Role of *Trichoderma asperellum* in mitigating NaCl stress in tomato substantiated by anti-oxidative enzymes

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Received: July 26, 2023/ Revised: Aug 12, 2023/Accepted: Aug 16, 2023
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Abstract

Salinity is a major abiotic stress that limits the growth and development of plants, and is no special to tomato. Scientists are in constant search for natural remedies against abiotic stress affecting crops worldwide. Beneficial microbes are abundantly available in nature, and if employed rationally, bound to benefit micro-biota of soil. *Trichoderma* sp. stimulates plant growth and resistance to a varied range of adverse environmental conditions. The present study demonstrates the effect of two *Trichoderma asperellum* isolates (T4 and T8) in alleviating the deleterious effects of varying concentrations of NaCl (0-200 mM) stress in tomato. Treatment with *T. asperellum* isolates reduced the effect of stress and tomato seedlings showed significant improvement in seed germination; shoot and root length, shoot and root fresh weight, plant dry weight and chlorophyll content along with decrease in seedling death as compared to control. The *T. asperellum* treatments in tomato plants under NaCl stress also significantly increased the soluble sugar and protein. The antioxidant enzymes, CAT, POX, SOD and APX in tomato seedlings also significantly increased with *T. asperellum* treatment, as compared to control. These results prove *T. asperellum* to be beneficial in imparting NaCl stress tolerance in tomato and a new paradigm for developing environmentally safe alternative for improvement of NaCl stress tolerance.

Keywords: *Trichoderma asperellum*, Tomato, Salinity, Plant Growth Promotion, Antioxidant Enzymes Activity.

Introduction

Food security is a fundamental need of all societies. Tomato is considered a universal fruit and vegetable, consumed in the highest proportion on a daily basis. Among threats to quality food production, abiotic and biotic stress contributes to agricultural productivity losses of about 50% and 30% respectively worldwide. Both stresses can either be natural or human-induced. Dominant abiotic stresses comprise temperature, drought, salinity and acidic conditions, heavy metal, light intensity, submergence, anaerobiosis and nutrient starvation (Grayson, 2013). Among environmental stresses, soil salinity is one of the most important abiotic stresses, which majorly affects plant growth; development and crop yield (Ahmad et al., 2015). Salinity results in poor microbial activity due to toxic effect of ions and osmotic stress leading to reduced plant growth and development.

The salinity affects about 5.2 billion hectares of fertile land and has formed a massive problem to farmers worldwide. Estimates recommend worldwide financial losses because of soil salinization of about US $ 27.3 billion per year (Qadir et al., 2014; Zhang et al., 2016). It distresses in about 45 million hectares of irrigated soils accounting for one-third of global food production (Machado & Serralheiro, 2017) and has been assessed that about 7 million hectares of land is enclosed by saline soil in India (Patel et al., 2011;
Tomato is an important horticultural crop that is used by human in terms of health and nutrition (Tang et al., 2008). Like other crop plants, tomato growth is negatively influenced by the prevalence of salinity in soil. Rhizospheric fungi and plant growth-promoting bacteria are useful microorganisms which can colonize the rhizosphere of plants and increase plants growth, enhance yield under abiotic and biotic environments (Singh et al., 2014; Nguyen et al., 2016; Khan et al., 2021). Among beneficial microbes for agriculture, Trichoderma is a genus of soil-borne filamentous moulds that comprises species extensively used as biological control agents in agriculture (Illescas et al., 2022). These Trichoderma species stand out for their capability to inhibit phytopathogens through several mechanisms such as competition, mycoparasitism, antibiotics, and stimulating plant defense; nevertheless, Trichoderma species are also able to trigger plant growth and induce resistance to abiotic stress (Lorito et al., 2010; Hermosa et al., 2012; Hermosa et al., 2013). These are filamentous fungi found in agricultural, native prairie, salt marsh, forest, and desert soils of all biomes (Waghund et al., 2016; Zhao et al., 2020) and have increased interest due to their high reproductive capacity, prolific producers of secondary metabolites, survival under unfavorable conditions, in addition, activates protective mechanisms to prevent the oxidative damage (Contreras-Cornejo et al., 2016; Shaw et al., 2016; Kashyap et al., 2017).

The rhizospheric fungi, Trichoderma sp. colonizes plants and produces certain compounds that provide tolerance to abiotic stresses and improve the branching capacity of the root (Yasmeen & Siddiqui, 2017; De Sousa et al., 2020). Trichoderma-host interactions improve seed germination, root growth, nutrient uptake, disease resistance and tolerance to abiotic stresses (Singh et al., 2016a). The direct mechanism contains the increase of nutrient uptake, production of phytohormones, and volatile or non-volatile compounds (Akash & Padmanabh, 2018; De Sousa et al., 2020; Ashraf et al., 2022). An indirect mechanism includes bio-control activity against phytopathogens and the capacity to communicate abiotic stress tolerance within the hosts (Pandey et al., 2016) and also increases photosystem II effectiveness and photosynthetic product under stress environments (Ruiz-Lozano et al., 2016). The presence of Trichoderma in a saline environment increased the activity of antioxidant enzymes (Yasmeen & Siddiqui, 2017; Poveda, 2020). The present study was conducted to analyse the ability of Trichoderma species in alleviating salt stress in mild to severe salt stress environments, substantiated by its effect on antioxidant enzymes in salt stress.

