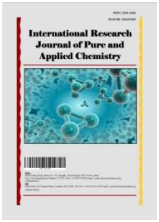

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Effect of *Trichoderma harzianum* and *Pseudomonas fluorescens* on the Enhancement of Drought Tolerance and Plant Growth in Tomato

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Authors' contributions

This work was carried out in collaboration among all authors. Author PA conducted research and performed the statistical analysis. Author RS designed the study and framed the protocol of experiments. Author HSV wrote the first draft of the manuscript and managed the analyses of the study. Authors AP and AKS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In the present study, different isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* were evaluated for the induction ofdrought tolerance and growth promotion in tomato plants. Four isolates of *Pseudomonas fluorescens* viz Pf 2 Pf 4, Pf 6 and Pf 28 and three isolates of *Trichoderma harzianum viz.* TH 08, IRRI 1 and TH 26 were evaluated by using seed biopriming as well as seedling/transplanted root dip method against drought stress and for their effect onplant growth promotion. Parametersincludingdrought tolerance capacity, water requirement, shoot length, root length, plant height, and chlorophyll content were recorded inall the treatments of different isolates along with the untreated control.Among the two methods of bioinoculation, seed biopriming was found more effective to transplanted root dip in all the treatments of isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens*. Among all the isolates, isolate IRRI 1 of *Trichoderma harzianum* was found highly effective in reducing the water stressbyincreasing thecumulative mean water stresstolerance duration of both methods of inoculation up to 14.83 days after the last

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irrigation with least mean water requirement of 13.92litres followed by isolate Pf28 of *Pseudomonas fluorescens* with mean water stress tolerance of 13.66 days and water requirement of 14.25 litres compared to the untreated control plants with least tolerance ofonly 10.66 days and highest water requirement of 16.25litres. Maximum cumulative mean shoot growth, root growth and chlorophyll content ofboth methods of inoculation of 76.25cm, 28.33cm and 41.20 SPAD units respectively were also recorded with IRRI 1 isolate followed by Pf 28 isolate with 67.17cm, 19cm, and 35.46 SPAD units respectively. Treatment with all other isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* also showed better water stress tolerance, less water consumption, increased shoot length, root length and chlorophyll content compared to the untreated control tomato plants.

Keywords: Trichoderma harzianum; Pseudomonas flourescens; seed biopriming; transplanted root dip; drought tolerance; water requirement; growth parameters.

ABBREVIATIONS

Pf - Pseudomonas fluorescens
TH - Trichoderma harzianum
SB - Seed biopriming
TR - Transplanted root dip
DAS - Days after sowing

1. INTRODUCTION

Drought is the major abiotic stress which has been increased over the past decades affecting world's food security. Its impact may range from moderate to severe form causing great loss to the crop yield [1]. It is expected to cause serious plant growth problems for more than 50% of the arable lands by 2050 [2].

Drought can directly induce a wide range of injury symptoms in plants, such as the inhibition of plant photosynthesis [3], increased oxidative stress [4] and changes in metabolism [5]. Furthermore this stress makes the plant to undergo a series of morphological and physiological adaptations. It influences the ratio of root and shoot biomass which is an important parameter of estimating drought resistance of plants [6,7,8]. There are currently several approaches that potentially reduce the impact of drought stress on crop production, such as cultivation of drought tolerant varieties, rain water harvesting, adopting agronomic practices like mulching, efficient irrigation systems like drip irrigation and sprinkler irrigation. However, none of these approaches are fully successful and needs to come up with a novel solution for crop management in reduced water supply situations.

One possibility to increase the drought tolerance is to use beneficial microorganisms as seed inoculants. Positive interactionsof these beneficial microorganisms like *Pseudomonas* spp., Arbuscular Mycorrhizal fungi and Trichoderma sp.etc with plants have been reported to stimulate drought tolerance [9]. The mechanisms of drought tolerance in plants through these beneficial organisms may include the production of phyto hormones like abscisic acid (ABA), gibberellic acid (GA), cytokinins(CK), indole-3-acetic acid (IAA),ACC deaminase which reduces the level of ethylene in the plant roots and induction of systemic tolerance to drought by different bacterial metabolites and exopolysaccharides. We therefore attempt to study the water stress alleviation potential of microbial species for their use in sustainable agriculture. So, our present study is focused upon screening different isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* against water stress *viz*. drought tolerant capacity and water requirement by inoculating them using seed biopriming and transplanted root dip methods in tomato plants. In addition to the water stress tolerance, effect of these isolates on plant growth promoting activities like increase in shoot length, root length and chlorophyll content in tomato plants will be studied which are indirectly responsible for drought stress tolerance.

