Environment and Ecology 41 (4C) : 2805—2813, October—December 2023
Article DOI: https://doi.org/10.60151/envec/ICQC4775
ISSN 0970-0420

Piriformospora Indica-Colonization in Tomato Seedlings Enhances Water Stress Tolerance by Inducing Antioxidant Enzyme Activities and Proline Accumulation

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Received 3 May 2023, Accepted 15 July 2023, Published on 15 December 2023

ABSTRACT

Cultivation of tomato under diverse climatic zones is often challenged by various biotic and abiotic stresses which ultimately results in poor productivity and yield. Among the abiotic stresses, water stress is the most common one limiting the crop productivity. Synergistic relationship of endophytic microorganisms *Piriformospora indica* is capable of colonizing the roots tomato and establishing symbiotic relationships. Study was conducted to understand the influence of *P. indica* on tomato (var Vellayani Vijai) seedlings, (under *in vitro* and *in vivo* conditions), under water stress simulated by the application of mannitol (0, 1, 2, 3 and 5%). A positive influence was observed on shoot and root length, fresh and dries weight as well as number of rootlets in *P. indica*-colonized seedlings under stressed *in vitro* condition compared to those under control. Similarly, under *in vivo* condition *P. indica* colonized seedlings were having better performance with respect to stem and root length, and fresh and dry weight. Activities of antioxidant enzymes such as peroxidase and super oxide dismutase and proline accumulation were enhanced in *P. indica*-colonized plants compared to the non-colonized plants. However, catalase activity was found less influenced by the fungal colonization in the roots of tomato.

Keywords Tomato, *Piriformospora indica*, Water stress, Growth.

INTRODUCTION

Plants in the natural ecosystem are subjected to various forms of biotic and abiotic stresses which affect their development and overall performance. Water stress is one of the most important factors limiting agricultural production and productivity (Ghobadi *et al.* 2013, Osakabe *et al.* 2014). It is also predicted that, most crucial difficulties faced by farming sector across the world would be ever increasing size of world population to feed and ever-expanding shortage in water availability (Bouman 2007). Water stress creates an imbalance between water supply to the roots and transpiration rates in the plants. This leads to several morphological, physiological, biochemical and molecular changes affecting the plant metabolism

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(Rao and Chaitanya 2016). Tomato (*Solanum lycopersicum* L.), is the most valuable vegetable crop worldwide after potato, and utilized in raw, cooked, and processed forms. Therefore, tomato has seen ever increasing demand globally (Chaudhary *et al.* 2019a). Cultivation of tomato is often challenged by various biotic and abiotic stresses and results in poor productivity and yield. This led to the adoption of greenhouse cultivation in the non-conventional areas (Chaudhary *et al*. 2019b). However, to cope up with the increasing population size, we have to rely on rainfed vegetable production to address the high demand of tomatoes. Therefore, enhancing stress tolerance in tomato cultivars through sustainable, ecofriendly and economical strategies are more desirable.

Under water scarce conditions, plants use several stress avoidance and tolerance mechanisms for adaptation (Lawlor 2013). In addition to the intrinsic plant protective mechanisms, plants do have association with various microorganisms such as mycorrhizal fungi, rhizobial and plant-growth-promoting rhizobacteria, endophytic fungi, which benefits in alleviating the effects of stress (Rodriguez 2008, Ali *et al.* 2014, Hashem *et al*. 2016). *Piriformospora indica* is a unique beneficial fungus capable of colonizing the roots of many plants and establishing symbiotic relationships. The fungus lacks host specificity, thus colonize all plantae. Moreover, the fungus can be axenically cultivated. It promotes plant growth especially in nutrient-deficient soils, confers tolerance to abiotic (salinity, drought, water, cold, high temperature and heavy metals) and biotic (root and foliar pathogens) stress (Varma *et al.* 1999, Oelmüller *et al.* 2009, Johnson *et al.* 2014, Gill *et al*. 2016).

In the present study, we studied the effect of *P. indica* on tomato seedlings (both *in vitro* and *in vivo* conditions), under water stress simulated by the application of mannitol. Work was mainly focused on biometric parameters of seedlings under *in vitro* and *in vivo* conditions and oxidative stress responses through the production of antioxidant enzymes and proline accumulation. This study provides valuable insights into the potential use of *P. indica* as a biological tool for improving tomato crop productivity and yield under water stress conditions, which is an important issue in many agricultural regions around the world.

