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Piriformospora indica Improves Drought Tolerance in Tomato Plants through Enhanced Nutrient Uptake and Antioxidant Enzymes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Piriformospora indica, a beneficial root endophytic basidiomyceteous fungus, has demonstrated the ability to enhance plant growth and increase tolerance to stressful conditions, particularly drought and salinity. This study aimed to examine the effectiveness of *P. indica* in mitigating the negative effects of drought stress in tomato plants. In a greenhouse pot experiment, tomato plants colonized with *P. indica* (P1) and non-inoculated with *P. indica* (P_2) were subjected to different water regimes, including well-watered conditions and varying levels of drought stress (D_1 - Control (100% Field Capacity - FC), D_2 - 75% FC, D_3 - 50% FC and D_4 - 25% FC). The study evaluated multiple parameters such as, yield per plant, anti-oxidant enzyme activities and nutrient uptake as influenced by *P. indica*-colonization and drought stress. The results revealed that as the drought stress increased, micro- and macro- nutrients uptake got reduced, leading to a reduction in yield. But this reduction was found to be less in *P. indica*-colonized plants compared to the control plants indicating the positive effect of colonization on nutrient uptake and yield per plant. Drought stress increased the accumulation of anti-oxidant enzymes and proline in tomato plants, which is a method of drought tolerance and mitigating mechanism. However, *P. indica*-colonization resulted in significantly higher anti-oxidants compared to control plants corresponding to same level of drought stress indicating the role of the endophyte in drought tolerance in tomato plants through improved antioxidant enzyme activities and proline content.

Keywords: Piriformospora indica; tomato; drought; nutrient uptake.

1. INTRODUCTION

Drought stress is a complex phenomenon that triggers various morpho-physiological, biochemical, and molecular alterations in plants, ultimately impeding their growth and development [1]. At the cellular level, this stress disrupts crucial processes, such as water homeostasis, metabolic functions, and hormonal balance. Moreover, it adversely affects chlorophyll synthesis, root differentiation, foliage growth, stomatal regulation, as well as water and mineral uptake, resulting in reduced plant yield and water use efficiency [2]. Additionally, drought induces the production of reactive oxygen species (ROS), leading to oxidative damage and disturbance in cellular redox regulation [3].

Plants have developed strategies to cope with water scarcity, aiming to increase water absorption from the soil and minimize water loss through transpiration [4]. Morphological adaptations, such as larger root size for enhanced soil exploration and increased surface absorption, can occur [5]. In response to drought, stomatal closure reduces transpiration but also hampers CO_2 diffusion and photosynthetic carbon assimilation [6]. Proline production, as a compatible organic solute, plays a crucial role in osmotic adjustment, ROS detoxification, and stabilization of cellular structures [4]. Additionally, plants activate antioxidant defense systems to counteract oxidative stress under drought, employing enzymes like catalase, ascorbate peroxidase, and dehydro-ascorbate reductase to scavenge reactive oxygen species and regenerate ascorbate [7].

Tomato (*Solanum lycopersicum* L.) is a globally significant horticultural crop. However, its susceptibility to water deficit has led to extensive efforts in developing drought-resistant tomato varieties. The plant microbiome plays a crucial role in enhancing plant growth and stress tolerance, with potential applications in crop production [8].

Beneficial interactions between plants and soil microorganisms, such as endophytic fungi, can mitigate the harmful impacts of environmental stresses and enhance plants' ability to withstand adverse conditions [9]. *Piriformospora indica*, an endophytic basidiomycete, has the capacity to colonize the roots of various plant species and has been reported to enhance plant tolerance to challenging conditions, including salinity stress [10]. Studies have demonstrated that inoculating plants with *P. indica* can enhance nutrient absorption, modify plant metabolites, and promote plant growth [11,12]. Various studies have indicated that mycorrhizal fungi play a role in modulating the expression of aquaporins, which are involved in water transport, under drought [13] and salinity [14] stress. According to a study by Abdelaziz et al. [15], *Arabidopsis* seedlings that were inoculated with *P. indica* demonstrated a noteworthy reduction in Na^+ uptake and an increase in K^+ concentration compared to non-inoculated plants. Enhanced nutrient uptake and growth promotion by *P.*

