

Microbial Science Archives (ISSN: 2583-1666)

An International Research Journal

Journal homepage: www.microbial.sciencearchives.org

<https://doi.org/10.47587/MSA.2023.3305>



Research Article

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

Narasimhamurthy Konappa¹, Soumya Krishnamurthy², Abijith M. Singh³, Divya, T⁴
Srinivas Chowdappa¹ and Udayashankar C Arakere⁵✉

¹Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Bangalore, Karnataka, India

²Field Marshal K.M. Cariappa College, A Constituent College of Mangalore University, Madikeri, Karnataka, India

³Department of Biotechnology, JSS College for Women, Saraswathipuram, Mysuru-570006, Karnataka, India

⁴Central Integrated Pest Management Centre, Jaivik Bhavan, Kannamangala Post, Bengaluru Karnataka-560067, India

⁵Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore -570 006, Karnataka, India

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

✉ Corresponding Author: acudays Shankar@gmail.com

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;

Mandal et al., 2018; Kumar and Sharma, 2020). According to estimation, 20% of total cultivated and 33% of irrigated farming lands globally are suffering by high salinity (Shrivastava & Kumar, 2015). The high temperature and low rainfall characteristics promote high salinity and have become a key factor limiting the development of salt-sensitive plants (Shahid et al., 2018). Salinity disturbs developments includes, seed germination, plant growth and development, water and nutrient uptake (Sofy et al., 2020), seedling growth and enzymes activity, photosynthesis, lipid metabolism, DNA, RNA, protein synthesis and mitosis and ecological balance (Seckin et al., 2009; Niu et al., 2013; Mishra et al., 2016; Machado & Serralheiro 2017). Under salt stress, reduction in plant root growth is connected to a weakening of endogenous levels of phytohormones such as IAA, salicylic acid, gibberellins, auxins and jasmonic acid (Alqarawi et al., 2014).

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, antibiosis, and stimulating plant defence; 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 200 mM 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 (Lichtenthaler, 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 peroxidase (Ahmad et al., 2015), Catalase (Cakmak & Marschner, 1992), Peroxidase (Zhang, 1992; Lavanya et al., 2012; 2018), Ascorbate peroxidase (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, 125mM, 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

Trichoderma isolates	Radial growth (mm)					
	NaCl Concentrations					
	0 mM	75 mM	100 mM	125 mM	150 mM	200 mM
T1	72.56±2.68abc	71.20±3.12bcd	65.67±2.16a	62.66±2.28a	52.09±1.78bc	41.89±1.57b
T3	73.67±3.12bc	72.65±3.16cd	71.22±3.05de	65.99±2.33cd	54.2±1.56de	39.20±1.58a
T4	74.07±3.45de	73.90±2.68cde	72.09±2.56ef	69.87±2.1f	56.76±1.66ef	47.19±0.86ef
T5	73.83±2.57cde	72.12±3.32cd	70.87±3.03cde	63.00±1.98b	51.09±1.57a	42.30±1.23bc
T6	72.32±2.33abc	71.38±2.69bcd	70.56±2.66cd	65.87±2.13cd	53.24±1.33cd	41.00±1.12b
T8	74.31±2.48de	73.11±2.42cde	71.52±3.57de	68.72±2.37e	56.11±2.58ef	47.48±0.75ef
T9	72.0±3.16abc	71.22±2.66ab	69.44±2.68bc	63.23±2.21b	55.32±1.98de	43.11±1.16cd
T10	71.20±3.37a	70.09±2.54a	68.98±2.32b	64.67±2.56bc	52.57±1.56bc	43.98±0.94d

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.

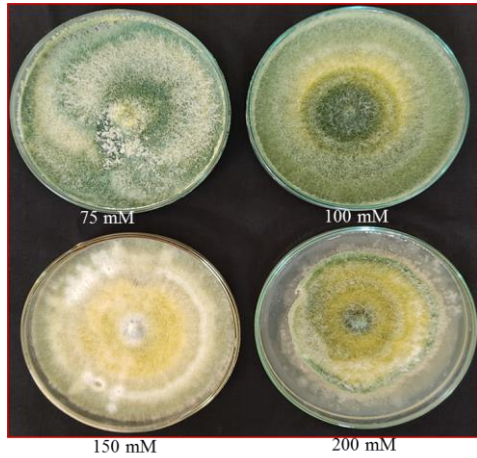


Fig. 1 Colonies of *Trichoderma* isolates tested for NaCl tolerance

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, 125mM, 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 75mM NaCl, 77% at 100 mM NaCl, 73% at 125mM NaCl, 69% at 150 mM NaCl and 63% at 200 mM NaCl concentration respectively (Fig. 2).

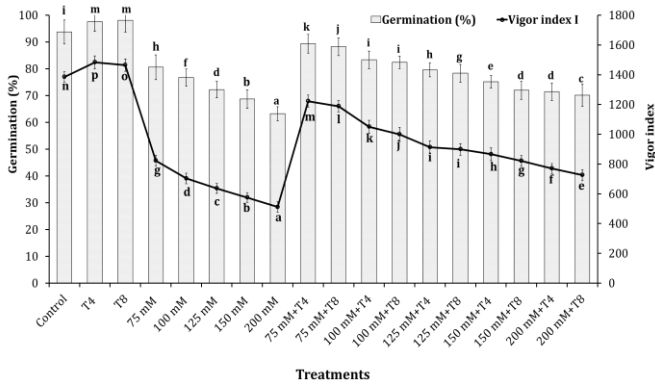


Fig. 2 Effect of *Trichoderma asperellum* isolates on germination and seedling vigour index in tomato. Each value is the mean of three replicates (n=3) and vertical bar lines indicates standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan’s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

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.