MATERIALS AND METHODS

The study was conducted at the Department of Vegetable Science, College of Agriculture, Vellayani during 2018-19. It comprised two experiments : One under *in vitro* and another under *in vivo* conditions. Both experiments were designed in Factorial-CRD, adopting five levels of water stress conditions (including control) simulated by the application of mannitol, and two types of tomato seedlings (*P. indica* colonized and non-colonized), each with five replications.

Growth conditions of fungus and inoculum preparation

The beneficial fungal root endophyte, *P. indica* from Department of Plant Pathology, College of Agriculture, Vellayani was maintained in potato dextrose agar (PDA) medium. This was routinely cultured by transferring fungal disc from actively growing margin of two weeks-old culture of *P. indica* to Petri plates containing PDA/conical flasks containing PDB (potato dextrose broth) and incubated in dark at room temperature. Subculturing was done once in fifteen days.

Co-cultivation of *P. indica* with tomato in MS media

To investigate the potential of the root endophytic fungus *P. indica* to enhance plant tolerance to drought, we conducted a simple and rapid *in vitro* experiment in jam bottles (Fig. 1). We prepared MS media with varying concentrations of mannitol (1%, 2%, 3%, and 5%) along with a control, and sterilized them in jam bottles before allowing them to solidify. Mycelial discs (5 mm) from a two-week-old culture of *P. indica* were placed in the center of jam bottles containing solidified medium and incubated in the dark. In half of the bottles, *P. indica* was omitted to serve as a control.

We surface-sterilized tomato seeds (var Vellayani Vijai) in 0.1% mercuric chloride for 7 minutes, followed by 3 washes in sterile water and drying. The seeds were germinated in Petri plates containing MS medium, and the resulting seedlings were transferred to each jam bottle containing MS medium with or without mannitol and *P. indica*. We confirmed root

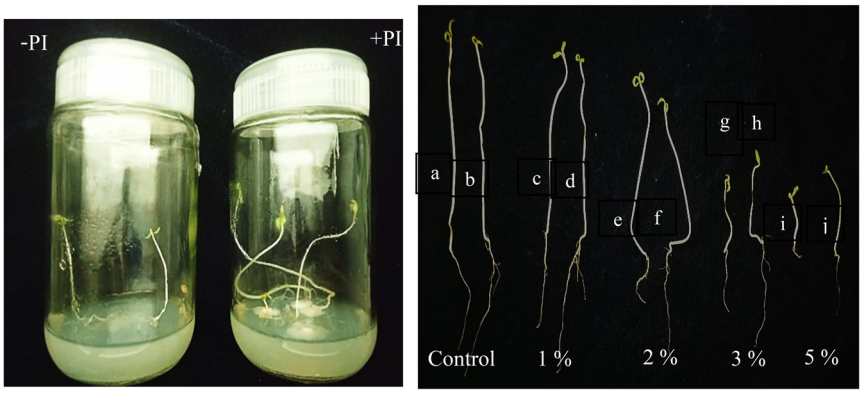

Fig. 1. *P. indica* colonized (+PI) and non-colonized (-PI) under different concentration of mannitol stress (a, c, e, g and i: with *P. indica;* b, d, f, h and j: without *P. indica*).

colonization by *P. indica* by observing root bits under a microscope (Leica - ICC50 HD, USA) five days after co-cultivation. Fifteen days after co-cultivation, observations were recorded and a two-factor analysis was conducted. The first factor examined colonization with or without *P. indica*, and the second factor looked at different levels of mannitol concentration (0%, 1%, 2%, 3% and 5%).

Co-cultivation of *P. indica* with tomato in vermiculite-perlite medium

To conduct the *in-vivo* studies, the potting medium was sterilized by moistening it up to field capacity and heating it at 121°C for 1 hr for two consecutive days. *P. indica* was cultured in 250 ml conical flasks using potato dextrose broth. The fungal mycelium was then harvested by filtering it through double layered muslin cloth and washed twice with sterile water. The resulting mycelial mass was mixed with sterile potting medium at a concentration of 1% (w/v), filled in trays, covered, and allowed to grow. After one week, surface-sterilized tomato seeds were placed in small pots filled with the medium and kept under controlled temperature and humidity conditions to ensure uniform germination. Normal sterilized potting medium without *P. indica* was used to maintain control plants. Root colonization by *P. indica* was confirmed by observing root bits under a microscope (Leica - ICC50 HD, USA). To compare the colonized and non-colonized plants, biometric observations were taken after 25 days and t-test was conducted to compare the means. To study the antioxidant activities in colonized and non-colonized plants during stress, mannitol (0, 1, 2, 3 and 5%) was applied to both *P. indica*-colonized and control plants at 25 days after sowing, observations on peroxidase, catalase, SOD and proline were estimated after 5 days and two factor analysis was conducted.