indica colonization was also reported in rice [16]. Drought and salinity stress share common characteristics in terms of their impact on plants. Both conditions trigger osmotic stress and oxidative damage at an early stage, resulting in reduced growth, stomatal closure, and nutrient deficiencies [17] Hence, plants may employ similar mechanisms to adapt to both drought and salinity stresses. These mechanisms include growth inhibition, accumulation of compatible solutes like proline, heightened levels of antioxidants and protective proteins, suppression of energy-intensive pathways, and regulation of gene expression [18].

Therefore, the primary aim of this study was to assess the effectiveness of *P. indica* in enhancing the growth and drought tolerance of tomato plants. To achieve this goal, various physiological and biochemical parameters such as chlorophyll content, nutrient uptake, proline levels, antioxidant enzyme activities, and antioxidant capacity were examined in the tomato plants.

2. MATERIALS AND METHODS

2.1 Maintenance of the Fungal Root Endophyte *P. indica*

The *P. indica* culture obtained from the Department of Plant Pathology, College of Agriculture, Vellayani, Kerala, India, was maintained on potato dextrose agar (PDA) medium. The fungus was regularly propagated by transferring a fungal disc from the actively growing edge of a two-week-old *P. indica* culture onto Petri plates containing PDA or conical flasks containing potato dextrose broth (PDB). The cultures were then incubated in the dark at room temperature. Subculturing was performed every fifteen days to maintain the fungus.

2.2 Co-Cultivation of *P. indica* with Tomato var. Vellayani Vijai in Potting Mixture

P. indica was cultured in sterilized potting mixture by following the method developed by Jojy et al. [19]. The potting mixtures containing *P. indica* and those without it were transferred to separate protrays after being sterilized. The seeds of tomato variety Vellayani Vijai were also sterilized with a 0.1% solution of mercuric chloride for 10 seconds and then washed twice with sterile water. These seeds were then planted firmly in the protrays and kept in a dark room for 2 days to promote the strength and vitality of the growing seedlings. In the following days, the protrays with the seedlings were kept under controlled conditions of temperature and humidity to ensure consistent germination and growth. They became ready for transplantation after 25 days.

To investigate the impact of *P. indica* inoculation on tomato plants under drought stress, a bioassay with two factors was conducted: *P. indica* inoculation (inoculated or uninoculated plants) and Drought induced by limiting irrigation (D_1-Control (100% Field Capacity (FC), D_2-75% FC, D_3-50% FC, D_4-25% FC). 45 seedlings (inoculated and non-inoculated) were individually transplanted into pots filled with 4.5 kg soil. Root colonization by *P. indica* was confirmed by observing root bits under microscope (Leica - ICC50 HD, USA) before transplanting and 15 days after transplanting.

2.3 Gravimetric Method for Simulating Drought Stress

In the gravimetric method [20] for simulating drought stress, each pot was filled with 4500 g of soil, while an additional 4500 g of soil was dried in an oven for 7 days to obtain the "dry weight" (WD). During the initial two weeks after transplanting, all plants were subjected to a well-watered condition for acclimatization. Following this period, four watering treatments were implemented for duration of two weeks: D_1-Control (100% FC), D_2-75% FC, D_3-50% FC, D_4-25% FC. In the well-watered regime, plants were irrigated with water equivalent to 100% of the soil's water holding capacity. In the drought stress treatment, plants were irrigated with water equivalent to only 75% FC,50% FC, and 25% FC of the soil's water holding capacity. The pots with tomato plants were weighed daily and watered to reach 100%, 75%, 50% and 25% of field capacity. To determine the soil water holding capacity or field capacity, an extra pot filled with 4500 g of soil was saturated with water, drained until reaching a constant weight, and recorded as the "wet weight" (WW). The pots' weights were assessed daily to maintain the relative soil water content (RSWC), with water replenished if any pot weight fell below the target weight (10 g threshold). Additionally, the total plant fresh weight (WP) was estimated by determining shoot and root weights of two extra pots (Figure 1). The target weight (WT) for each treatment (pot) was calculated by using equation.

$$W\,T = WD \times WP \times RSWC \times (WW - WD)$$

(WD - Oven dry weight of soil, WP - total plant fresh weight, RSWC - Relative soil water content, WW- Wet weight (weight of water saturated soil +pot))

2.4 Assessment of Yield per Plant

Yield per plant was taken and used to compare the differences between the yield from colonized by *P. indica* and non-colonized seedling under different drought conditions and control.