2. MATERIALS AND METHODS

Our present study was carried out during 2018-2019 in pots arranged in the polyhouse at Department of Plant Pathology, SVPUAT, Meerut(U.P),India. The tomato (*Solanum lycopersicon*) variety "Pusa ruby"of IARI, New delhi which is an indeterminate and early maturing variety was selected for carrying our experiments.

2.1 Source and Maintenance of *T. harzianum* and *P. fluorescens* Cultures

Four isolates of *P. fluorescens* (Pf 2 Pf 4, Pf 6 and Pf 28), two isolates of *T. harzianum* (TH 08,

TH 26) which were isolated from different locations at Crop Research Centre (CRC) SVPUAT, Meerutand one isolate (IRRI-1) which was obtained from International Rice Research Institute (IRRI) were used in this experiment which were being maintained in the bio-control laboratory, Department of Plant Pathology, SVPUAT, Meerut. These isolates were mass multiplied in a conical flask containing *Sorghum*grains and later powdered by using grinding machine and used for seed bio-priming as well as in transplanted root dip method which were carried in separate experiments.The treatment details of seed biopriming as well as transplanted root dip methods are given below in the Tables 1 and 2. Total eight treatments with three replications each, including untreated (control) were maintained in both experiments of seed bio-priming as well as transplanted root dip.

2.2 Seed Bio-priming and Transplanted Root Dip

Seeds of tomato (*Solanum lycopersicon*) were surface sterilized with 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and dried. The dried tomato seeds were primed by hydrating thin film of water and taken in the Petriplates which were treated with the 1g of *P. fluorescens* or *T. harzianum* for 30 seeds based on the treatments mentioned in the Tables 1 and 2. Seeds soaked in distilled water were served as control (Fig. 1a). Initially ten seeds were sown and later only one well established seedling is maintained per each pot of a replication by thinning out other seedlings.

In case of transplanted root dip, tomato seedlings were raised separately in the pro tray nursery. Twelve day old seedlings were uprooted from the nursery and kept it in a beaker containing suspension of biocontrol agents@ 10gm/lit for about 2hrsbefore transplanting and three seedlings per each potwere transplanted and later well established seedlings were thinned maintaining only one plant in each pot of a replication (Fig. 1b).

2.3 Maintaining Drought Stress in Plants

Bulk surface soil (0-15 cm) was collected from the CRC (Crop Research Center) at SVPUAT, Meerut. The soil was air dried, mixed thoroughly and passed through 2-mm sieve. Seeds were sown in plastic pots (2.5 kg capacity) filled with 2.0 kg autoclaved soil along with 100 grams of farm yard manure. The plant populations weremaintained as above mentioned and for all treatmentswith isolates including control, watering was provided for 3 weeks without any stress exposure. Moisture was maintained by applying 500 ml of water per pot every alternate day with the help of measuring beaker up to 3 weeks after sowing. After three weeks, watering was stopped for subsequent days until the plants attaining physiological wilting stages due to drought exposure and water is provided to all the plants of treatments including control only after the appearance of wilting and leaf rolling symptoms due to physiological stress by providing 1litre of water after 21 DAS with the help of measuring cylinder. Here the one litre of water is fixed for the entire experiment during drought exposure stages.Five parameters were recorded 1) Drought tolerance capacity, 2) Water requirement, 3) Root length, 4) shoot length, 5) Chlorophyll content.

2.4 Drought Tolerance Capacity

It was assessed by counting the maximum mean number of days withstand by plants after 21DAS between the last irrigation and till theplants showed the appearance of physiological wilting till end of the experiment.