Biometric parameters

In the *in vitro* study, we harvested five plants (replicates) per treatment from the bottles after 15 days of co-cultivation and measured their shoot and root length, number of rootlets, fresh weight, and dry weight. The plant material was oven-dried at 60°C for three days to constant weight and dry weight was measured. Under *in vivo* conditions, we compared *P. indica* colonized and non-colonized seedlings by recording biometric observations 25 days after sowing in the potting medium. We measured shoot and root length, number of rootlets, as well as the fresh and dry weight of the shoot and roots.

Enzymatic antioxidant activity

To study the stress response in tomato seedlings grown under *in vivo* conditions under water stress, the antioxidant enzymes, viz. peroxidase, catalase and SOD were estimated after subjecting the seedlings to stress treatment using different concentrations of mannitol for 5 days. The PO activity was determined using a protocol described by Srivastava (1987), while the catalase activity was determined by following the

procedure described by Luck (1974). For quantification of SOD activity, the method described by Kakker *et al.* (1984) was followed.

Proline estimation

Proline is precipitated as a protein sulpho salicylic acid complex during extraction of tissue with sulphosalicylic acid. The extracted proline is made to react with ninhydrin under acidic conditions to form a red color which is measured calorimetrically at 520 nm.

Five hundred mg of tissue was homogenized in a pestle and mortar with 10 ml of 3% aqueous sulpho salicylic acid and filtered through Whatman No. 2 filter paper. Extraction and pooling the filtrate was repeated. To 2 ml of filtrate, 2 ml each of glacial acetic acid and ninhydrin were added and mixed. It was then kept in boiling water bath for 1 hr and then reaction was terminated by placing on ice bath. 4 ml of toluene was added and mixed vigorously for 20-30 seconds. The toluene (chromophore) layer was aspirated and warmed to room temperature. The absorbance of red color was measured at 520 nm against a blank reagent. The amount of proline in the sample was calculated using a standard curve prepared from pure proline (Range 0.1—36 µmole) and expressed on fresh weight basis of sample.

$$\mu M \text{ of proline/g tissue} = \frac{(\mu g \text{ of proline}) \times \text{ml of toluene} \times 5}{115.5 \times \text{ g of sample}}$$

RESULTS

Biometric parameters of *in vitro* grown tomato seedlings

The growth of *in vitro* grown tomato seedlings was significantly affected by both the colonization of *P. indica* and the stress induced by the application of mannitol. The presence of *P. indica* increased shoot length by 11% compared to non-colonized seedlings, regardless of the concentration of mannitol in the growth media. As the concentration of mannitol increased from 0 to 5%, the mean shoot length decreased from 6.16 cm to 4.11 cm. However, the interaction between *P. indica* colonization and water stress caused by mannitol had no significant effect on the shoot length of tomato seedlings. Furthermore, *P. indica* colonization, mannitol mediated water stress and their interaction had a significant impact on the root length and number of rootlets. In this study, the root length was reduced by 67% in non-colonized plantlets when exposed to 5% mannitol, whereas *P. indica* colonized seedlings only had a 44% reduction under the same conditions. The mean number of rootlets was also higher in *P. indica* colonized seedlings (2.97) compared to non-colonized seedlings (1.67). *P. indica* colonized seedlings maintained a higher number of rootlets even when exposed to 4% mannitol, with only a 3% reduction in rootlets compared to nearly 15% in non-colonized seedlings. However, at 5% mannitol stress, the number of rootlets was significantly reduced in colonized seedlings as well (Table 1) Fresh weight and dry weight was also varied significantly by colonization with *P. indica* and colonization resulted in higher fresh and dry weight at all levels of mannitol concentration (Fig. 2). The results indicates that *P. indica* may help plants adapt to water stress by promoting root growth and development, which can ultimately help plants better absorb water and nutrients from the soil.