The activities of antioxidant enzymes, including peroxidase (PO), catalase, and superoxide dismutase (SOD), were assessed in tomato plants grown under different drought conditions. The activity of peroxidase was determined using a protocol described by Srivastava [21], while catalase activity was measured following the procedure outlined by Luck [22]. The superoxide dismutase (SOD) activity was carried out using the method described by Kakker et al. [23].

2.5 Nutrient Uptake

The plant samples were subjected to mineral element analysis, and the concentrations of various elements including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), iron (Fe), manganize (Mn), Copper (Cu), sulfur (S), and zinc (Zn) were determined. The analysis was performed in three replicates to ensure accuracy and reliability of the results. The plant samples were first ground into a fine powder and prepared for nutrient analysis. To determine the nutrient contents, the samples were digested using a di-acid mixture of HNO_3:$HClO_4$ (10:4). The digested samples were then analyzed for the concentrations of Zn, Fe, Mn, Cu, using an ICP-MS. Phosphorus, potassium, calcium and sulfur were determined using specific methods. Phosphorus was determined using the vanadomolybdo phosphoric yellow color method. Potassium and calcium levels were analyzed using a flame photometer following the procedure described by Jackson [24]. Sulfur content was measured using the turbidimetric method developed by Chesnin and Yien [25]. The nitrogen content in the plant samples was determined using the Kjeldahl method as outlined by Jackson [24]. To calculate the nutrient uptake by the tomato crop, the nutrient content values were multiplied by the corresponding yield.

2.6 Statistical Analysis

The experiment followed a factorial design within a completely randomized framework. It was replicated four times, and each calculated mean was based on four independent replicates. Statistical analysis was conducted using KAU-GRAPES online and mean comparisons were performed using a least significant difference (LSD) test with a significance level of $P < 0.05$.

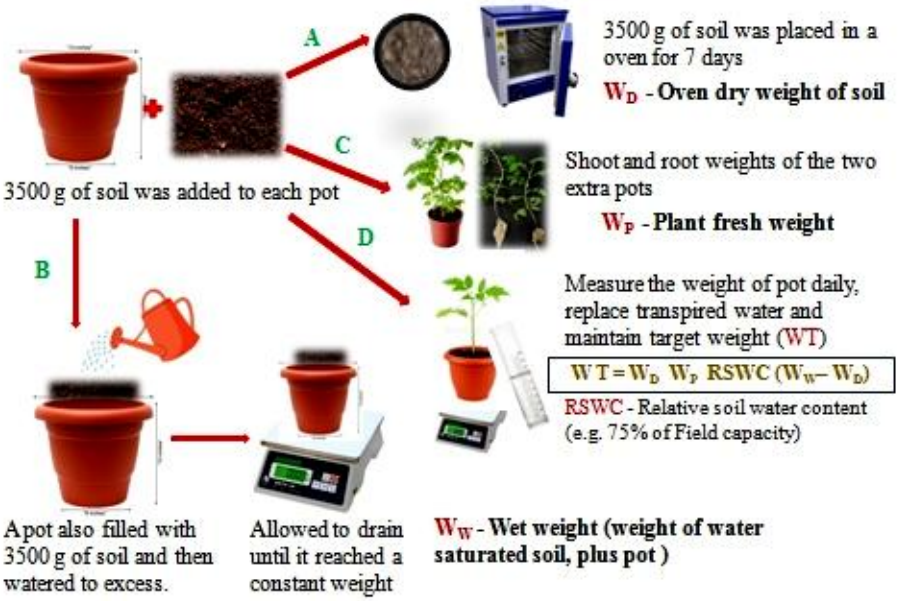

Fig. 1. Gravimetric method for creating drought stress

3. RESULTS AND DISCUSSION

3.1 Detection of *P. indica* in Inoculated Plant Root

Root colonization by *P.indica* was confirmed by observing root bits under microscope (Leica - ICC50 HD, USA) before transplanting as well as 15 days after transplanting (Fig. 2)