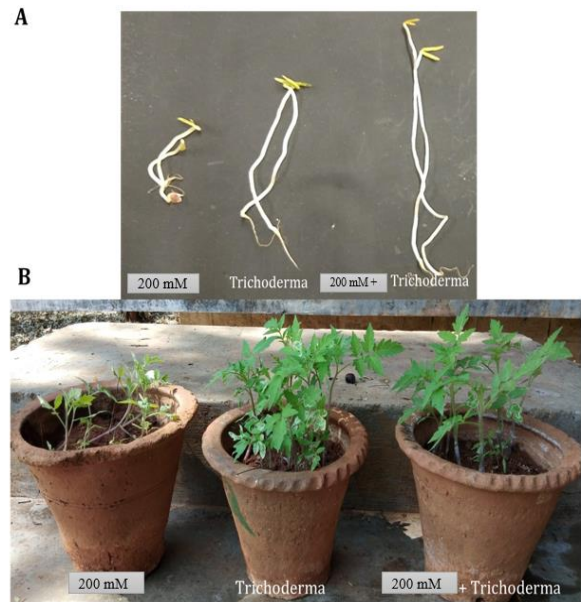


Fig. 3 Germination of tomato seeds and seedling growth under NaCl stress. (A) Effect of *Trichoderma asperellum* on tomato seed germination and seedling vigour index under laboratory conditions, (B) Effect of *Trichoderma asperellum* on plant growth promotion under greenhouse conditions.

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 29.45cm), root length (15.12 cm and 14.94 cm), shoot fresh weight (33.89g 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 43.33cm), shoot length (17.98 cm and 17.43 cm), root length (9.14 cm and 8.68 cm), shoot fresh weight (20.54g and 20.87 g), root fresh weight (3.73 g and 3.56 g), dry biomass (3.1g and 2.98g) 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

Treatments	Plant height (cm)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Dry weight (g)
Control	49.84±0.89 ^{ij}	23.56±0.43 ^h	12.32±0.33 ^f	28.75±0.46 ⁱ	5.93±0.12 ^{ij}	4.87±0.14 ^s
T4	69.56±1.26 ^m	29.87±0.57 ^k	15.12±0.65 ⁱ	33.89±0.57 ^j	7.87±0.33 ^k	6.12±0.33 ⁱ
T8	67.89±1.57 ^l	29.45±0.66 ^k	14.94±0.57 ⁱ	33.25±0.66 ^j	7.28±0.26 ^k	5.98±0.21 ⁱ
75 mM	47.34±1.12 ^{gh}	20.14±0.84 ^{ef}	10.87±0.33 ^e	21.93±0.84 ^{ef}	4.89±0.21 ^{ef}	3.93±0.10 ^d
100 mM	45.46±0.89 ^{ef}	19.63±0.66 ^{de}	10.34±0.32 ^{de}	21.56±0.56 ^{de}	4.67±0.23 ^{ef}	3.87±0.13 ^{de}
125 mM	42.58±0.66 ^c	18.16±0.57 ^{bc}	9.66±0.31 ^{cd}	18.98±0.78 ^b	4.23±0.36 ^{cd}	3.23±0.16 ^c
150 mM	39.76±0.74 ^b	17.43±0.33 ^b	9.18±0.34 ^{cd}	17.54±0.34 ^a	3.89±0.12 ^b	2.86±0.12 ^{ab}
200 mM	34.14±0.66 ^a	15.67±0.67 ^a	8.13±0.33 ^a	16.66±0.45 ^a	3.15±0.11 ^a	2.76±0.11 ^a
75 mM+T4	50.83±0.98 ^k	25.76±0.87 ^j	14.56±0.57 ⁱ	26.66±0.66 ^h	5.98±0.25 ^j	4.98±0.25 ^h
75 mM+T8	50.36±1.14 ^{jk}	24.87±0.39 ⁱ	14.33±0.66 ^{hi}	26.32±0.78 ^h	5.69±0.28 ^{ij}	4.54±0.23 ^{fg}
100 mM+T4	48.89±1.45 ⁱ	24.54±0.89 ^j	13.66±0.76 ^{gh}	25.88±0.9 ^{gh}	5.45±0.32 ^{hi}	4.26±0.26 ^{ef}
100 mM+T8	48.57±1.18 ^h	24.22±0.57 ^{hi}	13.38±0.84 ^g	24.97±0.54 ^g	5.18±0.14 ^{gh}	4.21±0.21 ^{ef}
125 mM+T4	46.89±0.93 ^s	21.76±0.43 ^s	10.87±0.33 ^e	22.76±0.7 ^f	4.98±0.2 ^{fg}	3.94±0.19 ^d
125 mM+T8	45.65±0.84 ^f	20.76±0.67 ^f	10.63±0.42 ^e	22.34±0.66 ^{ef}	4.45±0.19 ^{de}	3.86±0.16 ^d
150 mM+T4	44.87±.96 ^{ef}	19.34±0.6 ^{de}	9.89±0.53 ^{cd}	21.89±0.8 ^{ef}	4.23±0.16 ^{cd}	3.23±0.2 ^c
150 mM+T8	44.34±.067 ^{de}	18.87±0.66 ^{cd}	9.66±0.23 ^c	21.66±0.46 ^{de}	3.98±0.11 ^{bc}	3.11±0.14 ^{bc}
200 mM+T4	43.86±0.66 ^d	17.98±0.34 ^{bc}	9.14±0.33 ^{bc}	20.54±0.66 ^c	3.73±0.13 ^{ab}	3.1±0.12 ^{abc}
200 mM+T8	43.33±0.57 ^c	17.43±0.33 ^b	8.68±0.36 ^{ab}	20.87±0.57 ^{cd}	3.56±0.21 ^{ab}	2.98±0.16 ^{ab}