Table 1. Treatment details of seed biopriming

T1=seeds bio primed with *Pseudomonas fluorescens* isolate Pf-2@1g/30 seeds for 2 hours
T2= seeds bio primed with *Pseudomonas fluorescens* isolate Pf-4@1g/30 seeds for 2 hours
T3= seeds bio primed with *Pseudomonas fluorescens* isolate Pf-6@1g/30 seeds for 2 hours
T4= seeds bio primed with *Pseudomonas fluorescens* isolate Pf-28@1g/30 seeds for 2 hours
T5= seeds bio primed with *Pseudomonas fluorescens* isolate TH08@1g/30 seeds for 2 hours
T6= seeds bio primed with *Trichoderma harzianum* isolate IRRI-1@ 1g/30 seeds for 2 hours
T7= seeds bio primed with *Trichoderma harzianum* isolate TH-26@ 1g/30 seeds for 2 hours
T8= seeds without anytreatment (distilled water) (Control)

Table 2. Treatment details of transplanted root dip

T1= seedlings treated with *Pseudomonas fluorescens* Pf-2@10gm/lit suspensionfor 2 hours
T2= seedlings treated with *Pseudomonas fluorescens* Pf-4@ 10gm/lit suspension for 2 hours
T3= seedlings treated with *Pseudomonas fluorescens* Pf-6@ 10gm/lit suspension for 2 hours
T4= seedlings treated with *Pseudomonas fluorescens* Pf-28@ 10gm/litsuspension for 2 hours
T5= seedlings treated with *Pseudomonas fluorescens* TH08@ 10gm/lit suspension for 2 hours
T6= seedlings treated with *Trichoderma harzianum* IRRI-1@10gm/lit suspension for 2 hours
T7= seedlings treated with *Trichoderma harzianum* TH-26@10gm/lit suspension for 2 hours
T8= seedlings without anytreatment (distilled water) (Control)

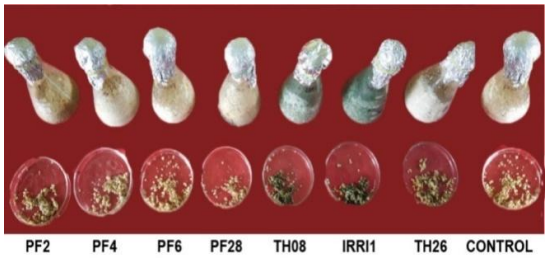


Fig. 1a. Seed biopriming with respective isolates of biocontrol agents of *Pseudomonas fluorescens* and *Trichoderma harzianum*

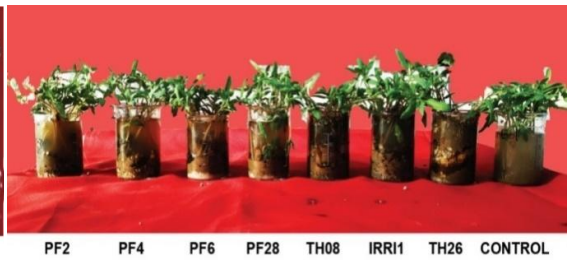


Fig. 1b. Seedling/transplanted root dip with respective isolates of biocontrol agents of *Pseudomonas fluorescens* and *Trichoderma harzianum*

2.5 Water Requirement

Water requirement was calculated by using measuring cylinder. 500ml of water is given equally in all treatments including control every alternate day up to 21DAS. After 21DAS, one liter water was given to each treatment per pot including control only after the appearance of physiological wilting till the end of the experiment. Cumulative mean of water requirement was calculated from sowing till the harvest in liters.

2.6 Root and Shoot Length

Root and shoot length were measured with the help of ruler from each pot in each replication after the harvest (pull off) of the plants.

2.7 Total Chlorophyll Content

The SPAD (Soil Plant Analytical Development) chlorophyll meter (Minolta^{TM}), portable chlorophyll meter was used to acquire rapid estimation of chlorophyll content in SPAD units [10]. The measurement was done in between 11 am to 12 noon to avoid droplets content on leaf surface.

