was narrowly followed by T2 (2.2 fold).

Total phenolics content

As far as phenolics content was concerned, it was increased substantially with an increase in salt stress level. Effect of treatment and salt stress was observed in the total pool of penolics content (Table 2). Significantly higher phenolics content was attained in plants raised from *Trichoderma* bio-primed seeds as the salt stress increased from 0 to 8 dSm^{-1} as compared to untreated plants (control). Considering mean at salt stress, highest phenolics content was recorded in treatment T2 (mean at salt stress = 139.59 µg g^{-1}) with salt tolerance index of 1.70 followed by the treatments T3 (128.97 µg g^{-1}) with the index of 1.57, while the untreated plants in treatment T5 (control) showed lowest phenolics

Table 4 Effect of different treatments (*Trichoderma* colonized and non-colonized plants) in suppressing leaf blast disease of finger millet grown under various salt stress levels

Treatment	Leaf blast disease (G)					Host response
	0 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	8 dSm^{-1}	Mean	
T1: TRU-21	1.33	1.67	1.67	1.33	1.50	R
T2: TRU-14	0.67	1.00	1.33	0.67	0.92	HR
T3: TRU-33	2.33	2.00	2.33	1.67	2.08	MR
T4: TRU-176	2.67	3.00	2.67	2.67	2.75	MR
T5: Control	3.67	4.00	4.33	4.00	4.00	S
	SE(d)					
S	0.202					
T	0.226					
S X T	0.451					

S Salt stress (0, 4, 6 and 8 dSm^{-1}), *T* Treatment (T1, T2, T3, T4 and T5)

R Resistant, *HR* Highly Resistant, *MR* Moderately Resistant, *S* Susceptible

content (mean at salt stress = 81.98 µg g^{-1}) with the lowest salt tolerance index of 1.00 (Table 3).

Leaf electrolyte leakage

The highest rate of electrolyte leakage was evident in plants exposed to 8 dSm^{-1}. However, *Trichoderma* treatments lowered down the electrolyte leakage than untreated plants under both normal and stressed conditions (Fig. 4a). The results accomplished a severe damage in untreated plants at higher salt levels of 6 dSm^{-1} (22.03%) and 8 dSm^{-1} (28.03%). However, maximum decrease in leaf electrolyte leakage was induced by the treatment T2 (mean at salt stress = 12.35%) followed by T3 (12.78%). All the *Trichoderma* biopriming treatments were effective in decreasing electrical conductivity of leaf leachates with indices in the range of 0.64–0.71 whereas highest index (1.00) was noticed in untreated control (Table 3).

H_2O_2 content

Concentration of H_2O_2 content increased significantly amongst all the treatments as the salt stress level increased from 0 to 8 dSm^{-1}. Treatment T5 (control) showed maximum H_2O_2 content under both normal (salt stress level of 0 dSm^{-1}) as well as stressed conditions viz. salt stress levels of 4, 6 and 8 dSm^{-1} with maximum salt tolerance index of 1.00 at mean value of salt stress (Fig. 4b). However, *Trichoderma* treatments suppressed the H_2O_2 concentration and this fluctuation was less dramatic in plants treated with *Trichoderma* with lowest concentration observed in treatment T2 (mean at salt stress level = 1.18 µmol/g fr. wt.) followed by T3 (mean at salt stress level = 1.43 µmol/g fr. wt.) and T1 mean at salt stress level = 1.79 µmol/g fr. wt.) with indices of 0.44, 0.54 and 0.67, respectively (Table 3).

Superoxide dismutase (SOD) activity

Among all the treatments, treatment T2 was found best to enhance SOD activity at all salt stress levels (Fig. 4c). Our results show that different *Trichoderma* isolates increased the SOD activity significantly in salt stressed finger millet plants as compared to untreated plants in T5 (mean at salt stress = 0.585 U/mg protein). In present study, SOD activity increased substantially with increasing trend of salt stress in all the treatments up to 4 dSm^{-1}. At 6 dSm^{-1} salt stress level, SOD content decreased in all the plants raised from *Trichoderma* pretreated seeds. However, at 8 dSm^{-1}, *Tricoderma* treated plants maintained a slight enhance in SOD activity as compared to 6 dSm^{-1}. The maximum decrease in SOD activity at higher salt stress level i.e. 8 dSm^{-1} was recorded in T5 (0.480). The highest salt tolerance index of 5.90 was exhibited by the treatment T2 followed by T1 (index 3.89) which was narrowly followed by T3 (index 3.88) while T5 showed the lowest salt tolerance index (1.00) as depicted in Table 3.