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.

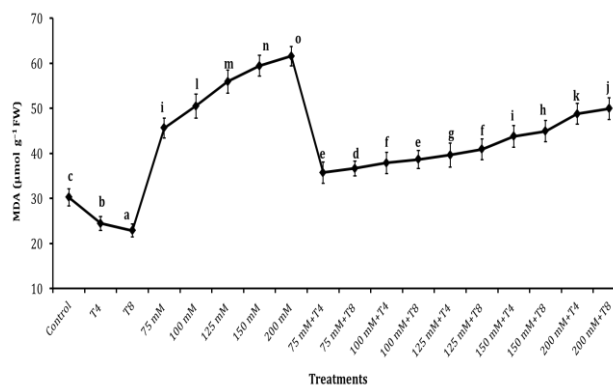


Fig. 4 Effect of *Trichoderma asperellum* isolates on Malondialdehyde (MDA) in tomato under different salt stress conditions. Each value is the mean of three replicates (n=3) and vertical bar lines indicates standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan's multiple range test at p ≤ 0.05 using one-way ANOVA.

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

Treatments	Total Chlorophyll (mg/g/ FW)	Soluble sugar (mg/g/FW)	Soluble protein (mg/g/FW)
Control	2.37±0.074 ^{fgh}	15.56±0.66 ^d	12.98±0.33 ^{def}
T4	2.8±0.057 ⁱ	20.24±0.57 ^{ghij}	16.12±0.57 ^g
T8	2.72±0.098 ⁱ	19.79±0.68 ^{fgh}	15.95±0.66 ^g
75 mM	2.07±0.068 ^{c-g}	15.96±0.84 ^c	10.98±0.43 ^c
100 mM	1.94±0.056 ^{bcde}	15.11±0.45 ^{bc}	10.56±0.48 ^{bc}
125 mM	1.82±0.043 ^{bcd}	14.93±0.57 ^b	10.15±0.36 ^{bc}
150 mM	1.68±0.032 ^{ab}	13.46±0.66 ^a	9.28±0.66 ^b
200 mM	1.46±0.036 ^a	12.98±0.34 ^a	8.49±0.57 ^a
75 mM+T4	2.43±0.068 ^{hi}	19.12±0.89 ^{efg}	13.45±0.48 ^{de}
75 mM+T8	2.37±0.054 ^{hi}	18.96±0.68 ^{ef}	13.21±0.6 ^d
100 mM+T4	2.25±0.076 ^{gh}	19.45±0.54 ^{ef}	13.96±0.38 ^{def}
100 mM+T8	2.2±0.066 ^{fgh}	19.63±0.57 ^{de}	13.75±0.93 ^{def}
125 mM+T4	2.09±0.057 ^{e-g}	19.95±0.66 ^{fghi}	14.14±0.68 ^{def}
125 mM+T8	2.05±0.048 ^{d-g}	19.78±0.78 ^{efg}	14.62±0.89 ^{ef}
150 mM+T4	1.81±0.034 ^{abcd}	20.91±0.46 ^{ij}	14.73±0.57 ^f
150 mM+T8	1.76±0.065 ^{abc}	20.76±0.66 ^{hij}	14.54±0.68 ^{ef}
200 mM+T4	1.67±0.054 ^{ab}	20.98±0.89 ^{ij}	14.86±0.73 ^{def}
200 mM+T8	1.64±0.038 ^{ab}	20.86±0.74 ^j	14.89±0.66 ^d

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 125mM, 28.26% and 27.15% at 150 mM and 23.50% and 22.27% at 200mM 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.