Table 1. Effect of *P. indica* on shoot length, root length and number of roots lets of tomato grown under different levels of mannitol concentration (*in vitro*). (D- Mean under different mannitol concentration, P- Mean under different *P. indica* treatments, Different letters within the same row represent significant differences).

Treatments	Shoot length (cm)			Root length (cm)			Number of rootlets		
	-*P. indica*	+*P. indica*	Mean (D)	-*P. indica*	+*P. indica*	Mean (M)	-*P. indica*	+*P. indica*	Mean (D)
Control	6.02	6.30	6.16^{A}	1.56	1.99	1.77^{A}	2.00	3.30	2.65^{A}
1% Mannitol	5.21	6.17	5.69^{B}	1.39	1.80	1.59^{B}	2.00	3.20	2.60^{A}
2% Mannitol	5.24	6.10	5.67^{B}	1.19	1.66	1.42^{C}	1.43	3.37	2.40^{A}
3% Mannitol	5.10	5.83	5.47^{C}	1.09	1.43	1.26^{D}	1.70	3.20	2.45^{A}
5% Mannitol	3.92	4.29	4.11^{D}	0.91	1.11	1.01^{E}	1.20	1.77	1.48^{B}
Mean (P)	5.10^{B}	5.74^{A}		1.23^{B}	1.60^{A}		1.67^{B}	2.97^{A}	
CD (D×P)		N/A			0.14			0.40	

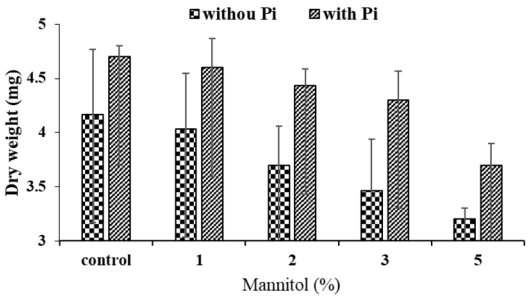

Fig. 2. Effect of *P. indica* on dry weight of tomato seedlings grown *in vitro* under mannitol stress.

Biometric and physiological parameters of tomato seedlings under *in vivo* condition

Influence of P. indica on growth parameters

The colonization of *P. indica* had a significant positive impact on several biometric parameters. Specifically, the colonized plants had significantly longer shoot and root lengths and a higher number of rootlets compared to non-colonized plants (Fig. 3). The average shoot length was 7.08 cm for colonized plants, whereas non-colonized plants had an average shoot length of only 5.40 cm. Similarly, the average root length was 11.10 cm for colonized plants, whereas non-colonized plants had an average root length of only 6.78 cm. In addition, colonized seedlings had an average of 30 rootlets, while non-colonized plants had only 22.33 rootlets. Furthermore, *P. indica* colonization led to a significant increase in both shoot and root fresh and dry weight. Specifically, colonized plants had a shoot fresh weight of 3.88 g and a shoot dry weight of 0.504 g, while non-colonized plants had a shoot fresh weight of 2.89 g and a shoot dry weight of 0.390 g. Colonized plants also had a root fresh weight of 1.39 g and a root dry weight of 0.12 g, while non-colonized plants had a root fresh weight of 0.82 g and a root dry weight of 0.068 g (Table 2) .The increase in shoot and root length, number of rootlets, and fresh and dry weights of both shoot and root indicate that *P. indica* may enhance the ability of the plant to uptake nutrients and water from the soil, leading to improved growth and biomass accumulation.