3.2 Yield per Plant

Colonizing tomato plants with *P. indica* (P_1) led to a significantly higher yield per plant compared to non-colonized tomato plants (P_2) which had a lower yield. Among the drought stress treatments, the control group (D_1) with regular irrigation had the highest yield (565.2g). As the level of drought stress increased, the yield decreased to 493.1g at 75%FC, 254.0g at 50% FC, and 129.6g at 25% FC, respectively. Regarding the treatment combinations, the group with *P. indica* colonization under 100% FC (P_1 D_1) had the highest yield (582.1g), while the group without *P. indica* colonization and subjected to the most severe drought stress, i.e., 25% FC (P_2 D_4) had the lowest yield (69.68g). The results suggest that *P. indica* colonization positively influences tomato plant growth and yield, and drought stress negatively affects plant productivity, with the severity of impact increasing with the reduction in irrigation levels (Fig. 3).

Colonizing tomato plants with *P. indica* resulted in higher yields compared to non-colonized plants. The control group with regular irrigation had the highest yield, while increased drought stress levels led to decreased yields. The results indicate that *P. indica* positively influences tomato plant growth and yield, while drought stress has a negative impact on plant productivity, with greater severity of impact as irrigation levels decrease (Fig. 3). The increased yield by the inoculation with *P. indica* was also reported in rice [26], maize [27], and in millets [28] under water stress. Recent studies have reported that *P. indica* exhibits promising potential for enhancing crop yields through improved uptake of phosphorus (P) and other essential nutrients [29].

3.3 Effect of *P. indica* on Anti-Oxidant Activity

The superoxide dismutase (SOD) activity in tomato plants was compared between different treatments, including *P. indica* colonization and varying levels of drought stress. In D_1 (control conditions), the SOD activity was 218.23mg g^{-1} fw for colonized plants (P_1) and only 210.64 mg g^{-1} fw in non-colonized plants (P_2). As the severity of drought stress increased, the SOD activity also increased. At 75% field capacity (D_2), the SOD activity was 247.33mg g^{-1} fw for colonized plants (P_1) and 233.61 mg g^{-1} fw for non-colonized plants (P_2). Further elevation in SOD activity was observed as the drought stress increased to 50% field capacity, i.e., 279.32mg g^{-1} fw for colonized plants (P_1) and 244.37mg g^{-1} fw for non-colonized plants (P_2). The highest SOD activity was recorded at D_4 (25% field capacity) indicating a substantial impact of severe drought stress on the activation of SOD in both *P. indica*-colonized and non-colonized tomato plants. From the table it is clear that the combination of colonized plants (P_1) with different levels of drought had a higher SOD activity than the corresponding combinations with non-colonized plants (P_2) indicating the role of *P. indica* on enhancing the SOD activity (Table 1).

Table 1. Influence of *P. indica* on anti-oxidant activities in tomato var. Vellayani Vijai under drought condition

Treatments	SOD (mg g^{-1}fw)	PO (min $^{-1}$ g $^{-1}$ fw)	CAT (units min^{-1} g^{-1} fw)
P_1 D_1 (+*P. indica*, 100%FC)	218.23±2.4	24.73±0.9	252.44±6.1
P_1 D_2 (+*P. indica*, 75%FC)	247.33±4.3	28.50±1.4	268.53±7.6
P_1 D_3 (+*P. indica*, 50%FC)	279.32±3.5	33.12±3.7	322.23±7.5
P_1 D_4 (+*P. indica*, 25%FC)	295.38±4.5	37.23±0.5	468.67±7.7
P_2 D_1 (-*P. indica*, 100%FC)	210.64±2.1	23.75±1.7	181.59±5.9
P_2 D_2 (-*P. indica*, 75%FC)	233.61±5.7	26.61±1.1	200.46±3.8
P_2 D_3 (-*P. indica*, 50%FC)	244.37±5.8	29.69±1.0	252.89±3.7
P_2 D_4 (-*P. indica*, 25%FC)	245.97±5.9	30.35±0.9	288.91±5.9
C.D	9.59	2.97	14.99
SEm(±)	3.17	0.98	4.96