Materials and method

Isolation and identification of microorganisms

Trichoderma sp. was isolated from different locations in apparently saline soils/fields. The collected soil were spread onto potato dextrose agar medium (PDA), and incubated for 7-days in incubation room with 12/12h light and near-ultraviolet light. Plates observed for Trichoderma colonies were initially identified morphologically followed by molecular identity by ITS sequencing. The isolates were identified as Trichoderma longibrachiatum UNS11 T1(MK611663), T. harzianum UNS10 T3 (MK611661), T. asperellum T4(KF679342), T. harzianum UNS35 T5 (MK611662), T. longibrachiatum UNS11 T6 (MK611664), T. asperellum T8 (KF679343), T. viride UNS42 T9 (MK886526) and T. viride UNS35 T10 (MK886525) (Narasimhamurthy et al., 2013a; b; 2018, 2020). The isolates were subjected to in vitro screening for NaCl tolerance to be used for seed treatment to study its ability to withstand salt stress (Fig. 1).

In vitro screening for NaCl tolerance

Mycelial discs of Trichoderma sp. were placed onto PDA medium supplemented with different concentrations of NaCl viz., 0 mM, 75 mM, 100 mM, 125 mM, 150 mM and 200 mM. The plates were incubated for five days at 28 °C and growth on NaCl amended media was recorded (Singh et al., 2015). PDA plates without salt were used as control. Each treatment was replicated thrice. The radial growth of mycelia was recorded and results were analysed statistically. The two selected T. asperellum isolates were identified by molecular method as T. asperellum T4 (KF679342) and T. asperellum T8 (KF679343).

Seed germination and seedling growth under NaCl stress

Healthy and uniform-sized tomato seeds (Arka Meghali) were surface sterilized with 1% sodium hypochlorite solution for 2 min, after which they were repeatedly washed with distilled water. Germination tests were analysed in plastic plates (85 mm × 15 mm) with three moist filter paper discs. Salt stress condition was prepared by treating seeds with NaCl solution to final concentrations of 0 mM, 75mM, 100mM, 125mM 150mM and 200mM NaCl respectively. Twenty-five tomato seeds were placed in each petri plate and incubated at 28 °C for ten days. The parameters studied were germination percent (%), vigour index (Abdul Baki & Anderson, 1973), shoot length, root length, seedling length, seedling fresh weight and seedling dry weight.

Plant growth promotion under different salt stress conditions

The surface sterilized seeds were grown in plastic seedling trays filled with sterilized soil and coconut pith compost. At 20 days after
sowing, seedlings were subsequently transplanted to pots (25×25 cm), filled with autoclaved soil and vermicompost (2:1). The soil was autoclaved for 40 min at 121 °C (at 15 psi pressure), cooled down and then distributed among pots. The seedlings were maintained in greenhouse under day/night cycle of 16/8h and 28/20°C and 65% humidity. All the plants in the pots were given 100 mL of 10% Hoagland’s nutrient solution (Hoagland & Arnon, 1950; Sun et al., 2020). After 21 days of planting, the seedlings were subjected to salt stress was given to plants by dissolving NaCl in 10% Hoagland’s nutrient solution to different concentrations such as 0 mM, 75 mM, 100 mM, 125 mM, 150 mM and 200 mM NaCl for one week. The experiment was conducted under a randomized block design with three replications. The fully expanded third leaves of each plant were collected, immediately frozen in liquid nitrogen and stored at -80 °C for analysis. The tomato plants were allowed to grow, to be assessed for various physiological parameters and antioxidant enzyme activities after 60 days of seedling transplantation. The plants were removed very carefully from plots, the shoot, root lengths plant biomass data were measured (Narasimhamurthy et al., 2016; 2018). The whole plant was dried at 110 °C for 24 h to measure plant biomass and the entire experiment was repeated thrice.

Estimation of total chlorophyll, soluble sugar and protein content

To substantiate the positive effect of T. asperellum on tomato against salt stress, parameters such as leaves total chlorophyll content (Lichtenenthaler, 1987), chlorophyll concentration (expressed as mg/g tissue fresh weight), total soluble sugars (Naguib, 1963; 1964), total soluble proteins (Lowry et al., 1951) and lipid peroxidation (Heath and Packer, 1968) was determined by measuring the amount of malondialdehyde (MDA) produced by the thiobarbituric acid reaction were analysed.

Antioxidant enzymes activities

The leaves from tomato plants raised from seeds treated with T. asperellum and stressed with various concentrations of NaCl were collected to study the effect on enzymes in alleviating salt stress. The plant extract used to study enzyme activities viz., ascorbate peroxidise (Ahmad et al., 2015), Catalase (Cakmak & Marschner, 1992), Peroxidase (Zhang, 1992; Lavanya et al., 2012; 2018), Ascorbate peroxidise (Nakano & Asada, 1981) and Superoxide dismutase (Rao and Sresty, 2000).