Antioxidant enzyme activities

The effect of *P. indica*-colonization on the enzymatic

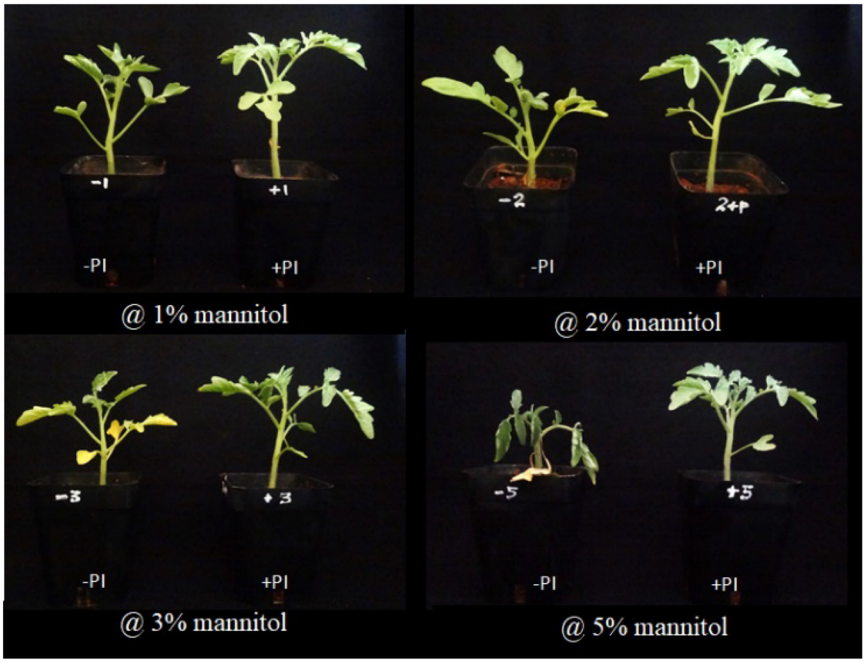

Fig. 3. *In-vivo* grown seedlings after stress treatment with mannitol (With the increase in concentration of mannitol, control plants showed severe wilting symptoms whereas *P. indica* colonized plants remained healthy).

Table 2. Effect of *P. indica* on growth parameters of *in vivo* grown tomato seedlings (25 days after germination). (Different letters within the same column represent significant differences).

Treatments	Shoot length (cm)	Root length (cm)	Number of rootlets	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root fresh weight (mg)	Root dry weight (mg)
-*P. indica*	5.40^{B}	6.78^{B}	22.33^{B}	2.68^{B}	0.35^{B}	0.82^{B}	0.08^{B}
+*P. indica*	7.08^{A}	11.10^{A}	30.00^{A}	3.88^{A}	0.50^{A}	1.39^{A}	0.12^{A}
CD	0.70	0.83	1.09	0.24	0.11	0.31	0.01

antioxidative mechanism of tomato seedlings grown *in vivo* after mannitol application was studied. Peroxidase (PO), Superoxide dismutase (SOD) and Catalase (CAT) activities were determined on leaves collected from *P. indica*-colonized and non-colonized plants after stress treatment. Coonization with *P. indica*, different levels of stress created by mannitola and their interaction had a significant influence on PO, SOD and CAT activities. The antioxidant activity was found to be increased with the increase in level of water stress in both colonized and non-colonized plants. With the increase in water stress, PO activity increase by 84% in colonized plants as against only 71% in non-colonized plants compared to the control. Similarly, when the concentration of mannitol reached 3%, SOD activity was increased by 106% in non-colonized plants compared to the control as against an increase of 150% in colonized plants. Catalase activity was increased by 31% in non-colonized plants whereas in colonized plants, 45% increment was noticed in colonized plants (Table 3).

Accumulation of proline

Proline accumulated significantly when the concentration of mannitol was increased in both colonized and non-colonized plants. Proline accumulation was not significantly influenced by the endophyte up to 2% mannitol stress, whereas the fungus played a vital role at 3 and 5% concentration levels by enhancing the accumulation of the same by 42 and 59% respectively compared to the control.

DISCUSSION

Endophytic fungi are well known to contribute to better plant performance and impart the plants with ability to tolerate biotic and abiotic stresses, including drought (Rodriguez and Redman 2008). Several mechanisms have been proposed to explain drought tolerance of plants colonized by endophytes. Photosynthesis stimulation (Ghabooli *et al.* 2013), osmo-regulation and stomatal regulation, increases in antioxidant levels (Sun *et al*. 2010) and up regulation of stress related plant genes (Sherameti *et al.* 2005 Oelmüller *et al.* 2009, Ghabooli *et al.* 2013) were involved in stress tolerance mediated by endophytes.

In the present study, both *P. indica* colonized and non-colonized plants experienced a significant decline in performance as water stress increased. The reduction in plant growth due to water stress has been well documented in previous studies of various crop plants, including tomato (Kıran *et al.* 2014, Alp and

Table 3. Influence of *P. indica* colonization and water stress on antioxidant enzymes viz. peroxidase, SOD and catalase activities. (D- Mean under different mannitol concentration, P- Mean under different *P. indica* treatments, Different letters within the same row represent significant differences).