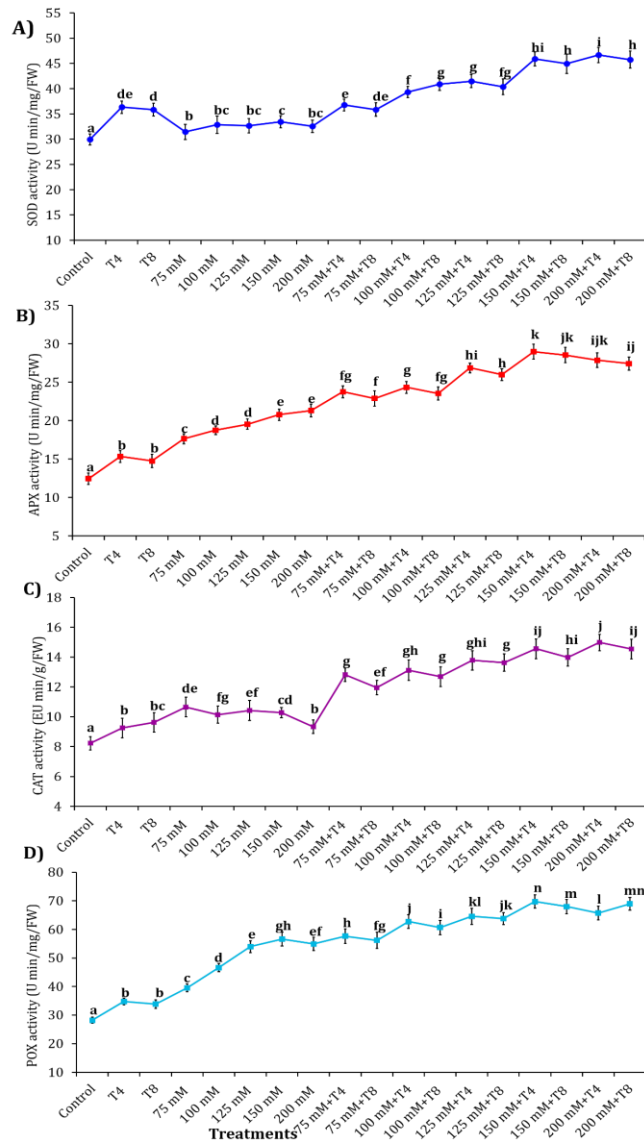


Fig. 5 Effect of *Trichoderma asperellum* isolates on (a) superoxide dismutase (SOD) (b) ascorbate peroxidase (APX) (c) catalase (CAT), and (d) peroxidase (POX) of tomato under different salt stress conditions. Each value is the mean of three replicates (n=3) and vertical bar lines indicates standard error. Different lowercase letters indicate significant difference between treatments and control by Duncan's multiple range test at $p \leq 0.05$ 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 capability 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 antioxidant enzymes activity, increasing the plant tolerance to abiotic stresses (Karuppiyah 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.

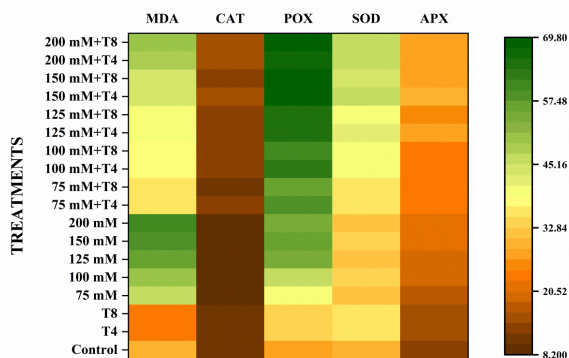


Fig. 6 Accumulation of malondialdehyde (MDA) content and antioxidant enzymes activities in tomato plants with or without stressed raised from *Trichoderma* treatments. Time-course induction of Catalase (CAT), peroxidase (POX), Superoxide dismutase (SOD), and Ascorbate peroxidase (APX) in tomato plants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

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.

References

Abdul Baki, A. A. & Anderson, J. D. (1973). Vigour determination in soybean seed by multiple criteria. *Crop Science*, 13, 630–633.

Ahmad, P., Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., John, R. & Egamberdieva, D. (2015). Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L) through antioxidative defense system. *Frontiers in Plant Science*, 6, 868.

Akash, H. & Padmanabh, D. (2018). Plant Responses to *Trichoderma* spp. and their tolerance to abiotic stresses: A review. *Journal of Pharmacognosy and Phytochemistry*, 7, 758–766.

Alqarawi, A. A., Abd Allah, E. F., Hashem, A., Al Huqail, A. A., Abdulaziz, A. & Al Sahli, A. A. (2014). Impact of abiotic salt stress on some metabolic

activities of *Ephedra alata* Decne. *Journal of Food, Agriculture and Environment* 12, 620–625.

Anshu, A., Agarwal, P., Mishra, K. (2022). Synergistic action of *Trichoderma koningiopsis* and *T. asperellum* mitigates salt stress in paddy. *Physiology and Molecular Biology of Plants*, 28, 987–1004.

Arakere, U. C., Jagannath, S., Krishnamurthy, S., Chowdappa, S. and Konappa, N., 2022. Microbial bio-pesticide as sustainable solution for management of pests: achievements and prospects. *Biopesticides*, pp.183-200.

Ashraf, S. A., El-Sayed, Hanan, E., Dief, El Sayed, A., Hashem, Ahmed, M., Desouky, Zamarud Shah, Salwa Fawzan. (2022). Fungal biopriming increases the resistance of wheat to abiotic stress. *Journal of Plant Biotechnology*, 49, 107-117.

Azarmi, R., Hajieghrari, B. & Giglou, A. (2011). *Trichoderma* isolated on tomato seedling growth response and nutrient uptake. *African Journal of Biotechnology* 10, 5850–5855.