Efficacy of *Trichoderma* treatments in control of leaf blast disease

The symptoms of leaf blast disease appeared on leaves in the form of spindle shaped spots with yellowish margin and grayish centered which later on became ash colored. The results revealed the potential ability of *Trichoderma* treatments in controlling the incidence of leaf blast disease caused by *M. grisea* when compared to untreated plants. The data demonstrated that increase in salt stress levels from 0 to 8 dSm^{-1} did not impose any significant influence on the leaf blast disease incidence. However, the data depicted in Table 4 clearly revealed the potential of *Trichoderma*

isolates in reducing the leaf blast disease incidence caused by *M. grisea* as compared to untreated plants under both normal (0 dSm^{-1}) and various salinity stress levels. All the *Trichoderma* treatments showed a significant difference in the suppression of disease occurrence when compared to control, however, plants previously bioprimed with TRU-14 showed maximum potential in lowering blast disease (0.92 G, considering mean at salt stress) with HR (Highly Resistant) response followed by T1 (mean at salt stress = 1.50 G) with R (Resistant) response. Maximum disease (4.00 G) was recorded in T5 (control) with S (Susceptible) response followed by T4 (2.75 G) with MR (Moderately Resistant) response.

Discussion

Salinity stress is one of the most widespread constraints that restricts crop growth and yield of most of the crops grown in marginal lands. Among various strategies used to improve crop health under salinity stress, application of microbial inoculants such as salinity tolerant *Trichoderma* is an efficient and easily adaptive strategy. The results of this study provided evidence that seed biopriming with salinity tolerant *Trichoderma* isolates encouraged germination, vegetative growth and modulated physiological and biochemical responses in finger millet plants in order to adapt to salinity stress and thereby reducing deleterious effects of the same as compared to those plants raised from untreated seeds in control. The present investigation thus led to demonstrate a highly significant impact of seed biopriming with TRU-14, a salinity tolerant isolate of *Trichoderma* spp. to alleviate salt stress and to develop new options of salt tolerance in finger millet plants and at the same time, came out as an alternative, effective and sustainable resource for the management of leaf blast disease under both normal as well as salinity stress conditions.

In the present research, salt stress induced a substantial reduction in germination percentage, root and shoot lengths, fresh and dry weights of shoot and root in untreated plants. Because of the reduced water potential and the resulting slower imbibition rate, salt stress decreased the rate of germination. These findings are consistent with previous studies produced by Ghoulam et al. (2002) and Jamil et al. (2007), which demonstrated a pronounced decrease in germination and plant growth parameters under salt stress. Hadas (1977) reported the disturbances in the ionic balance of plant cells and imbalances in plant nutrients due to high levels of NaCl, which not only affected percent germination but also plant growth. Seed biopriming with *Trichoderma* increases the output of seeds and allows seeds to germinate even under unfavorable soil conditions (Rawat et al. 2012).

The root and shoot lengths as well as fresh and dry weights are the most important characteristics for salt stress tolerance, as roots are in direct contact with the soil and absorb water and nutrients for shoot supply. For this reason, the lengths and weights of the root and shoot provide a significant clue to plants' response to salt stress. In our study, seeds bioprimed with salinity tolerant *Trichoderma* isolates proved to be effective as compared to untreated seeds in inducing salt tolerance at the germination stage and further at vegetative growth in finger millet at all stress levels by exhibiting higher shoot and root length. shoot and root fresh as well as dry weight. *Trichoderma* strains produce plant growth hormones like cytokinin-like molecules, e.g., zeatin and gibberellins GA3 or GA3-related. *Trichoderma's* symbiotic colonization enhances deep roots, which help in increased water acquisition thereby increasing plant's ability to withstand abiotic stresses (salt, drought etc.) and uptake of nutrients (Howell 2003; Benitez et al. 2004). The proposed mechanisms, among many others, involved in *Trichoderma*-induced plant growth promotion include increased nutrient uptake efficiency due to improved nutrient availability through solubilization and chelation of minerals (Harman et al. 2004a). The addition of salts to water decreases its osmotic potential, resulting in lower supply of water to the roots and exposing plants to secondary osmotic stress. This implies that salt stress evokes all the physiological responses which are associated with drought stress. Enhanced rooting by *Trichoderma* provides increased surface area for water absorption. Longer root penetrates deeper into soil, which, even under water stress conditions, can absorb deep rooted water and increase plant standing (Malinowski and Belesky 2000). The physical existence of *Trichoderma* mycelial mass in the rhizosphere in itself functions as appendages to the normal plant rhizosphere or contributes to the creation of a relationship of plant fungus similar to that defined for mycorrhizal fungi (Barea et al. 2002).