Treatments	Peroxidase (unit/min/g)			SOD (unit/min/mg)			Catalase (unit/min/g)		
	-*P. indica*	+*P. indica*	Mean (D)	-*P. indica*	+*P. indica*	Mean (D)	-*P. indica*	+*P. indica*	Mean (D)
Control	20.6	20.5	20.5^{D}	206.9	216.9	211.9^{E}	263.2	248.7	255.9^{E}
1% Mannitol	24.2	25.1	24.7^{C}	302.6	327.3	314.9^{D}	260.9	241.0	251.0^{D}
2% Mannitol	27.6	34.1	30.9^{B}	347.7	403.7	375.7^{C}	250.2	271.2	260.7^{C}
3% Mannitol	33.2	36.7	34.9^{A}	427.0	542.4	484.7^{B}	322.0	295.4	308.7^{B}
5% Mannitol	35.2	37.7	36.4^{A}	620.8	660.5	640.6^{A}	346.8	360.4	353.6^{A}
Mean (P)	28.2^{B}	30.8^{A}		381.0^{B}	430.2^{A}		288.6^{A}	283.3^{B}	
CD (D×P)		2.60			9.95			10.91	

Kabay 2017). The effect of mannitol-mediated water stress on growth parameters has also been reported in broccoli sprouts (Kiani *et al*. 2018). Several factors, such as the unavailability of soil water, impaired mineral uptake and further reduction in water uptake, may be responsible for the decrease in plant growth under water-stressed conditions (Jatav *et al.* 2014).

The present study demonstrated that colonization of tomato seedlings with *P. indica* had a significant positive effect on shoot length, root length and number of rootlets under *in vitro* conditions, regardless of the level of water stress. The root length and number of rootlets were also positively affected by colonization at different levels of water stress. Furthermore, the fresh weights of the shoots and roots increased by 45% and 70%, respectively, compared to the control group. These findings are consistent with previous studies, such as Abdelaziz *et al.* (2019), who reported improved growth performance of *P. indica*-colonized tomato plants. Other studies have also reported that *P. indica* can increase the biomass of colonized plants by up to 100% (Waller *et al.* 2005) or 20% (Peskan-Berghöfer *et al.* 2004).

Ghorbani *et al.* (2018) reported that *P. indica* can mediate stress alleviation and improve plant growth in tomato. In the present study, increasing levels of mannitol stress from 0 to 5% resulted in a 22% reduction in fresh weight for *in vitro* grown seedlings. However, *P. indica*-colonized plants exhibited an 18% increase in fresh weight over non-colonized plants at 4% mannitol stress, and a 10% increase at 5% mannitol stress. Similar growth promotion activities of *P. indica* have been reported previously in other studies, such as Fakhro *et al.* (2010), Jogawat *et al.* (2013) and Abdelaziz *et al.* (2019).

In this study, it was found that *P. indica* colonization led to a significant increase in the number of rootlets in tomato seedlings grown under *in vitro* conditions, with an 88% increase observed at 3% mannitol stress and a 47% increase at 5% mannitol stress when compared to non-colonized plants. Additionally, colonized plants exhibited 34% more roots than the control group when grown under *in vivo* conditions. The fresh weights of both the shoot and root were also improved by 45% and 70%, respectively, in colonized plants under *in vivo* conditions. These findings are consistent with previous research, such as Xu *et al.* (2017), who reported enhanced shoot and root biomass in *P. indica*-colonized maize plants.

Under unfavorable conditions, plants often produce reactive oxygen species (ROS) and free radicals that can damage plant cells. To counteract this, plants have developed defense mechanisms to increase stress tolerance (Tuteja 2007). In this study, it was observed that antioxidant activities such as PO, SOD, and catalase were not significantly different between colonized and non-colonized tomato plants under normal conditions. However, under stressed conditions, colonization by *P. indica* significantly enhanced antioxidant activities, with PO activity increasing by 6%, SOD activity by 10%, and catalase activity by 4% compared to non-colonized plants at the highest level of mannitol stress (i.e. 5%). These elevated antioxidant levels may have contributed to the better survival of *P. indica*-colonized tomato seedlings under *in vitro* and *in vivo* conditions, even at the highest levels of stress induced by mannitol (Hosseini *et al.* 2017). A similar increase in antioxidant activities under stressed conditions was also documented by Abdelaziz *et al.* (2019) in tomato plants colonized by *P. indica*, although catalase activity was not significantly affected by colonization.