Azevedo-Neto, A. D., Prisco, J. T., Enéas-Filho, J., Abreu, C. E. B. & Gomes-Filho, E. (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environmental and Experimental Botany*, 56, 87–94.

Bartels, D. & Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*, 24, 23–58.

Bharti, N., Pandey, S. S., Barnawal, D., Patel, V. K. & Kalra, A. (2016). Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Scientific Reports*, 6, 34768.

Boamah, S., Zhang, S., Xu, B., Li, T., Calderón-Urrea, A. & John Tiika R. (2022). *Trichoderma longibrachiatum* TG1 increases endogenous salicylic acid content and antioxidants activity in wheat seedlings under salinity stress. *Peer Journal*, 10:e12923.

Boamah, S., Zhang, S., Xu, B., Li, T., Calderón-Urrea, A. (2021) *Trichoderma longibrachiatum* (TG1) enhances wheat seedlings tolerance to salt stress and resistance to *Fusarium pseudograminearum*. *Frontiers in Plant Science*, 12, 741231.

Bomle, D. V., Kiran, A., Kumar, J. K., Nagaraj, L. S., Pradeep, C. K., Ansari, M. A., Alghamdi, S., Kabrah, A., Assaggaf, H., Dablood, A. S., Murali, M., Amruthesh, K.N., Udayashankar, A.C. and Niranjana, S.R. 2021. Plants Saline Environment in Perception with Rhizosphere Bacteria Containing 1-Aminocyclopropane-1-Carboxylate Deaminase. *International Journal of Molecular Sciences* 22, 11461.

Cakmak, I. & Marschner, H. (1992). Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiology*, 98, 1222–1227.

Contreras-Cornejo, H. A., Macías-Rodríguez, L., Del-Val, E. & Larsen, J. (2016). Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. *FEMS Microbiology Ecology*, 92, fiw036

De Sousa, T. P., Chaibub, A. A., da Silva, G. B. & de Filippi, M. C. C. (2020). *Trichoderma asperellum* modulates defense genes and potentiates gas exchanges in upland rice plants. *Physiological and Molecular Plant Pathology*, 112, 101561. doi: 10.1016/j.pmp.2020.101561

Doni, F., Fathurrahman, F., Mispan, M. S., Suhaimi, N. S. M., Yusoff, W. M. W. & Uphoff, N. (2019). Transcriptomic profiling of rice seedlings inoculated with the symbiotic fungus *Trichoderma asperellum* SL2. *Journal of Plant Growth Regulation* 38, 1507–1515. doi: 10.1007/s00344-019-09952-7

Estévez-Geffriaud, V., Vicente, R., Vergara-Díaz, O., Narváez Reinaldo, J. J. & Trillas, M. I. (2020). Application of *Trichoderma asperellum* T34 on maize (*Zea mays*) seeds protects against drought stress. *Planta* 252, 1–12. doi: 10.1007/s00425-020-03404-3

Fu, J. & Huang, B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*, 45, 105–114.

Grayson, M. (2013). Agriculture and drought. *Nature* 501:S1. doi: 10.1038/501S1a

Hameed, A., Egamberdieva, D., Abd-Allah, E. F., Hashem, A., Kumar, A. & Ahmad, P. (2014). Salinity stress and arbuscular mycorrhizal symbiosis in plants. In: Miransari, M. (Ed.), Use of microbes for the alleviation of soil stresses. Springer, New York. 139–159.

Hashem, A., Abd-Allah, E. F., Alqarawi, A. A., Al Huqail, A. A. & Egamberdieva, D. (2014). Alleviation of abiotic salt stress in *Ochradenus baccatus* (Del.) by *Trichoderma hamatum* (Bonord.) Bainier. *Journal of Plant Interactions*, 9, 857–868.

Heath, R. L. & Packer, L. 1968. Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125, 189–198.

Hermosa, R., Rubio, M. B., Cardoza, R. E., Nicolás, C., Monte, E. & Gutiérrez, S. (2013). The contribution of *Trichoderma* to balancing the costs of plant growth