Relative water content (RWC) refers to the absorption of water by the roots as well as the loss of water by transpiration. Salt stress has been reported to reduce RWC at the seedling stage in wheat cultivars (Slama et al. 2015). When subjected to water stress, Nayyar and Gupta (2006) have also reported significant reductions in RWC and water capacity of leaves. In present study, RWC decreased gradually with salt stress increase, however, *Trichoderma* isolate TRU-14 was found most potent with highest RWC and highest salt tolerance index amongst all the treatments. The results suggest that application of TRU-14 through seed biopriming turned out to be a promising step to overcome the damage pertaining to reduction in RWC in leaves of finger millet. Less is known about the mechanism by which *Trichoderma* increases the water deficit resistance of plants. However, considering that the contact between the plant and the fungus occurs predominantly in the rhizosphere, such a process

is likely to be associated with an increase in the efficiency of water absorption, which is likely to be associated with an increase in the volume of the root, resulting in an increase in water absorption (Shukla et al. 2014).

Salt stress induces a disturbance in membrane permeability expressed by an increase in leakage of electrolytes (Deshmukh et al. 1991). The results of leaf electrolyte leakage showed a substantially rising pattern with the increase in salt concentration. In present study, maximum leaf electrolyte leakage was recorded in untreated plants at all stress levels, suggesting significant membrane damage in plants raised from untreated seeds, probably due to increased accumulation of H_2O_2 and lipid peroxidation under salt stress as reported by Dionisio-See and Tobita (1998). Our study reported relatively lower leakage in all *Trichoderma* – treated plants, with lowest leakage in TUR-14 treated plants and that might be due to induction of antioxidant responses triggered by TUR-14 inoculation that might have given protection to the plant from oxidative damage under salt stress. Plant resistance to stress factor is related to their possible antioxidant potential, and the expanded degrees of the antioxidant constituents may prevent stress damage before it becomes lethal (Khan and Panda 2008).

The reduction in chlorophyll content under stress has been considered to be a typical symptom of oxidative stress and could be the result of pigment photo-oxidation and chlorophyll degradation (Shukla et al. 2012). The perusal of present study data revealed that there was reduction in total chlorophyll content (TCC) with increased salinity. The results are in agreement with the study reported by many previous workers (Hamada and El-Elnany 1994; Rawat et al. 2016) showing decrease of chlorophyll content in salt susceptible plants under salinity stress. NaCl stress decreases the TCC of the plant by increasing the activity of chlorophyllase, chlorophyll degrading enzyme, causing destruction of the chloroplast structure and destabilizing pigment protein complexes (Singh and Dubey 1995; Rawat et al. 2012). Our findings showed that the treatment T2 (TRU-14) showed maximum TCC while the least amount of TCC was noticed in untreated plants. Findings of Bae et al. (2009) has suggested enhanced chlorophyll content and greenness in *T. hamatum* DIS 219b-colonized seedlings.

Maximal quantum yield of PS II (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 T5 (control) among all the treatments. However, reduction in physiological response viz., chlorophyll fluorescens (CF) was less pronounced in *Trichoderma* treatments when compared to untreated plants. The reduction of CF is associated with an increase of Na accumulation (Dionisio-Sese and Tobita 1998). Salt stress reduces the performance of photosynthesis (Ashraf and Shahbaz 2003). Our findings indicate that the application of *Trichoderma* via seed biopriming improved CF at all levels of stress in comparison to untreated control. Higher CF was maintained in *T. harzianum* colonized plants in both normal and stressed conditions (Pandey et al. 2016).