Statistical analysis

The statistical analysis was performed by analysis of variance (ANOVA) using the SPSS package (SPSS 20.0 for Windows) followed by Duncan’s Multiple Range Test (DMRT). Each value is the mean ± SE for 3 replicates in each group. P ≤ 0.05 were considered as significant.

Results and discussion

In vitro screening for NaCl tolerance

Ten Trichoderma sp. isolates were screened for salt tolerance at different concentrations viz., 0 mM, 75 mM, 100 mM, 125 mM, 150 mM and 200 mM at 28±1 °C. All the isolates growth covered full petriplate (71.20 to 74.31 mm) at 0 mM (control). At high NaCl concentration of 200mM, Trichoderma asperellum T8 isolate grew up to 47.48 mm followed by T. asperellum T4(47.19 mm), T. viride UNS35 T10 (43.98 mm), T. viride UNS42 T9(43.11 mm), T. harzianum UNS35 T5(42.30 mm), T. longibrachiatum UNS11 T1 (41.89 mm), T. longibrachiatum UNS11 T6 (41.00 mm) and T. harzianum UNS10 T3(39.20 mm) respectively. The mean radial growth at all NaCl concentrations, T. asperellum (T4 and T8) isolates exhibited maximum stress tolerance in comparison to other isolates (Fig. 1 and Table 1).

Table 1. Radial mycelia growth of T. asperellum isolates at different salt (NaCl) concentrations

<table>
<thead>
<tr>
<th>Trichoderma isolates</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
<th>T10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl Concentrations</td>
<td>0 mM</td>
<td>75 mM</td>
<td>100 mM</td>
<td>125 mM</td>
<td>150 mM</td>
<td>200 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>72.56±2.68abc</td>
<td>72.09±2.66abc</td>
<td>71.20±3.12bcd</td>
<td>68.99±2.37cd</td>
<td>65.99±2.38cd</td>
<td>52.09±1.78bc</td>
<td>50.72±0.89cd</td>
<td>51.09±1.66cd</td>
<td>52.09±1.78bc</td>
<td>50.72±0.89cd</td>
</tr>
<tr>
<td>75 mM</td>
<td>71.20±3.12bcd</td>
<td>73.83±2.37de</td>
<td>73.67±2.68abc</td>
<td>72.12±3.05de</td>
<td>70.87±1.98bc</td>
<td>68.99±2.37cd</td>
<td>66.56±2.16ab</td>
<td>66.56±2.16ab</td>
<td>68.99±2.37cd</td>
<td>66.56±2.16ab</td>
</tr>
<tr>
<td>100 mM</td>
<td>69.44±2.87de</td>
<td>68.99±2.37cd</td>
<td>65.99±2.38cd</td>
<td>62.66±2.28a</td>
<td>54.2±1.56de</td>
<td>56.76±1.66ef</td>
<td>51.09±1.66cd</td>
<td>51.09±1.66cd</td>
<td>54.2±1.56de</td>
<td>56.76±1.66ef</td>
</tr>
<tr>
<td>125 mM</td>
<td>62.66±2.28a</td>
<td>62.66±2.28a</td>
<td>62.66±2.28a</td>
<td>62.66±2.28a</td>
<td>56.76±1.66ef</td>
<td>51.09±1.66cd</td>
<td>51.09±1.66cd</td>
<td>51.09±1.66cd</td>
<td>56.76±1.66ef</td>
<td>51.09±1.66cd</td>
</tr>
<tr>
<td>150 mM</td>
<td>56.11±2.58ef</td>
<td>52.09±1.78bc</td>
<td>51.09±1.66cd</td>
<td>51.09±1.66cd</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
</tr>
<tr>
<td>200 mM</td>
<td>56.11±2.58ef</td>
<td>56.11±2.58ef</td>
<td>52.09±1.78bc</td>
<td>52.09±1.78bc</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
<td>47.19±0.86ef</td>
</tr>
</tbody>
</table>

The data represent mean ±SE of replicates (n = 3). Data with different superscript letters indicate significantly different between treatments and control by Duncan’s multiple range tests at p ≤0.05. T= Trichoderma isolates.
Seed germination and seedling growth under NaCl stress

The effect of salinity on seed germination of tomato were evaluated using NaCl concentrations of 75 mM, 100 mM, 125 mM, 150 mM and 200 mM. The seed germination decreased with increasing concentrations of NaCl compared to control. The observed seed germination was 94% in control and 81% at 75 mM NaCl, 77% at 100 mM NaCl, 73% at 125 mM NaCl, 69% at 150 mM NaCl and 63% at 200 mM NaCl concentration respectively (Fig. 2).