- and defense. *International Journal of Microbiology*, 16, 69–80.
- Hermosa, R., Viterbo, A., Chet, I. & Monte, E. (2012). Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*, 158, 17–25.
- Hoagland, D. R. & Arnon, D. I. (1950). The water culture method of growing plants without soil. California Agricultural Experiment Station, University of California, Berkeley, College Agriculture Circular, No. 1950, 347.
- Illescas, M., Morán-Diez, M. E., Martínez de Alba, Á. E., Hermosa, R. & Monte, E. (2022). Effect of *Trichoderma asperellum* on Wheat Plants' Biochemical and Molecular Responses, and Yield under Different Water Stress Conditions. *International Journal of Molecular Sciences*, 23, 6782.
- Karupiah, V., Sun, J., Li, T., Vallikkannu, M. & Chen, J. (2019). Co-cultivation of *Trichoderma asperellum* GDFS1009 and *Bacillus amyloliquefaciens* 1841 causes differential gene expression and improvement in the wheat growth and biocontrol activity. *Frontiers in Microbiology*, 10 (2019), 1068.
- Kashyap, P. L., Rai, P., Srivastava, A. K. & Kumar, S. (2017). *Trichoderma* for climate resilient agriculture. *World Journal of Microbiology and Biotechnology*, 33, 1–18.
- Khan, N., Ali, S., Shahid, M. A., Mustafa, A., Sayyed, R. Z. & Curá, J. A. (2021). Insights into the Interactions among Roots, Rhizosphere and Rhizobacteria for Improving Plant Growth and Tolerance to Abiotic Stresses: A Review. *Cells*, 10, 1551.
- Khoshmanzar, E., Aliasgharzad, N., Neyshabouri, M. R., Khoshru, B., Arzanlou, M. & Asgari Lajayer, B. (2020). Effects of *Trichoderma* isolates on tomato growth and inducing its tolerance to water-deficit stress. *International Journal of Environmental Science and Technology*, 17, 869–878.
- Kumar, P. & Sharma, P. K. (2020). Soil Salinity and Food Security in India. *Frontiers in Sustainable Food Systems*, 4, 533781
- Lavanya, S. N., Niranjan Raj, S., Udayashankar, A. C., Kini, K. R., Amrutesh, K. N., Niranjana, S. R. and Shetty, H. S. (2012). Comparative analysis of activities of vital defense enzymes during induction of resistance in pearl millet against downy mildew. *Archives of Phytopathology and Plant Protection* 45 (11) 1252-1272.
- Lavanya, S. N., Udayashankar, A. C., Niranjan Raj, S., Mohan, C. D., Gupta, V. K., Tarasatyavati, C., Srivastava, R. and Nayaka. (2018). Lipopolysaccharide-induced priming enhances NO-mediated activation of defense responses in pearl millet challenged with *Sclerospora graminicola*. 3 Biotech 8: 475.
- Lawson, T. & Flexas, J. (2020). Fuelling life: recent advances in photosynthesis research. *Plant Journal*, 101, 753-755.
- Li, Y. 2009. Physiological responses of tomato seedlings (*Lycopersicon esculentum*) to salt stress. *Modern Applied Science*, 3, 171–176.
- Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350–382.
- Liu, S., Dong, Y., Xu, L. & Kong, J. (2014). Effects of foliar applications of nitric oxide and salicylic acid on salt-induced changes in photosynthesis and antioxidative metabolism of cotton seedlings. *Plant Growth Regulations* 73, 67–68.
- Lorito, M., Woo, S. L., Harman, G. E. & Monte, E. (2010). Translational research on *Trichoderma*: From omics to the field. *Annual Review of Phytopathology* 48, 395–417.
- Lowry, O. H., Rosebrough, N. J., Farr, L. A., and Randall, R. J. (1951). Protein measurements with the folin-phenol reagent. *Journal of Biological Chemistry*, 193, 265–275.
- Machado, R. M. A., and Serralheiro, R. P. (2017). Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*, 3, 30
- Mandal, S., Raju, R., Kumar, A., Kumar, P. & Sharma, P. C. (2018). Current status of research, technology response and policy needs of salt-affected soils in India – a review. *Indian Society of Coastal Agricultural Research*, 36, 40–53.
- Mastouri, F., Björkman, T. & Harman, G. E. (2010). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology*, 100, 1213–1221.
- Mastouri, F., Bjorkmann, T. & Harman, G. E. (2012). *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Molecular Plant-Microbe Interactions*, 25, 1264–1271
- Mishra, M. K. & Prakash, V. (2010). Response of non-enzymatic antioxidants to zinc induced stress at different pH in Glycine max L. cv. Merrill. *American Journal of Plant Sciences*, 1, 1–10
- Mishra, N., Khan, S. S. & Sundari, S. K. (2016). Native isolate of *Trichoderma*: a biocontrol agent with unique stress tolerance properties. *World Journal of Microbiology and Biotechnology*, 32, 130.
- Montesinos, B. S., Fernando Diáñez., Alejandro Moreno-Gavira., Francisco J. Gea & Mila Santos. (2019). Plant Growth Promotion and Biocontrol of *Pythium ultimum* by Saline Tolerant *Trichoderma* Isolates under Salinity Stress. *International Journal of Environmental Research Public Health* pp. 2053.