Proline plays a critical role in protein protection and osmoregulation and a positive relationship between proline accumulation and salt tolerance has been recorded in many plant species (Rawat et al. 2012). The accumulation of proline is proposed to play a significant role in defending against oxidative damage and stabilizing cell membranes. There is also clear evidence that salt stress induces cytosol proline synthesis, which may lead to osmoregulation (Slama et al. 2015). Our results indicate that proline accumulation in finger millet seedlings obtained from *Trichoderma* treated seeds was comparatively higher (1.4–2.0 folds, considering mean at salt stress) as compared to control (untreated seeds). The higher concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of the leaf and, thus, in the osmotic adjustment. The maximum proline content in the TRU-14 treated plants under salt stress might have helped in maintaining structure and function of cellular macromolecules. Proline regulation in *Trichoderma buchenaui* under water and salinity stress was found consistent with its role as a compatible organic solute in ionic balance, preserving protein structure and activity, and deactivation of hydroxyl radicals and reactive chemicals (Hassine et al. 2008).

The level of malondialdehyde (MDA) content accumulation has been reported to be an indicative of the rate of lipid peroxidation due to salt stress (Bor et al. 2003; Meloni et al. 2003). Since lipid peroxidation is the symptom often attributed to oxidative damage, it is sometimes used as an indication of increased cellular and molecular damage and plays a crucial signaling function during abiotic stresses. Lipid peroxidation is the key index for the increase in active free radicals, and the major by-product of the lipid peroxidation process is MDA (Khan and Panda 2008; Mittler 2002). In present study, the degree of accumulation of MDA in untreated plants was greater and this increase was less pronounced in *Trichoderma* treated plants at all stress levels with TRU-14 showing most promising response at all stress levels in finger millet. The lowest MDA accumulation in *Trichoderma* colonized plants might be due to increased expression of stress related proteins such as, glutathione S-transferase (GST), glutathione-dependent formaldehyde dehydrogenase (FALDH) and peroxidase (Hernandez et al. 2000; Harman et al. 2004b) as plant resistance to stress factors is associated with increased levels of their antioxidant constituents that prevent stress damage (Shukla et al. 2012).

In the current study, positive influence was observed in plants previously treated by *Trichoderma* isolates with respect to total phenolics content as an increase in the content of total phenolics in salt stress conditions was of

higher magnitude in *Trichoderma* treated finger millet plants when compared to untreated plants with maximum phenol content appeared in plants previously bioprimed with TRU-14 along with highest salt tolerance index. Our results are in line with the previous results, which showed that root colonization by *Trichoderma harzianum* resulted in increased plant enzyme levels, including different peroxidases, chitinases and compounds such as phytoalexins and phenolics (Hoitink et al. 2006; Gachomo and Kotchoni 2008). *Trichoderma* strains, thus, not only directly produce metabolites, but also actively induce plants to produce defensive compounds of their own. The addition of *Trichoderma* led to a transient increase in phenolic glucoside levels in cucumber (Benitez et al. 2004). In addition to having antifungal, antibacterial and antiviral functions, phenolic compounds often have antioxidant properties and thus serve as activated free radical scavengers.

Many ROS are unstable and are converted to H_2O_2, which in the presence of metal ions, is converted to hydroxyl radicals and that begin chain reactions leading to membrane lipid peroxidation (Aust et al. 1985), resulting in loss of membrane integrity and also damage to other macromolecules. Results of present investigation showed significant reduced H_2O_2 content in TRU-14 treated plants followed by other *Trichoderma* treatments though maximum content was reported in untreated plants. The inoculation of *Trichoderma* strain T22 in maize plants has had a similar effect on H_2O_2, leading to an increased concentration of antioxidant enzymes counteracting H_2O_2 (Dixon et al. 2002). The mechanisms whereby *Trichoderma* spp. induces such changes are still unknown; however, increased ROS level may serve as a signal to regulate expression of some of the associated genes resulting in elevated protection from the oxidative damage and play a central role in protecting cell from oxidative damage (Shukla et al. 2012).