(P_1 - P. indica-colonized tomato; P_2 - Non-colonized tomato; D_1-Control; D_2-75% FC; D_3-50% FC; D_4-25% FC)

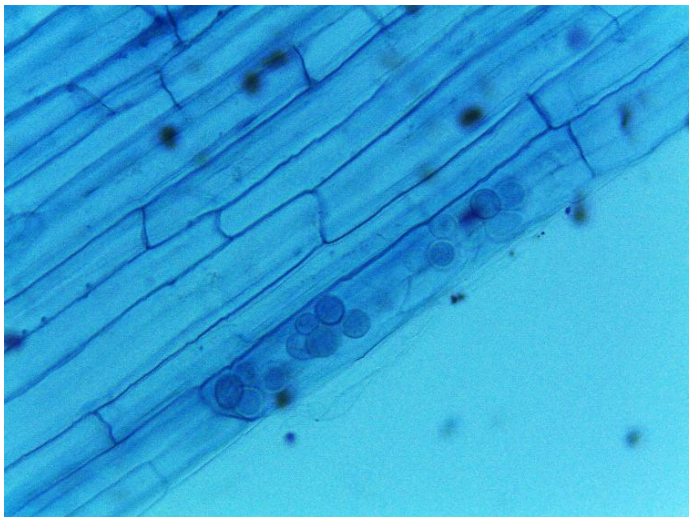

Fig. 2. Root colonization of *P. indica* in tomato @15 days after co-cultivation

The effect of *P. indica* colonization and drought stress on peroxidase activity (PO) in tomato plants are represented in Table 1. The results revealed that PO activity varied across the treatment combinations. PO activity in *P. indica*-colonized plants exposed to different water stress conditions was 24.73 min $^{-1}$ g $^{-1}$ fw at 100% FC, 28.50 min $^{-1}$ g $^{-1}$ fw at 75% FC, 33.12 min $^{-1}$ g $^{-1}$ fw at 50% FC and 37.23min $^{-1}$ g $^{-1}$ fw at 25% FC. The combination of *P. indica* colonized plants with different levels of field capacities had significantly higher PO activities than the corresponding combinations with non-colonized plants. This suggests that *P. indica* colonization may have a positive effect on peroxidase activity under drought stress conditions. Furthermore, the PO activity increased gradually as the drought stress levels intensified irrespective of colonization with or without *P. indica* (P1 or P2). This indicates that higher levels of drought stress may lead to an increase in peroxidase activity, and the presence of *P. indica* colonization might further enhance this response.

The results showed significant variations in CAT activity across different treatments (Table 1). For *P. indica* colonized plants (P_1) under different levels of drought stress, the CAT activity was significantly higher compared to non-colonized plants (P_2) indicating the positive effect of *P. indica* colonization on CAT activity. Under control conditions (D1), *P. indica* colonized plants (P_1) had a CAT activity of 252.44 units min^{-1} g^{-1}fw, whereas non-colonized plants (P_2) had a lower CAT activity of only 181.59units min^{-1} g^{-1}fw. In both colonized plants (P_1) and non-colonized plants (P_2), the CAT activity increased with increasing severity of drought stress. As the drought level increased to 25% FC (D_4), the CAT activity increased drastically in both colonized plants (P_1) and non-colonized plants (P_2). In colonized plants (P_1), the CAT activity increased from 252.44 units min^{-1} g^{-1}fw at 100% FC (D_1) to 468.67 units min^{-1} g^{-1}fw at 25% FC (D_4). Similarly, in non-colonized plants (P_2), the CAT activity increased from 181.59units min^{-1} g^{-1}fw at 100% FC (D_1) to 288.91 units min^{-1} g^{-1}fw at 25% FC (D_4).