- Naguib, M. I. (1963). Colorimetric estimation of plant polysaccharides. *Zucker* 16:15–18
- Naguib, M. I. (1964). Effect of sevin on carbohydrate and nitrogen metabolism during germination of cotton seeds. *Indian Journal of Experimental Biology*, 2, 149–152.
- Nakano, Y. & Asada, K. (1981). Hydrogen-peroxide is scavenged by ascorbate-specific Peroxidase in spinach-chloroplasts. *Plant Cell Physiology*, 22, 867–880.
- Narasimha Murthy, K., Krishnamurthy, S., Arakere, U. C. (2020). Efficacy of indigenous plant growth-promoting rhizobacteria and *Trichoderma* strains in eliciting resistance against bacterial wilt in a tomato. *Egyptian Journal of Biological Pest Control*, 30, 106.
- Narasimhamurthy, K., Malini, M., Fazilath, U., Soumya, K. K., Chandra, N. S., Niranjana, S. R. & Srinivas, C. (2016). Lactic acid bacteria mediated induction of defense enzymes to enhance the resistance in tomato against *Ralstonia solanacearum* causing bacterial wilt. *Scientia Horticulturae*, 207, 183–192.
- Narasimhamurthy, K., Nirmala Devi, D. & Srinivas, C. (2013b). Efficacy of *Trichoderma asperellum* against *Ralstonia solanacearum* under greenhouse conditions. *Annals of Plant Sciences*, 2, 342–350
- Narasimhamurthy, K., Soumya, K., Chandranayak, S., Niranjana, S. R. & Srinivas, C. (2018). Evaluation of biological efficacy of *Trichoderma asperellum* against tomato bacterial wilt caused by *Ralstonia solanacearum*. *Egyptian Journal of Biological Pest Control*, 28, 1-11.
- Narasimhamurthy, K., Uzma, F. & Srinivas, C. (2013a). Induction of systemic resistance by *Trichoderma asperellum* against bacterial wilt of tomato caused by *Ralstonia solanacearum*. *International Journal of Advanced Research*, 1, 181–194.
- Nguyen, D., Rieu, I., Mariani, C. & van Dam, N. M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Molecular Biology*, 91, 727–740.
- Niu, G. D., Rodriguez, D., Dever, J. & Zhan, J. (2013). Growth and physiological responses of five cotton genotypes to sodium chloride and sodium sulfate saline water irrigation. *Cotton Science*, 17(2), 233–244.
- Pandey, V., Ansari, M. W., Tula, S., Yadav, S. & Sahoo, R. K. (2016). Dose-dependent response of *Trichoderma harzianum* in improving drought tolerance in rice genotypes. *Planta*, 243, 1251.
- Patel, B. B., Patel, Bharat, B. & Dave, R. S. (2011). Studies on infiltration of saline-alkali soils of several parts of Mehsana and Patan districts of north Gujarat. *Journal of Applied Sciences in Environmental Sanitation*, 1(1), 87–92.
- Poveda, J. (2020). *Trichoderma parareesei* favors the tolerance of rapeseed (*Brassica napus* L.) to salinity and drought due to a chorismate mutase. *Agronomy*, 10, 116.
- Qadir, M., Quillerou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R. J. (2014). Economics of salt-induced land degradation and restoration. *Natural Resources Forum*, 38, 282–295.
- Radwan, D. E. M. (2012). Salicylic acid induced alleviation of oxidative stress caused by clethodim in maize (*Zea mays* L.) leaves. *Pesticide Biochemistry and Physiology*, 102, 182–188.
- Rao, K. V. M. & Sresty, T. V. S. (2000). Antioxidant parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. *Plant Science*, 157, 113–128.
- Rasool, S., Ahmad, A., Siddiqi, T. O. & Ahmad, P. (2013). Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiologiae Plantarum*, 35, 1039–1050.
- Resende, M. P., Jakoby, I. C. M. C., dos Santos, L. C. R., Soares, M. A., Pereira, F. D., Souchie, E. L. & Silva, F. G. (2014). Phosphate solubilization and phytohormone production by endophytic and rhizosphere *Trichoderma* isolates of guanandi (*Calophyllum brasiliense* Cambess.). *African Journal of Microbiology Research*, 8, 2616–2623.
- Rubio, M. B., Hermosa, R., Vicente, R., Gómez-Acosta, F. A., Morcuende, R., Monte, E. & Bettioli, W. (2017). The combination of *Trichoderma harzianum* and chemical fertilization leads to the deregulation of phytohormone networking, preventing the adaptive responses of tomato plants to salt stress. *Frontiers in Plant Science*, 8, 29.
- Rubio, M. B., Quijada, N. M., Pérez, E., Domínguez, S., Monte, E. & Hermosa, R. (2014). Identifying beneficial qualities of *Trichoderma parareesei* for Plants. *Applied and Environmental Microbiology*, 80, 1864–1873.
- Ruchi, T., Chetan, K. & Rashmi, T. (2021). *Trichoderma Koningii* enhances tolerance against thermal stress by regulating ROS metabolism in tomato (*Solanum lycopersicum* L.) plants. *Journal of Plant Interactions*, 16, 116-125.