The effect of T. asperellum isolates on seed germination and vigour index was studied under laboratory condition after 14 days of incubation (Fig. 3A). Among isolates, seeds treated with T. asperellum (T4) and T. asperellum (T8) recorded maximum germination of 97.54% and 97.98%, respectively, with vigour index of 1483.63 and 1464.85 respectively.

Fig. 2 Effect of Trichoderma asperellum isolates on germination and seedling vigour index in tomato. Each value is the mean of three replicates (n=3) and vertical bar lines indicates standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan’s multiple range test at p ≤ 0.05 using one-way ANOVA. T4 and T8= Trichoderma asperellum isolates.

Fig. 3 Germination of tomato seeds and seedling growth under NaCl stress. (A) Effect of Trichoderma asperellum on tomato seed germination and seedling vigour index under laboratory conditions, (B) Effect of Trichoderma asperellum on plant growth promotion under greenhouse conditions.

Plant growth promotion under different salt stress conditions

A significant increase in plant height, roots and shoot length, shoot and root fresh weight and dry biomass was observed in plants treated with T. asperellum isolates as compared to control; 60 days post-transplantation under salt stress condition (Fig. 3B). Maximum plant height (69.56 cm and 67.89 cm), shoot length (29.87 cm and 29.45 cm), root length (15.12 cm and 14.94 cm), shoot fresh weight (33.89 g and 33.25 g), root fresh weight (7.87 g and 7.28 g), dry biomass (6.12 g and 5.98 g) respectively were recorded in seedlings treated with T4 and T8 isolates as compared to control (Table 2). Increased plant height (43.86 cm and 43.33 cm), shoot length (17.98 cm and 17.43 cm), root length (9.14 cm and 8.68 cm), shoot fresh weight (20.54 g and 20.87 g), root fresh weight (3.73 g and 3.56 g), dry biomass (3.1 g and 2.98 g) respectively were recorded when seedlings were treated with T4 and T8 isolates under high salt stress (200 mM NaCl).
Table 2. Effect of *T. asperellum* isolates on tomato plant growth promotion under different salt stress conditions

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Shoot fresh weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>49.84±0.89a</td>
<td>23.56±0.43b</td>
<td>12.32±0.33c</td>
<td>28.75±0.46c</td>
<td>5.93±0.12c</td>
<td>4.87±0.14e</td>
</tr>
<tr>
<td>T4</td>
<td>69.56±1.26c</td>
<td>29.87±0.57e</td>
<td>15.12±0.65d</td>
<td>33.89±0.57d</td>
<td>7.87±0.33d</td>
<td>6.12±0.33e</td>
</tr>
<tr>
<td>T8</td>
<td>67.89±1.57f</td>
<td>29.45±0.66d</td>
<td>14.94±0.57c</td>
<td>33.25±0.66c</td>
<td>7.28±0.26b</td>
<td>5.98±0.21b</td>
</tr>
<tr>
<td>75 mM</td>
<td>47.34±1.12g</td>
<td>20.14±0.84ef</td>
<td>10.87±0.33c</td>
<td>21.93±0.84d</td>
<td>4.89±0.21d</td>
<td>3.93±0.10i</td>
</tr>
<tr>
<td>100 mM</td>
<td>45.46±0.89ef</td>
<td>19.63±0.66d</td>
<td>10.34±0.32bc</td>
<td>21.56±0.56de</td>
<td>4.67±0.23de</td>
<td>3.87±0.13bc</td>
</tr>
<tr>
<td>125 mM</td>
<td>42.58±0.66e</td>
<td>18.16±0.57cd</td>
<td>9.66±0.31c</td>
<td>18.98±0.78e</td>
<td>4.23±0.36cd</td>
<td>3.23±0.16e</td>
</tr>
<tr>
<td>150 mM</td>
<td>39.76±0.74b</td>
<td>17.43±0.33b</td>
<td>9.18±0.34cd</td>
<td>17.54±0.34b</td>
<td>3.89±0.12b</td>
<td>2.86±0.12b</td>
</tr>
<tr>
<td>200 mM</td>
<td>34.14±0.66a</td>
<td>15.67±0.67a</td>
<td>8.13±0.33d</td>
<td>16.66±0.45f</td>
<td>3.15±0.11d</td>
<td>2.76±0.11a</td>
</tr>
<tr>
<td>75 mM+T4</td>
<td>50.83±0.98c</td>
<td>25.76±0.87f</td>
<td>14.56±0.57c</td>
<td>26.66±0.66b</td>
<td>5.98±0.25c</td>
<td>4.98±0.25b</td>
</tr>
<tr>
<td>75 mM+T8</td>
<td>50.36±1.14ik</td>
<td>24.87±0.39io</td>
<td>14.33±0.66f</td>
<td>26.32±0.78b</td>
<td>5.69±0.28i</td>
<td>4.54±0.23f</td>
</tr>
<tr>
<td>100 mM+T4</td>
<td>48.89±1.45j</td>
<td>24.54±0.89j</td>
<td>13.66±0.76j</td>
<td>25.88±0.9j</td>
<td>5.45±0.32je</td>
<td>4.26±0.26jef</td>
</tr>
<tr>
<td>100 mM+T8</td>
<td>48.57±1.18i</td>
<td>24.22±0.57j</td>
<td>13.38±0.84k</td>
<td>24.97±0.54h</td>
<td>5.18±0.14he</td>
<td>4.21±0.21he</td>
</tr>
<tr>
<td>125 mM+T4</td>
<td>46.89±0.93l</td>
<td>21.76±0.43g</td>
<td>10.87±0.33k</td>
<td>22.76±0.71h</td>
<td>4.98±0.22g</td>
<td>3.94±0.19he</td>
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<tr>
<td>125 mM+T8</td>
<td>45.65±0.84j</td>
<td>20.76±0.67i</td>
<td>10.63±0.42l</td>
<td>22.34±0.66f</td>
<td>4.45±0.19gel</td>
<td>3.86±0.16j</td>
</tr>
<tr>
<td>150 mM+T4</td>
<td>44.87±0.96k</td>
<td>19.34±0.66e</td>
<td>9.89±0.53h</td>
<td>21.89±0.84g</td>
<td>4.23±0.16he</td>
<td>3.23±0.2j</td>
</tr>
<tr>
<td>150 mM+T8</td>
<td>44.34±0.67l</td>
<td>18.87±0.66cd</td>
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<td>21.66±0.46bc</td>
<td>3.98±0.11bc</td>
<td>3.11±0.14bc</td>
</tr>
<tr>
<td>200 mM+T4</td>
<td>43.86±0.66d</td>
<td>17.98±0.34bc</td>
<td>9.14±0.33bc</td>
<td>20.54±0.66c</td>
<td>3.73±0.13bc</td>
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</tr>
<tr>
<td>200 mM+T8</td>
<td>43.33±0.57c</td>
<td>17.43±0.33b</td>
<td>8.68±0.36ab</td>
<td>20.87±0.57cd</td>
<td>3.56±0.21ab</td>
<td>2.98±0.16ab</td>
</tr>
</tbody>
</table>