In the present study, the anti-oxidant enzymes such as, SOD, PO and CAT showed a significantly higher activity in *P. indica* colonized plants compared to the non-colonized plants. As the stress level reaches 25%FC (D4), SOD was increased by 20%, PO by 22% and CAT activity by 62% in colonized plants as compared to the control plants (non-colonized).

Anti-oxidants play a vital role in safeguarding plants. When exposed to different environmental stressors, either individually or in combination, plants produce an excessive amount of reactive oxygen species (ROS). This surplus leads to oxidative stress and disrupts the balance of redox homeostasis [30]. However, an effective interplay between ROS production and the antioxidant defense system is crucial for shielding the photosynthetic apparatus, preserving the integrity of membranes, and averting harm to nucleic acids and proteins. It is worth mentioning that the antioxidant defense system not only eliminates ROS but also controls their levels for signaling purposes [31]. *P. indica* colonization in plants has been linked to enhanced levels of various antioxidant enzymes,

including catalase, ascorbate peroxidase, dehydro ascorbate reductase, monodehy droascorbate reductase, and glutathione reductase, providing protection against salt stress [32]. In maize plants, *P. indica* colonization was found to protect against the root pathogen *F. verticilloides* by increasing the activity of antioxidant enzymes, such as catalase, glutathione reductase, glutathione S-transferase, and superoxide dismutase. Colonized plants exhibited 23-fold, 3.8-fold, and 1.7-fold increases in the activity of CAT, GST, and SOD, respectively, compared to uncolonized diseased plants [33]. Chinese cabbage plants colonized by *P. indica* showed an improvement in photosynthetic efficiency under drought stress, attributed to the prevention of chlorophyll and thylakoid protein degradation. This effect was accompanied by increased activities of peroxidase, catalase, and superoxide dismutase [34]. *P. indica* induced systemic resistance against rice sheath blight by reducing hydrogen peroxide levels and increasing the activity of superoxide dismutase [35]. In Medicago truncatula plants subjected to salt stress, *P. indica* priming led to a 44% increase in peroxidase activity and a 38% increase in catalase activity [36]. *P. indica* priming in cowpea plants resulted in increased activity of defense enzymes, such as peroxidase and polyphenol oxidase, providing protection against Black eye cowpea mosaic virus [37]. Additionally, in rice seedlings exposed to water stress, the activity of catalase was upregulated upon inoculation with *P. indica* [26].

3.4 Effect of *P. indica* on Nutrient Uptake

The impact of different levels of drought stress on nutrient uptake in both *P. indica*-colonized and non-colonized plants was examined. It was observed that the imposing drought stress led to a decrease in nutrient concentration in leaves of the plants. However, the percentage reduction in macro nutrient concentration was found to be higher in non-colonized plants compared to *P. indica*-colonized plants (Table 2 and 3). Both *P. indica* colonization and drought treatment had a significant effect on the content of P, K, Ca, Mg, S, Mn, Zn and Cu (Table 2 and 3). With respect to Macronutrients (P, K, Ca), the treatment combination of*P. indica* with 25% FC was statistically equivalent to control plants without colonization under 100% FC (P_2D_1). The highest concentration was observed in colonized plants under 100% FC (P_1D_1) for elements P (0.29 g/Kg), K (3.64 g/Kg), Ca (2.74 g/Kg), Mg (0.83 g/Kg), and S (0.59 g/Kg). Colonized plants under 100% FC exhibited 20.8% more uptake of Phosphorus, 9.3% more uptake of Potassium, and 5.8% more uptake of Calcium compared to control plants (P_2D_1) Significantly higher concentrations of the micronutrients such as Manganese, Zinc, and Copper were observed in the colonized plants under 100% FC compared to all other treatments. Specifically, the concentration of Manganese was found to be 361 g/kg, Zinc was 62 g/kg, and Copper was 21.4 g/kg in the colonized plants under 100% FC (P_1D_1). Under drought stress conditions, the concentration of P, K, Ca, Mg, S, Mn, Zn and Cu significantly increased in plants that were colonized with *P. indica* compared to non-colonized plants. *P. indica* colonization had a significant effect on the P and Ca concentration, resulting in an increase in inoculated plants regardless of the water stress. On the other hand, the total N and Mn content were not significantly affected by any of the factors or their interaction (Table 2 and 3).