2.8 Statistical Analysis

The data were statistically analyzed using IBM SPSS Statistics Base 22.0 and Duncan's Multiple Range Test (DMRT) was used to compare the treatment means. Significance was evaluated at the 5 % ($P<0.05$) level.

3. RESULTS AND DISCUSSION

3.1 Drought Tolerant Capacity

Among all the isolates, IRRI 1 isolate of *T. harzianum* and Pf28 isolate of *Pseudomonas fluorescens* showed maximum mean cumulative drought tolerance capacity of both methods of inoculation after last irrigation of 14.83 days and 13.66 days with the increase of 38.11% and 28.14%respectivelyover control. The third most effective isolate was found to be Pf4 with (13.33 days) 25.04% increase over control which was closely followed by isolates TH 08 and TH 26 with (13.00 days) 21.95% increase over control. The isolate Pf 6 moderately mitigated the drought stress with (12.33 days) 15.66% over control. The lowest drought tolerant capacity was noticed in the isolate Pf 2 with just (11.00 days) 3.18% over control. While the control plants was recorded with only 10.66 days. Among the two modes of inoculation, seed biopriming significantly reduced the drought stress than transplanted root dip method (Fig. 2) the isolates enhanced better water stress tolerance ability to the tomato plants than untreated plants.

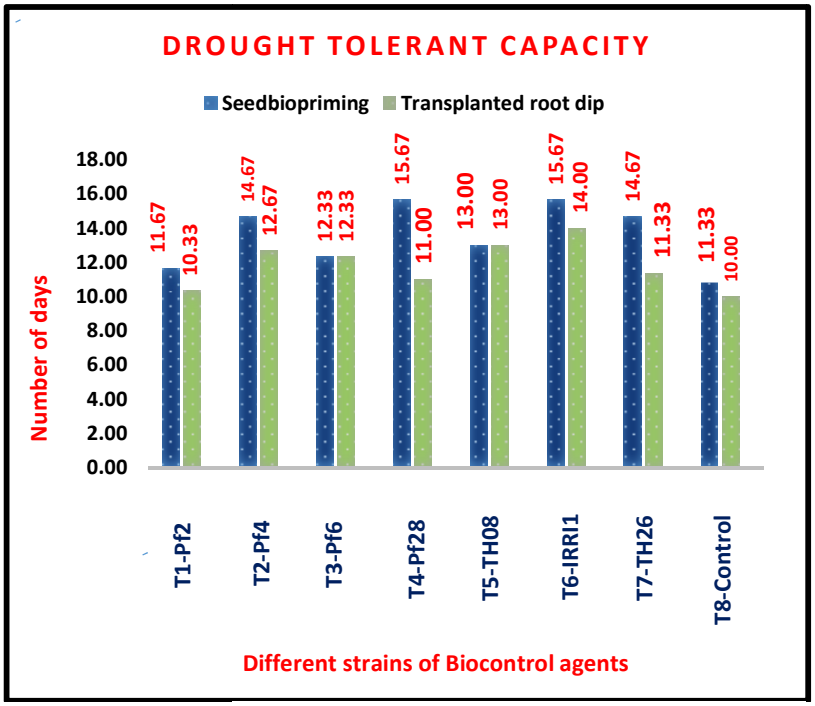


Fig. 2. Effect of seed biopriming and transplanted root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* on drought tolerant capacity (days)

The results were in agreement with shukla et al [11] where *Trichoderma*-colonized rice seedlings were slower to wilt in response to drought stress, where they reported that Th56 isolate of *Trichoderma harzianum* induced maximum drought tolerance even at 9 Days Drought Stress (DDS). Results were also similar with [12] as they reported that seed biopriming of wheat with powdered formulation of drought tolerant isolates of *Trichoderma harzianum* enhanced tolerance against water stress condition and the isolates Rani Th-14, Rani Th-25 and Rani Th-30 performed better (< 60%) to stand the plants against drought stress even up to 13 Days Drought Stress (DDS).