Data analysis indicated that seed biopriming with *Trichoderma* isolates showed increased levels of SOD content in leaves at all stress levels in comparison to control plants. Similar findings linked to increased SOD content in response to *Trichoderma* colonization have been documented in *Arabidopsis* and *Cucumis sativus* (Brotman et al. 2013), which reported that plants treated with *Trichoderma* prior to salt stress imposition showed an impact on the expression of antioxidant genes like SOD (Mn) and SOD (Cu) in roots. These detoxifying proteins activated by *Trichoderma* inoculation act as scavenging enzymes in response to ROS production. In present study, maximum increase in SOD activity at all stress levels was noticed in plants raised from TRU-14 bioprimed seeds, which further explain a potential role of TRU-14 in inducing salt tolerance in finger millet.

Currently, biopriming of seed has already been documented as an efficient method of bioinoculant application to protect seed against seed and soil-borne phytopathogens (Mahmood et al. 2016). In this study, seed biopriming with all *Trichoderma* isolates protected finger millet plant from leaf blast disease by manifesting resistant (R) response to moderately resistant (MR) response in pretreated plants whereas untreated plants were recorded with susceptible (S) response with maximum disease incidence. Our results suggest that TRU-14 exhibited multiple beneficial traits to the host plant and may act as a sustainable resource to reduce the usage of chemical fungicides for control of leaf blast disease on finger millet. Bioinoculants have been found to be effective in promoting cereal and legume development, biofortification of mineral nutrients in grains and as well as suppressing phytopathogens under salinity stress (Gopalakrishnan et al. 2016; Rawat et al. 2013).

From present investigation, seed biopriming with *Trichoderma* TRU-14 was found best by showing most consistent effect in terms of reducing the detrimental effects of salinity and suppressing leaf blast disease in finger millet. Plants pretreated with *Trichoderma* reacted to salinity stress by means of adjusting physiological and biochemical boundaries, which lead to the reclamation of cell homeostasis, detoxification of toxins and recuperation of growth. Our findings also support that, in addition to performing the process of osmoregulation, seed biopriming in finger millet with salinity tolerant *Trichoderma* isolates increased root vigour. It ameliorated salt stress by inducing physiological defense against oxidative damage in plants, due to increased ROS scavenging ability and increased SOD level, a mechanism that is expected to increase salt stress tolerance (Benitez et al. 2004). The present research confirms the potential of *Trichoderma t*o mitigate salt stress induced growth reduction and other salt injuries that may be attributed due to the fact that *Trichoderma* colonizes and penetrates root tissues and initiates a sequence of changes in the plant that are morphological and biochemical. The considerable improvement in growth, physiological and biochemical parameters under salt stress might have resulted from the overall positive effect of seed biopriming with *Trichoderma.* Evidence presented in this research indicates that *Trichoderma*- finger millet interaction improves salt stress tolerance via biochemical communication between the root of the host and mycelium of the fungus. However, to elucidate the cell-signaling network of physiological processes involved in salt stress response, the biochemical and molecular profiles of salt stress-related genes/proteins is needed to be explored further.

Conclusion

It could be concluded from the present investigation that seed biopriming with *Trichoderma* isolate TRU-14 enhanced finger millet's ability to successfully develop under saline conditions, and the salt tolerance index revealed heterogeneity

among various *Trichoderma* isolates for salt tolerance. The *Trichoderma* isolate TRU-14 was submitted to Indian Type Culture Collection (biological repository), New Delhi, India and was characterized as *Trichoderma asperellum* (ITCC-7903). The results of the study thus present a novel insight into a vital possible role of *Trichoderma asperellum* (ITCC-7903) in imparting salt stress tolerance and at the same time proving natural & durable resistance against leaf blast disease in finger millet under normal as well as salinity stress conditions. The present research, thus, offers a novel approach and merits further attention and may also pave the way for the use of *Trichoderma* application through seed biopriming in plants for enhanced salt and disease tolerance. This study constitutes a first phase to use this potential *Trichoderma* strain (TRU-14) further in natural saline areas for improving growth and suppressing leaf blast disease of this important crop within the context of organic farming in general, and of sustainable crop production in particular.

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Availability of data and material In current study, data was generated from the conducted experiments & are available with the corresponding author and has not been published elsewhere.

Code availability Not Applicable.

Declarations

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethics approval Not Applicable.

Consent to participate Consents from all the authors have been taken.

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