Table 2. Effect of *P. indica and* drought on macro nutrient concentration (g/Kg DW) in leaves of tomato var. Vellayani Vijai

Treatments	N	P	K	Ca	Mg	S
P_1 D_1 (+*P. indica*, 100%FC)	3.73	0.29^{A}	3.64^{A}	2.74^{A}	0.83^{A}	0.59^{A}
P_1 D_2 (+*P. indica*, 75%FC)	3.67	0.27^{B}	3.63^{A}	2.73^{A}	0.81^{A}	0.55^{B}
P_1 D_3 (+*P. indica*, 50%FC)	3.63	0.27^{B}	3.48^{B}	2.64^{A}	0.76^{B}	0.46^{C}
P_1 D_4 (+*P. indica*, 25%FC)	3.6	0.24^{C}	3.30^{C}	2.51^{B}	0.62^{C}	0.40^{C}
P_2 D_1 (-*P. indica*, 100%FC)	3.22	0.24^{C}	3.33^{C}	2.59^{BC}	0.78^{C}	0.49^{D}
P_2 D_2 (-*P. indica*, 75%FC)	3.21	0.24^{D}	3.28^{CD}	2.54^{CD}	0.75^{D}	0.49^{D}
P_2 D_3 (-*P. indica*, 50%FC)	3.15	0.22^{E}	3.18^{D}	2.46^{D}	0.72^{D}	0.47^{E}
P_2 D_4 (-*P. indica*, 25%FC)	3.08	0.21^{F}	3.17^{D}	2.28^{E}	0.72^{E}	0.39^{E}
C.D	N/A	0.009	0.112	0.088	0.023	0.017
SEM(±)	0.027	0.003	0.037	0.029	0.008	0.006

(P_1 - P. indica-colonized tomato; P_2 - Non-colonized tomato; D_1-Control; D_2-75% FC; D_3-50% FC; D_4-25% FC; N-Nitrogen, P-Phosphorus, K-Potassium, Ca-Calcium, Mg-Magnesium, S-Sulfur)

Table 3. Effect of *P. indica and* drought on micro nutrient concentration (g/Kg DW) in leaves of tomato var. Vellayani Vijai

Treatments	Iron	Manganese	Zinc	Copper
P_1 D_1 (+*P. indica*, 100%FC)	387.0	361.0^{A}	62.0^{A}	21.4^{A}
P_1 D_2 (+*P. indica*, 75%FC)	370.1	220.0^{B}	59.9^{AB}	19.7^{B}
P_1 D_3 (+*P. indica*, 50%FC)	357.0	163.1^{C}	59.1^{B}	19.2^{C}
P_1 D_4 (+*P. indica*, 25%FC)	340.2	137.3^{C}	47.8^{C}	17.2^{C}
P_2 D_1 (-*P. indica*, 100%FC)	370.1	223.0^{C}	56.4^{CD}	20.6^{C}
P_2 D_2 (-*P. indica*, 75%FC)	366.2	232.0^{D}	55.3^{CD}	19.5^{D}
P_2 D_3 (-*P. indica*, 50%FC)	358.3	217.3^{E}	55.2^{D}	18.4^{D}
P_2 D_4 (-*P. indica*, 25%FC)	352.0	204.4^{F}	53.9^{E}	18.2^{E}
C.D	N/A	8.48	2.48	0.68
SEM(±)	5.291	2.805	0.823	0.227

(P_1 - P. indica-colonized tomato; P_2 - Non-colonized tomato; D_1-Control; D_2-75% FC; D_3-50% FC; D_4-25% FC)