3.2 Water Requirement

The mean cumulative water consumption of two methods of inoculation by different bio-inoculants treated tomato plants varied between 14.58 to 15.58 litres. However the treated plants showed less water consumption than the untreated plants. Among different treatments, IRRI 1 isolate of *Trichoderma harzianum* treated plants recorded the lowest mean cumulative water consumption of both methods of inoculation which consumed only 14.58 litres which is 10.27% lower(-10.27%)than the untreated plants. The plants treated with the isolates Pf28, Pf4, Pf6, Th08 and Th26 performed well with regard to water consumption than control which were statistically similar with each other with water consumption of14.91litres(-8.24%),14.75 litres (-9.23%), 14.75 litres(-9.23%), 15.08 litres(-7.20%) and 15.25 litres(-6.15%) respectively. The highest water consumption was noticed in the untreated plants which were recorded with 16.25 litres. Among two modes of inoculation, seed biopriming significantly reduced the water consumption than transplanted root dip method (Fig. 3).

The results are in concurrence with Rodriguez et al. [13] who reported that uninoculated wheat plants wilt much earlier (6 to 10 days) than the inoculated plants. Remarkably, inoculated plants consume less water (30–50%) than un-inoculated plants. They noticed that plants treated with bioinoculants having improved water use efficiency during abiotic stress. Nayyar and Gupta [14] also reported that the interaction between the plant and the fungus occurs mainly at the rhizosphere, such a mechanism is likely related to an increase in the water absorption efficiency, which presumably is related to the increased root volume leading to increased water absorption.