The data represent Mean ±SE of replicates (n = 3). Data with different superscript letters indicate significantly different between treatments and control by Duncan’s multiple range tests at p ≤0.05. T4 and T8 = *T. asperellum* isolates.

**Total chlorophyll, soluble sugar and protein contents**

The results pertaining to the effect of NaCl and *Trichoderma* on chlorophyll content is represented in Table 3. Leaf chlorophyll contents were lower in plants under salt stress without *T. asperellum* isolates. Moreover, the total chlorophyll content increased with the application of *T. asperellum* isolates without salt stress as compared to control seedlings. Treatment with *T. asperellum* (T4 and T8) isolates increased by 12.56% and 10.79% in total chlorophyll content respectively at 200 mM as compared to the 200 mM NaCl treatments alone (Table 3).

**Soluble sugar and protein content**

Application of *T. asperellum* isolates increased soluble sugar and protein contents in tomato seedlings grown under salt stress, compared to control. However, the content of soluble sugar and protein in tomato seedlings significantly decreased after the treatment of NaCl alone (Table 3). Nevertheless, *T. asperellum* (T4 and T8) isolates alone caused an increase in soluble sugar of 18.54% and 17.06% respectively and protein content of 14.86 and 14.17% respectively as compared to control. The treatment with *T. asperellum* isolates (T4 and T8) increased soluble sugar content by 13.10% and 12.52% at 75 mM, 14.39% and 14.3% at 100 mM, 20.12% and 19.57% at 125 mM, 28.75% and 28.33% at 150 mM and 30.79% and 30.47% at 200 mM NaCl respectively, as compared to the NaCl stressed plants. The treatment of *T. asperellum* isolates (T4 and T8) increased protein content by 13.38% and 12.24% at 75 mM, 17.93% and 17.02% at 100 mM, 20.84% and 22.78% at 125 mM, 27.62% and 26.91% at 150 mM and 32.07% and 32.17% at 200 mM respectively, as compared to the NaCl stressed plants (Table 3). Malondialdehyde (MDA) content.

Fig. 4 Effect of *Trichoderma asperellum* isolates on Malondialdehyde (MDA) in tomato under different salt stress conditions. Each value is the mean of three replicates (n=3) and vertical bar lines indicate standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan’s multiple range test at p ≤0.05 using one-way ANOVA.
In the presence of *T. asperellum* isolates (T4 and T8), lipid peroxidation varied significantly. A significant reduction in malondialdehyde (MDA) was observed in plants exposed to salt stress irrespective of *T. asperellum* (T4 and T8) isolates treatment (Fig. 4).