Accumulation of proline is a well-known response to a broad range of stress conditions such as water stress, salinity, high temperatures and elevated light intensity. In this study, it was observed that *P. indica*-colonized tomato plants had a significantly higher accumulation of proline under higher levels of mannitol stress. This finding suggests that the accumulation of proline may play a role in enhancing water tolerance in *P. indica*-colonized tomato plants. Similar findings were reported in *P. indica*-colonized plants of maize (Xu *et al.* 2017) and fin rice (Saddique *et al.* 2018), where higher accumulation of proline was also observed. *P. indica* colonization has the potential to positively impact plant growth, even under stress conditions, by increasing the number of roots and rootlets, as well as enhancing shoot and root fresh weights. These findings may have important implications for the development of sustainable and eco-friendly agricultural practices. The results

of this study suggest that *P. indica* colonization can positively impact the growth of tomato seedlings under *in vitro* conditions, even in the presence of water stress. These findings support previous research demonstrating the growth-promoting effects of *P. indica* colonization in plants.

CONCLUSION

The results showed that *P. indica* colonization had a positive effect on various growth parameters, including shoot and root length, fresh and dry weight, and the number of rootlets, under stressed *in vitro* conditions compared to the control. Similarly, *in vivo* experiments demonstrated that *P. indica*-colonized seedlings had improved performance in terms of stem and root length, as well as fresh and dry weight. *P. indica* colonization resulted in enhanced activities of antioxidant enzymes, such as peroxidase and superoxide dismutase as well as increased proline accumulation, compared to non-colonized plants. These findings suggest that *P. indica* colonization can enhance drought tolerance in tomato plants by improving growth and activating stress-related biochemical pathways.

REFERENCES

Abdelaziz ME, Abdelsattar M, Abdeldaym EA, Atia MA, Mahmoud AWM, Saad MM, Hirt H (2019) *Piriformospora indica* alters Na+/K+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Scientia Horticulture* 256 : 108532. 10.1016/j.*scienta*.2019.05.059.

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Pl Physiol Biochem* 80 : 160—167.

Alp Y, Kabay T (2017) The effect of drought stress on plant development in some landraces and commercial tomato genotypes. *Yüzüncü Yil Üniversitesi J Agricult Sci* 27(3) : 387—395.

Bouman BAM (2007) A conceptual framework for the improvement of crop water productivity at different spatial scales. *Agricult Syst* 93 (1-3) : 43—60.

Chaudhary J, Alisha A, Bhatt V, Chandanshive S, Kumar N, Mir Z, Deshmukh R (2019a) Mutation breeding in tomato: Ad vances, applicability and challenges. *Plants* 8(5) : 128.

Chaudhary J, Khatri P, Singla P, Kumawat S, Kumari ARV, Vikram A, Jindal SK, Kardile H, Kumar R, Sonah H, Deshmukh R (2019b) Advances in omics approaches for abiotic stress tolerance in tomato. Biology 8 (4) : 90. https://doi.org/10.3390/biology8040090.

Fakhro A, Andrade-Linares DR, von Bargen S, Bandte M, Büttner C, Grosch R, Franken P (2010) Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* 20 (3) : 191—200.

Ghabooli M, Khatabi B, Ahmadi FS, Sepehri M, Mirzaei M, Amirkhani A, Salekdeh GH (2013) Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley. *J Proteomics* 94 : 289—301.

Ghobadi M, Taherabadi S, Ghobadi ME, Mohammadi GR, Jalali-Honarmand S (2013) Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. *Industrial Crops Prod* 50 : 29—38.

Ghorbani A, Razavi SM, Omran VG, Pirdashti H (2018) *Piriformospora indica* alleviates salinity by boosting redox poise and antioxidative potential of tomato. *Russian J Pl Physiol* 65 (6) : 898—907.

Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, Ansari AA, Johri AK, Prasad R, Pereira E, Varma A (2016) *Piriformospora indica*: Potential and significance in plant stress tolerance. Front Microbiol 7: 332.

Hashem A, Abd_Allah EF, Alqarawi AA, Al-Huqail AA, Wirth S, Egamberdieva D (2016) The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. *Front Microbiol* 7 : 1089.