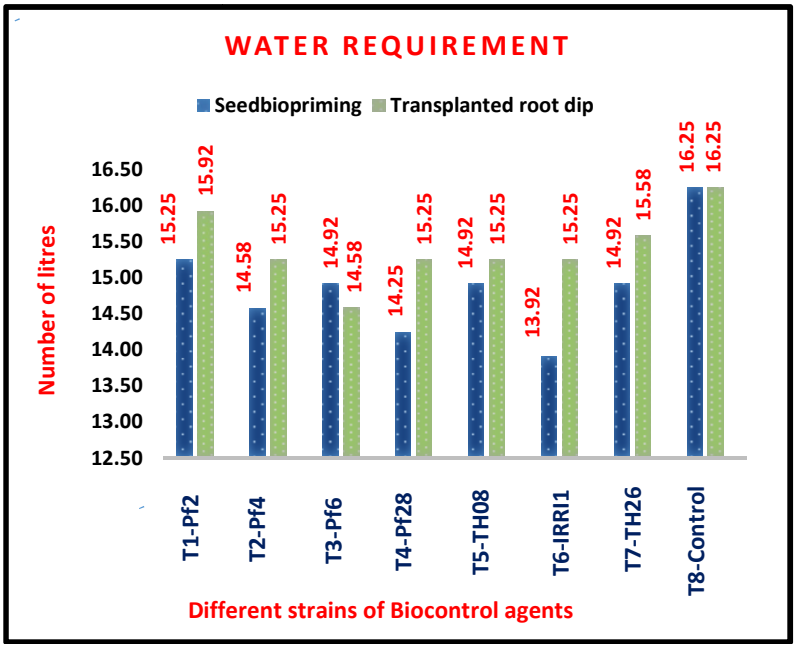


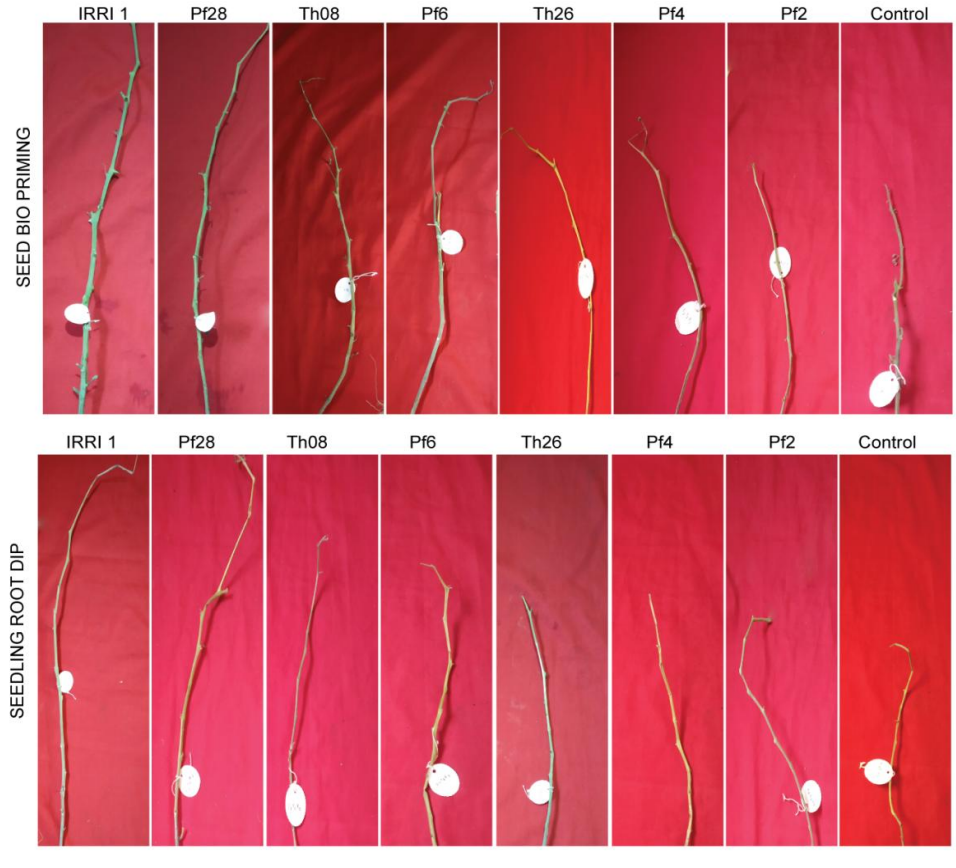
Fig. 3. Effect of seed biopriming and transplanted root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonasfluorescens* on water requirement (litres)

3.3 Shoot and Root Length

Significant results have been achieved with the methods of inoculation, where seed biopriming increased the shoot length more effectively than the transplanted root dip (Fig. 4a and 4b).The cumulative mean of both methods of inoculation found that maximum shoot length was recorded in the plants treated with IRRI 1 isolate which gave 32.60% (76.25cm) increase over control followed by the isolates Pf 28, TH08 and Pf 6 with mean root lengths of 67.17cm,63.16cm and 63cm increasing shoot lengthsby 16.80%, 9.84% and 9.56% respectively over control. The plants treated with TH 26 also positively influenced the shoot length with an increase of 8.10% (62.16 cm) over control. The isolate Pf 4 also had negligible impact on improvement in shoot length with 4.92% (60.33 cm) increase than control. The remaining isolate Pf 2 showed negative effect on shoot length with 29% (57.33 cm) decrease compared to the control recording only 57.50 cm.

Although methods of inoculation by seed biopriming as well as transplanted root dipwere statistically similar with each other, seed biopriming was found superior to transplanted root dip. However the significant improvement in root length was achieved in the plants treated with IRRI 1 isolate with maximum mean cumulative root length of both methods of inoculation of 28.33 cm with 146.34% increase over control. The isolate IRRI 1 was followed by the isolates Pf 28 and TH08 which were statistically similar with each other with the increase of root length by 65.21% (19.00 cm) and 57.91% (18.16cm) respectivelyover control. The remaining isolates like TH 26, Pf 6, Pf 4 and Pf 2 were statistically similar with each other enhancing the root length by 53.56% (17.66 cm), 43.47% (16.50 cm), 46.34% (16.83%) and 36.17% (15.66 cm) over control respectively. Whereas mean of root length of both methods of inoculation in untreated plants (control) was recorded with only 11.50 cm (Fig. 5 a,b). It was observed that the maximum root length increase was negatively correlated with water consumption *i.e* the plants recorded with maximum root length consumed less water.

Similar results were obtained by (Shukla et al., [11]) where they reported that rice seed bioprimed with *Trichoderma* isolates such as Th 56 and Th 75 had increased the shoot and root length than the untreated plants. The results were also in agreement with [15,16,17] who reported that tomato seedlings treated with *Trichoderma harzianum* (OTPB3) enhanced the shoot and root length with 38.53% and 32.04% increase as compared to the control seedling.