**Table 3. Effect of *T. asperellum* treatment on chlorophyll, soluble sugar and protein in tomato under different salt stress conditions**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total Chlorophyll (mg/g FW)</th>
<th>Soluble sugar (mg/gFW)</th>
<th>Soluble protein (mg/gFW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.37±0.074</td>
<td>15.56±0.66</td>
<td>12.98±0.33</td>
</tr>
<tr>
<td>T4</td>
<td>2.8±0.057i</td>
<td>20.24±0.57ghi</td>
<td>16.12±0.57</td>
</tr>
<tr>
<td>T8</td>
<td>2.72±0.098i</td>
<td>19.79±0.68fghi</td>
<td>15.95±0.66</td>
</tr>
<tr>
<td>75 mM</td>
<td>2.07±0.068c-g</td>
<td>15.96±0.84c</td>
<td>10.98±0.43c</td>
</tr>
<tr>
<td>100 mM</td>
<td>1.94±0.056bcde</td>
<td>15.11±0.45bc</td>
<td>10.56±0.48bc</td>
</tr>
<tr>
<td>125 mM</td>
<td>1.82±0.043bcd</td>
<td>14.93±0.57b</td>
<td>10.15±0.36bc</td>
</tr>
<tr>
<td>150 mM</td>
<td>1.68±0.032ab</td>
<td>13.46±0.66a</td>
<td>9.28±0.66b</td>
</tr>
<tr>
<td>200 mM</td>
<td>1.46±0.036a</td>
<td>12.98±0.34a</td>
<td>8.49±0.57a</td>
</tr>
<tr>
<td>75 mM+T4</td>
<td>2.43±0.068hi</td>
<td>19.12±0.89efghi</td>
<td>13.45±0.48de</td>
</tr>
<tr>
<td>75 mM+T8</td>
<td>2.37±0.054hi</td>
<td>18.96±0.68ef</td>
<td>13.21±0.6d</td>
</tr>
<tr>
<td>100 mM+T4</td>
<td>2.25±0.076gh</td>
<td>19.45±0.54ef</td>
<td>13.96±0.38def</td>
</tr>
<tr>
<td>100 mM+T8</td>
<td>2.24±0.066fg</td>
<td>19.63±0.57d</td>
<td>13.75±0.93def</td>
</tr>
<tr>
<td>125 mM+T4</td>
<td>2.09±0.057e-g</td>
<td>19.95±0.66fgghi</td>
<td>14.14±0.68def</td>
</tr>
<tr>
<td>125 mM+T8</td>
<td>2.05±0.048d-g</td>
<td>19.78±0.078efg</td>
<td>14.62±0.89ef</td>
</tr>
<tr>
<td>150 mM+T4</td>
<td>1.81±0.034abcd</td>
<td>20.91±0.46ij</td>
<td>14.73±0.57f</td>
</tr>
<tr>
<td>150 mM+T8</td>
<td>1.76±0.065abc</td>
<td>20.76±0.66hij</td>
<td>14.54±0.68ef</td>
</tr>
<tr>
<td>200 mM+T4</td>
<td>1.67±0.054ab</td>
<td>20.98±0.89ij</td>
<td>14.86±0.73def</td>
</tr>
<tr>
<td>200 mM+T8</td>
<td>1.64±0.038ab</td>
<td>20.86±0.74j</td>
<td>14.89±0.66d</td>
</tr>
</tbody>
</table>

The data represent Mean ±SE of replicates (n = 3). Data with different superscript letters indicate significantly different between treatments and control by Duncan’s multiple range tests at p ≤0.05. T4 and T8= *T. asperellum* isolates; FW=Fresh weight.

Treatment of *T. asperellum* isolates (T4 and T8) significantly reduced MDA content to 21.71% and 19.68% at 75mM, 24.99% and 23.46% at 100mM, 29.15% and 26.90% at 125mM, 26.36% and 24.39% at 150mM and 20.79% and 18.85% at 200mM respectively as compared to NaCl stressed plants. Hence *Trichoderma* sp. was greatly effective in declining MDA as compared to salt treated seedlings without *T. asperellum* isolates.