Hosseini F, Mosaddeghi MR, Dexter AR (2017) Effect of the fungus *Piriformospora indica* on physiological characteristics and root morphology of wheat under combined drought and mechanical stresses. *Pl Physiol Biochem* 118 : 107—120.

Jatav KS, Agarwal RM, Tomar NS, Tyagi SR (2014) Nitrogen metabolism, growth and yield responses of wheat (*Triticum aestivum* L.) to restricted water supply and varying potassium treatments. *J Ind Bot Soc* 93(3 and 4) : 177—189.

Jogawat A, Saha S, Bakshi M, Dayaman V, Kumar M, Dua M, Johri AK (2013) *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Pl Signal Behavior* 8(10) : e26891.

Johnson JM, Alex T, Oelmüller R (2014) *Piriformospora indica*: The versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. *J Trop Agric* 52 (2) : 103—122.

Kakker P, Das B, Viswanathan NP (1984) A modified spectrometric assay of superoxide dismutase. *Ind J Biochem Biophysics* 21: 130—132.

Kiani S, Babaeianjelodar N, Bagheri N, Najafizarrini H (2018) Effect of mannitol stress on morphological, biochemical and polyphenol parameters in broccoli sprouts (*Brassica oleracea* var. *italica*). *Appl Ecol Environm Res* 16 (2):In press.

Kıran S, Özkay F, Kuşvuran Ş, Ellİaltıoğlu ŞŞ (2014) Changes on some characteristics of tomato genotypes in different tolerance levels for salt stress under drought stress conditions. *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi* 31(3) : 41—48.

Lawlor DW (2013) Genetic engineering to improve plant performance under drought: Physiological evaluation of achievements, limitations and possibilities. *J Experim Bot*

64(1) : 83—108.

Luck H (1974) In: Methods in enzymatic analysis 2 (Ed Bergmeyer) Academic Press, New York, pp 885.

Oelmüller R, Sherameti I, Tripathi S, Varma A (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis* 49 (1) : 1—17.

Osakabe Y, Osakabe K, Shinozaki K, Tran LSP (2014) Response of plants to water stress. *Front Pl Sci* 5 : 86.

Peškan-Berghöfer T, Shahollari B, Giong PH, Hehl S, Markert C, Blanke V, Oelmüller R (2004) Association of *Piriformoformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. *Physiol Plantarum* 122 (4) : 465—477.

Rao DE, Chaitanya KV (2016) Photosynthesis and antioxidative defense mechanisms in deciphering drought stress tolerance of crop plants. *Biol Plantarum* 60 (2) : 201—218.

Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, Redman RS (2008) Stress tolerance in plants via habitat-adapted symbiosis. *The ISME J* 2(4) : 404—416.

Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *J Experim Bot* 59 (5) : 1109—1114.

Saddique MAB, Ali Z, Khan AS, Rana IA, Shamsi IH 2018) Inoculation with the endophyte *Piriformospora indica* significantly affects mechanisms involved in osmotic stress in rice. *Rice* 11(1) : 1—12.

Sherameti I, Shahollari B, Venus Y, Altschmied L, Varma A, Oelmüller R (2005) The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their pro moters. *J Biol Chem* 280 (28) : 26241—26247.

Sun C, Johnson JM, Cai D, Sherameti I, Oelmüller R, Lou B (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J Pl Physiol* 167(12) : 1009—1017.

Srivastava SK (1987) Peroxidasae and polyphenol oxidase in *Brassica juncea* plants infected with *Macrophomina phaseolina* (Tassai) and their implications in diseases resistance. *Phytopathol* 120 : 249—254.

Tuteja N (2007) Mechanisms of high salinity tolerance in plants. *Methods Enzymology* 428 : 419—438.

Varma A, Verma S, Sudha S, Sahay N, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant growth promoting root endophyte. *Appl Environ Microbiol* 65 : 2741—2744.

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M amp, Kogel KH (2005) The endophytic fungus *Piriformospora indica* reprograms barley to saltstresstolerance, disease resistance, and higher yield. Proc *Nat Acad Sci* 102 (38) : 13386—13391.

Xu L, Wang A, Wang J, Wei Q, Zhang W (2017) *Piriformospora indica* confers drought tolerance on *Zea mays* L. through. enhancement of antioxidant activity and expression of drought-related genes. *The Crop J* 5(3): 251—258.