- Ruiz-Lozano, J. M., Aroca, R., Zamarreño, Á. M., Molina, S., Andreo-Jiménez, B., Porcel, R., García-Mina, J. M., Ruyter-Spira, C. & López-Ráez, J.A. (2016). Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant Cell Environment*, 39, 441–452.
- Scudeletti, D., Crucioli, C. A. C., Bossolani, J. W., Moretti, L. G., Momesso, L., Servaz Tubaña, B., de Castro, S. G. Q., De Oliveira, E. F. & Hungria, M. (2021). *Trichoderma asperellum* Inoculation as a Tool for Attenuating Drought Stress in Sugarcane. *Frontiers in Plant Science*, 12, 645542
- Seckin, B., Sekmen, A. H. & Turkan, I. (2009). An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. *Journal of Plant Growth Regulation*, 28, 12–20.
- Segarra, G., Casanova, E., Bellido, D., Odena, M. A., Oliveira, E., and Trillas, I. (2007). Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. *Proteomics*, 7, 3943–3952. doi: 10.1002/pmic.200700173
- Shahid, S. A., Zaman, M. & Heng, L. (2018). Soil salinity: historical perspectives and a world overview of the problem, in *Guideline for Salinity Assessment, Mitigation and Adaptation using Nuclear and Related Techniques* (Cham: Springer), 43–53.
- Shaw, S., Le Cocq, K., Paszkiewicz, K., Moore, K., Winsbury, R. & de Torres Zabala, M. (2016). Transcriptional reprogramming underpins enhanced plant growth promotion by the biocontrol fungus *Trichoderma hamatum* GD12 during antagonistic interactions with *Sclerotinia sclerotiorum* in soil. *Molecular Plant Pathology* 17:1425–1441
- Shoresh, M., Mastouri, F. & Harman, G. (2010). Induced systemic resistance and plant responses to fungal biocontrol agents. *Annual Review of Phytopathology*, 48, 21–43.
- Shrivastava, P. & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, 22, 123–131.
- Siddiqui, Z. S., Cho, J. I., Hanpark, S., Kwon, T. R., Ahn, B. O., Lee, G. S., Jeong, M. J., Whankim, K., Konlee, S. & Chulpark, S. (2014). Phenotyping of rice in salt stress environment using high-throughput infrared imaging. *Acta Botanica Croatica*, 73, 149–158.
- Singh, R., Singh, P. & Sharma, R. (2014). Microorganism as a tool of bioremediation technology for cleaning environment: A review. *Proceedings of the International Academy of Ecology and Environmental Sciences* 4:1–6
- Singh, R. P., Jha, P. & Jha, P. N. (2015). The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. *Journal of Plant Physiology*, 184, 57–67.
- Singh, V., Upadhyay, R. S., Sarma, B. K., and Singh, H. B. (2016a). *Trichoderma asperellum* spore dose depended modulation of plant growth in vegetable crops. *Microbiological Research*, 193, 74–86.
- Singh, Y. P., Mishra, V. K., Singh, S., Sharma, D. K., Singh, D., Singh, U. S., Singh, R. K., Haefele, S. M. & Ismail, A. M. (2016b). Productivity of sodic soils can be enhanced through the use of salt tolerant rice varieties and proper agronomic practices. *Field Crops Research*, 190, 82–90
- Sofy, M. R., Elhawat, N. & Tarek, A. (2020b). Glycine betaine counters salinity stress by maintaining high K⁺/Na⁺ ratio and antioxidant defense via limiting Na⁺ uptake in common bean (*Phaseolus vulgaris* L.). *Ecotoxicology and Environmental Safety* 200, 110732.
- Tandon, A., Anshu, A., Kumar, S., Yadav, U., Mishra, S. K., Srivastava, S., Chauhan, P. S., Srivastava, P. K., Bahadur, L., Shirke, P. A. & Srivastava, M. (2022). *Trichoderma*-primed rice straw alters structural and functional properties of sodic soil. *Land Degradation & Development*, 33(5), 698–709.
- Tang, F. Y., Shih, C. J., Cheng, L. H., Ho, H. J. & Chen, H. J. (2020). Transcriptome analysis of heat stress and drought stress in pearl millet based on Pacbio full-length transcriptome sequencing. *BMC Plant Biol*, 20, 1–15.
- Tang, F. Y., Shih, C. J., Cheng, L. H., Ho, H. J. & Chen, H. J. (2008). Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. *Molecular Nutrition and Food Research*, 52, 646–654.
- Udayashankar, A. C., Rajini, S. B., Nandhini, M., Suhas, Y. S., Niranjana, S. R., Lund, O. S. and Prakash, H. S. 2016. Acute oral toxicity, dermal irritation and eye irritation study of *Eclipta alba* aqueous extract in sprague dawley rats and New Zealand white rabbits. *International Research Journal of Pharmacy* 7 (6): 103-109.
- Waghund, R. R., Shelake, R. M. & Sabalpara, A. N. (2016). *Trichoderma*: a significant fungus for agriculture and environment. *African Journal of Agricultural Research*, 11, 1952–1965.
- Wu, Q. S., Srivastava, A. K. & Zou, Y. N. (2013). AMF-induced tolerance to drought stress in citrus: a review. *Scientia Horticulturae*, 164, 77–87.
- Yasmeen, R. & Siddiqui, Z. S. (2017). Physiological responses of crop plants against *Trichoderma harzianum* in saline environment. *Acta Botanica Croatica*, 76, 154–162.
- Zhang, S., Gan, Y. & Xu, B. (2016). Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Frontiers in Plant Science*, 7, 1405
- Zhang, X. Z. (1992). The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: Zhang XZ (ed) *Research methodology of crop physiology*. Agriculture Press, Beijing, pp 208–211.
- Zhao, L., Wang, Y. & Kong, S. (2020). Effects of *Trichoderma asperellum* and its siderophores on endogenous auxin in *Arabidopsis thaliana* under iron-deficiency stress. *International Microbiology*, 23, 501–509.

How to cite this article

Konappa, N., Krishnamurthy, S., Singh, A. M., Divya, T., Chowdappa, S. and Arakere, U. C. (2023). Role of *Trichoderma asperellum* in mitigating NaCl stress in tomato substantiated by anti-oxidative enzymes. *Microbial Science Archives*, Vol. 3(3), 101-111. <https://doi.org/10.47587/MSA.2023.3305>

This work is licensed under a [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