**Antioxidant enzymes activities**

In comparison to untreated control seedlings, significant increase in terms of enzymatic activity was observed in plants treated with *T. asperellum*. Activity of antioxidant enzymes like CAT, POX, SOD and APX were determined at different NaCl concentrations with or without the *Trichoderma* sp. Maximum CAT activity was recorded in seedlings treated with both *T. asperellum* isolates (T4 and T8) as compared to control seedlings. Addition of *T. asperellum* isolates (T4 and T8) to salt stressed seedlings showed increase CAT activity of 16.86% and 13.95% at 75mM, 15.94% and 12.69% at 100mM, 16.90% and 15.99% at 125mM, 29.39% and 26.46% at 150mM and 37.71% and 36.05% at 200mM respectively, as compared to NaCl stressed plants (Fig. 5C). The POX activity increased in seedlings treated with *T. asperellum* isolates as compared to control seedlings. Treatment of *T. asperellum* isolates (T4 and T8) to salt stressed seedlings recorded increased POX activity of 31.41% and 29.58% at 75mM, 26.66% and 23.08% at 100mM, 16.38% and 15.37% at 125mM, 18.83% and 16.71% at 150mM and 16.44% and 18.33% at 200mM respectively, as compared to NaCl stressed controls (Fig. 5D). The SOD activity increased in seedlings treated with *T. asperellum* isolates as compared to control seedlings (Fig. 5A). Supplementation of *T. asperellum* isolates (T4 and T8) to salt stressed seedlings exhibited increase SOD activity of 14.44% and 12.32% at 75 mM, 16.44% and 17.12% at 100 mM, 21.28% and 19.09% at 125 mM, 27.04% and 25.52% at 150 mM and 30.26% and 28.88% at 200 mM respectively, as compared to NaCl stressed controls. Treatments of *T. asperellum* isolates (T4 and T8) to NaCl stressed seedlings exhibited APX activity increased by 27.02% and 24.24% at 75 mM, 22.92% and 20.31% at 100 mM, 27.27% and 24.78% at 125 mM, 28.26% and 27.15% at 150 mM and 22.27% at 200 mM respectively, as compared to NaCl stressed controls (Fig. 5B). It was observed that the addition of *T. asperellum* isolates in saline environment more over improved the activity of antioxidant enzymes as compared to seedlings with different saline conditions.
Fig. 5 Effect of *Trichoderma asperellum* isolates on (a) superoxide dismutase (SOD) (b) ascorbate peroxidase (APX) (c) catalase (CAT), and (d) peroxidase (POX) of tomato under different salt stress conditions. Each value is the mean of three replicates (n=3) and vertical bar lines indicates standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan’s multiple range test at p ≤ 0.05 using one-way ANOVA.

Soil salinity stress has become a serious roadblock for food production worldwide since it is one of the key factors affecting agricultural productivity. Salinity and drought are predicted to cause considerable loss of crops (Bomle et al., 2021). The whole world is dependent on food through agriculture. The use of fungicide and insecticide is inevitable to produce large quantity of food crops worldwide. Alternatives such as bio-control agents, bio-fertilizers and plant products are successful, if handled properly at right time of cropping (Udayashankar et al., 2016). The inappropriate and excessive use of modern agricultural pesticides has a major effect on the environment resulting in contamination of soils, health issues to human beings, flora and fauna, household animals and wild life making these synthetic chemicals environmentally intolerable (Arakere et al., 2022). *Trichoderma* sp. has an important role in metabolic processes of host plants that could impart tolerance against NaCl stress and capacity to induce systemic resistance, increase nitrogen use efficiency (Shoresh et al., 2010), increases water holding capacity (Hameed et al., 2014; Scudeletti et al., 2021; Anshu et al., 2022), helps in uptake of essential minerals and enhances photosynthetic efficiency (Hashem et al., 2014). The results of the present study reveal, under salt (NaCl) stress condition, increased germination percentage and vigour indices of tomato seeds was observed following treatment of *T. asperellum* isolates as compared to salt stressed and untreated control. In the present study, shoot and root length, fresh weight and dry weight was reduced under NaCl stress which may be endorsed to salt stress, deficiency of nutrients, and disturbance of various physiological and biochemical mechanisms. The treatment of *T. asperellum* isolates were significantly enhanced growth parameters and could easily
adaptive strategy to alleviate the negative impact of salt stress. Under salt stress the *Trichoderma* sp. Produces in plants such as plant growth hormones and has the capacity to enhance growth and development of plants (Kashyap et al., 2017). In the present study we found that shoot, root growth and dry weight were significantly improved after treatment with *T. asperellum* isolates in tomato seedlings either under salt stress conditions or *T. asperellum* treatment alone. Similarly, results have been reported with *T. parareesei* treatment which improved tomato lateral root growth and promotion under salt stress conditions (Rubio et al., 2014).

Mastouri et al. (2012) described *T. harzianum* treatments led to increased tomato root, shoot length and plant dry weight respectively as compared to NaCl stressed plants alone. Salt stress directly disturbs the chloroplast function, degrading enzymes, which results in substantial reduction in photosynthesis of plants (Siddiqui et al., 2014). However in present study revealed, treatment with *T. asperellum* isolates, and increased total chlorophyll content in tomato under different salt conditions as compared to uninoculated control. Ahmad et al., (2015) reported the *T. harzianum* restored the chlorophyll up to an appreciable level in saline condition. Similar outcomes were described in NaCl stressed soybean and cotton seedlings (Liu et al., 2014). Therefore, the chlorophyll contents were increased which might be due to phytohormone production in *Trichoderma* sp. treated plants (Resende et al., 2014). The increase in chlorophyll content by *Trichoderma* sp. colonization in plants may be due to inhibition of Na’ uptake. *T. asperellum* altered the concentration of Nitrogen, a nutrient directly intricate in the composition of chlorophyll (Lawson & Flexas, 2020; Boamah et al., 2021; Tandon et al., 2022).

Photosynthetic efficiency, the synthesis of photosynthetic pigments and phytohormones improved in tomato, due to inoculation of *T. harzianum* (Azarmi et al., 2011), which was also observed in our study. Montesinos et al. (2019) reported plant growth promotion and biocontrol of *Pythium ultimum* by saline tolerant *Trichoderma* isolates under salinity stress.