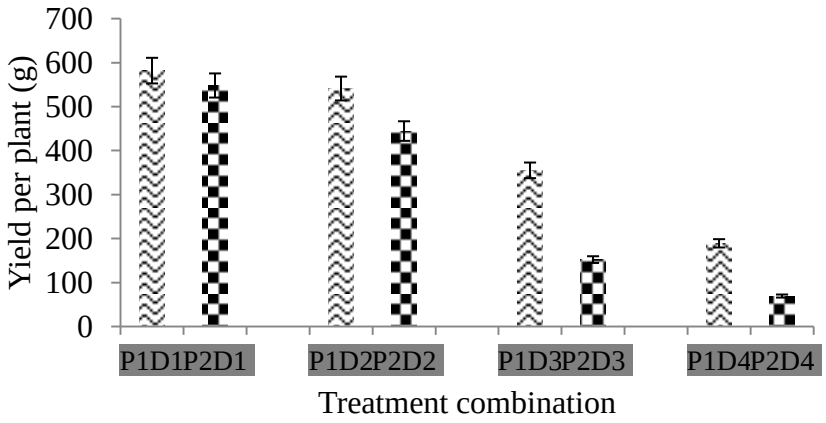

Fig. 3. Yield per plant as affected by colonization with *P. indica* and drought stress

The positive effects of fungal symbiosis on enhancing plant tolerance to drought stress are closely associated with improved nutrient absorption, which aligns with the findings of our study. Our results demonstrate that exposure to 25% FC treatment led to a reduction in the absorption of P, K, Ca, Mg, S, Mn, Zn and Cu content in the leaves of tomato plants compared to the control plants. However, when *P. indica* was inoculated, the absorption of N, P, and Ca decreased in comparison to non-inoculated plants under same treatment, although there was no significant difference in N and Mn content among the different inoculation treatments. Previous studies have also reported increased nutrient concentration facilitated by *P. indica* [38-40]. Phosphorus plays a crucial role in supporting plant growth and development, especially under unfavorable conditions. Adequate levels of P have been found to enhance salinity tolerance by promoting root growth into deeper soil regions and supplying an adequate supply of inorganic P for carbon assimilation [41]. *Piriformospora indica* contributes significantly to nutrient uptake through the action of an acid phosphatase located in the tips of its hyphae and the accumulation of organic acids [42,43]. The introduction of *P. indica* to tomato and Brassica nupus plants resulted in improved nutrient levels, as observed in studies conducted by Wang et al. [44] and Wu et al. [43]. Previous research conducted by Shahollari et al. [45] in Arabidopsis has shown that *P. indica* can enhance phosphorus absorption. Furthermore, *P. indica* inoculation has been reported to increase the accumulation of nitrogen (N) and calcium (Ca), which can mitigate the toxicity caused by salinity by regulating sodium (Na^+) uptake and maintaining optimal chlorophyll levels, thus promoting plant growth under saline conditions [46]. Research suggests that symbiotic fungi, such as *P. indica*, can enhance nutrient absorption in plant roots through changes in root architecture and increased root length facilitated by extra-radical hyphae. Notably, *P. indica* did not affect N uptake, and considering that N ions have lower toxicity compared to other ions [47], it can be inferred that the positive effects of *P. indica* on tomato growth are associated with an increased uptake phosphorus (P), potassium (K),

and calcium (Ca), or a reduced absorption of Na.

4. CONCLUSIONS

The colonization of tomato plants with *P. indica* increased plant yield by enhancing anti-oxidant activities and nutrient uptake, particularly under drought stress conditions. Drought stress negatively impacted plant productivity, with severe stress leading to decreased yields. However, *P. indica* colonization mitigated the effects of drought by improving antioxidant enzyme activity, such as SOD, PO, and CAT, and promoting nutrient absorption, including macro and micro nutrients. These findings suggest that *P. indica* colonization holds potential for enhancing crop growth, stress tolerance, and productivity, providing a promising avenue for sustainable agricultural practices in water-limited environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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