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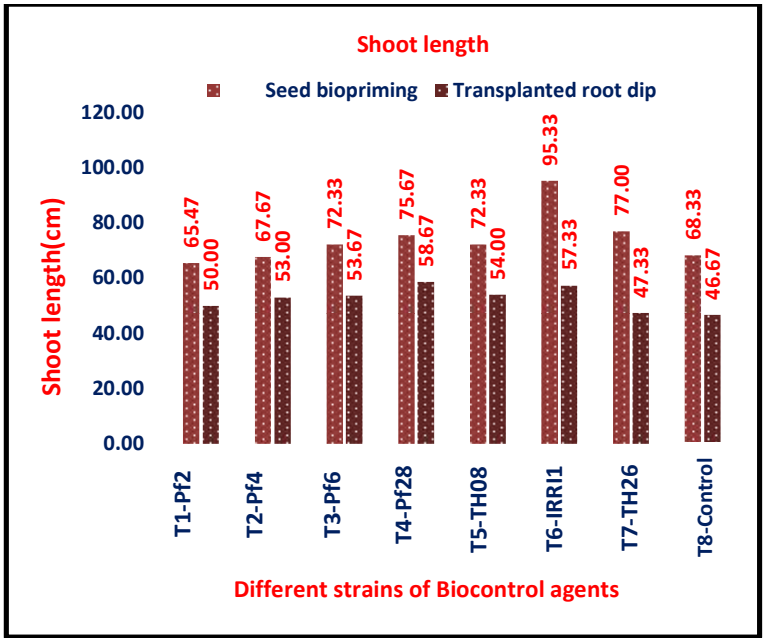

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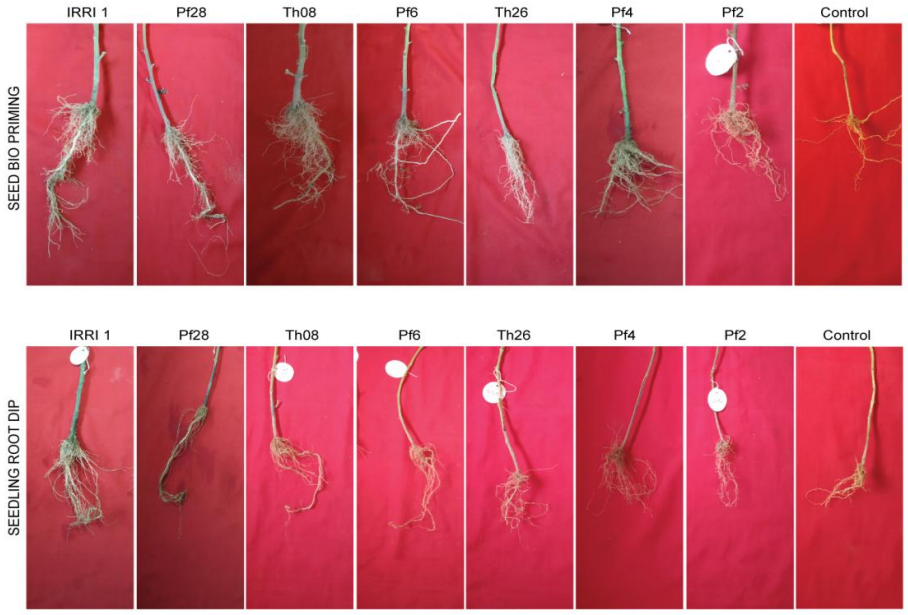
Fig. 4(a,b). Effect of seed biopriming and transplanted root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonasfluorescens* on shoot length (cm)