In the present study, the soluble sugars and proteins were increased in *T. asperellum* treated tomato seedlings as compared to control and NaCl stressed alone. It is extensively known that osmosis molecules, including soluble sugars and proteins, are significant indicators in response to abiotic stress (Azevedo-Neto et al., 2006). The accumulation of glucose and sucrose increased in plants usually indicates an extremely defensive mechanism against oxidative damage caused by high salinity in the plant environment (Bartels and Sunkar, 2005). Importance of soluble sugar content accumulation to play a significant role in increasing the internal osmotic pressure in plants (Wu et al., 2013). We found that *T. asperellum* had an extremely significant effect on the content of soluble sugar and protein in tomato seedlings under salt stress conditions.

In the present study, the MDA significantly increased in NaCl stressed tomato seedlings and decrease was observed in plants treated with *T. asperellum* as compared to NaCl stressed tomato plants. Li. (2009) reported that increased MDA content under salt stress condition in tomato plants The MDA is a main derivative of lipid peroxidation process that increases with an increase of salinity in plants and breakdown of polyunsaturated fatty acids of bio membranes. The expression of stress related proteins produced by *Trichoderma* sp. such as glutathione dependent formaldehyde dehydrogenase (FALDH), glutathione S-transferase (GST) and peroxidase, which could reduce the MDA (Hashem et al., 2014; Rasool et al., 2013). These investigations are reliable with earlier reported that *Trichoderma* spp. can improve quality and yield under abiotic stresses from different crops such as tomato (Khoshmanzar et al., 2020), rice (Doni et al., 2019; De Sousa et al., 2020), maize (Estévez-Geffriaud et al., 2020) and wheat (Zhang et al., 2016).

In the present study, results revealed that treatment of *T. asperellum* isolates in tomato seedlings induced the pathways responsible for the production of antioxidant enzymes as compared to control and stressed plants. Rubio et al. (2017) reported that the defense gene expression following the treatment of *T. harzianum* T34 under salinity conditions and revealed the expression of eight genes out of nine. *T. asperellum* is documented as a modulator of defense gene expression in interaction with their host, regulating the pathway of jasmonic acid and salicylic acid, both intricate in developing plant responses to environmental factors (Segarra et al., 2007; Mastouri et al., 2010; De Sousa et al., 2020; Ruchi et al., 2022). *Trichoderma* sp. Treated plants with POX generally involved in removing reactive oxygen species (Bharti et al., 2016). *Trichoderma* improved activities of antioxidant enzymes and reduced salt stress in plants (Singh et al., 2016b). *Trichoderma* sp. may be intricate in enhancing tolerance to abiotic stress in the regulation of resistance genes (De Sousa et al., 2020).

However, a protective mechanism comprising antioxidant enzymes machinery i.e., SOD is activated under stress conditions and destroys the ROS entities produced during stress conditions (Mishra et al., 2010; Boamah et al., 2022). The SOD enzyme of defense system dismutate the superoxide radicals generated after oxidative metabolism into H2O2 and O2 which acts as a first line of defense response in hosts (Radwan et al., 2012). Increased activity of SOD and CAT alleviates stress damage by the removal of H2O2 (Wu et al., 2013). The treated tomato seedlings with *T. harzianum* T22 has been found to increase the expression of cytosolic enzymes and chloroplast belonging to the ascorbate glutathione cycle (APX) and to produce an increase in SOD (Mastouri et al., 2012). Combination of *T. asperellum* and *Bacillus amyloliquefaciens* in wheat growth specified that *T. asperellum* induced the secretion of secondary metabolites, containing proline, and improved the antioxidative enzymes activity, increasing the plant tolerance to abiotic stresses (Karuppiah et al., 2019; Ruchi et al., 2022; Boamah et al., 2022).

The present study recommends that the NaCl tolerant *T. asperellum* isolates can show a significant role in conferring salt tolerance in plants. These isolates are able to inhabit root system, enhance plant growth under salt stressed condition. Antioxidant enzymes CAT, POX, SOD and APX induced by NaCl and *Trichoderma* sp. isolates further increasing the activity of antioxidative defense
system and protect tomato plants from salt stress (Fig. 6). The use of plant growth promoting fungi with salt tolerant properties holds a great potential for their use as biofertilizer in sodic soils and results into significantly increased plant growth, nutritional balance and ultimately higher yields and returns.

![Fig. 6 Accumulation of malondialdehyde (MDA) content and antioxidant enzymes activities in tomato plants with or without stressed raised from Trichoderma treatments. Time-course induction of Catalase (CAT), peroxidase (POX), Superoxide dismutase (SOD), and Ascorbate peroxidase (APX) in tomato plants under greenhouse conditions plotted using heatmap clustering.](image)

Acknowledgements

We are thankful for facilities provided by the Department of Microbiology and Biotechnology, Bangalore University, Bengaluru, under UGC-SAP-II (DRS-II) Reference No. F.4-8/2018/DRS-II (SAP-II), dated 23 April 2018.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

Funding support

The author declares that they have no funding support for this study.

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