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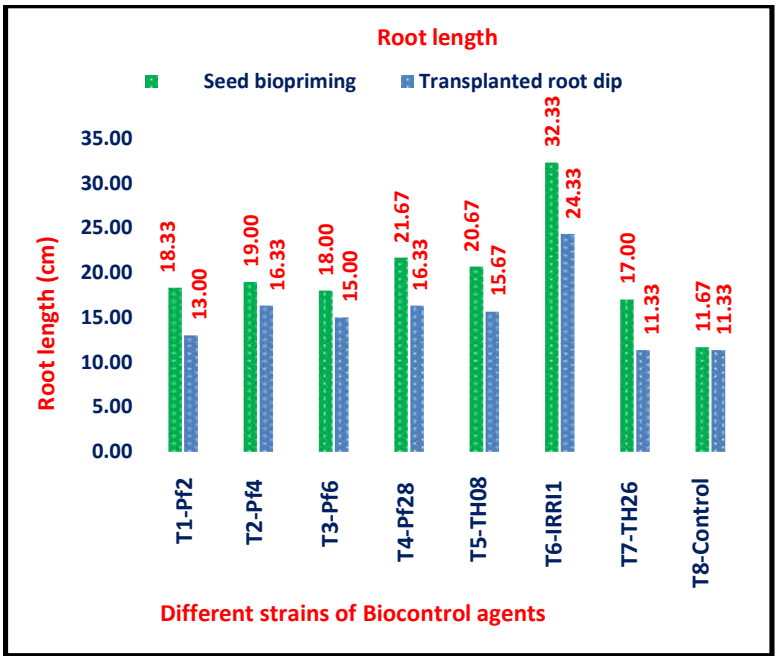

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Fig. 5(a,b). Effect of seed biopriming and transplanted root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonasfluorescens* on root length (cm)

3.4 Chlorophyll Content

The chlorophyll content measured in plants treated with all isolates except IRRI 1 showed non-significant relationship with each other. Similarly no significant differences were recorded between the modes of inoculation. However among different isolates, maximum mean cumulative SPAD readings of both methods of inoculation were obtained in the plants treated with IRRI 1 isolate which gave 39.75% (41.20 SPAD units) increase over control. The

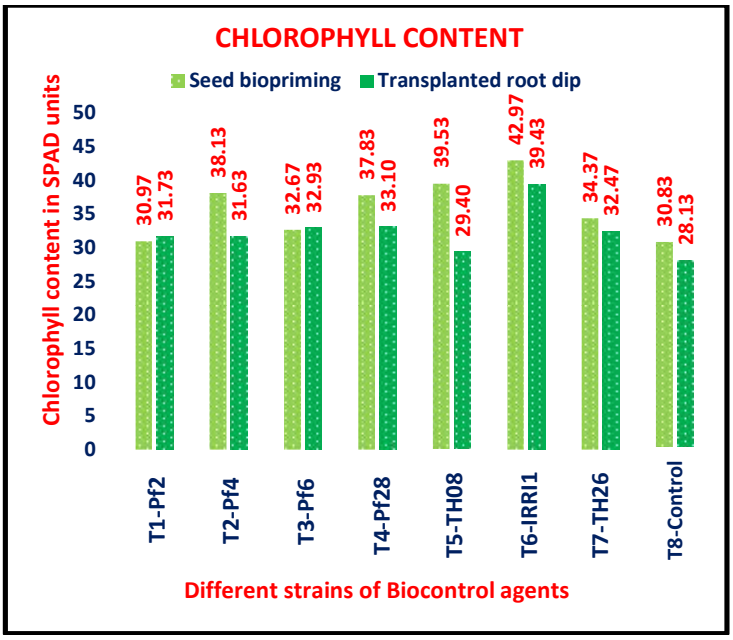

Fig. 6. Effect of seed biopriming and transplanted root dip with selected isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* on chlorophyll content

remaining isolates Pf 28, TH 26, Pf 6, Pf 2, TH 08 and Pf 4 were recorded with 20.28% (35.46 SPAD units), 18.31% (34.88), 16.89% (34.46 SPAD units), 16.72% (34.41 SPAD units) and 6.34% (31.35 SPAD units) respectively were statistically similar with each other which were higher than control (Fig. 6). The cumulative mean SPAD readings of methods of inoculation of untreated plants (control)were only 29.48 SPAD units. The maximum SPAD reading governing plants were positively correlated with the maximum tolerance during water stress and negatively correlated with the water consumption.

The above results are in agreement with the findings of Ahmad et al. [18] where they reported that application of *Trichoderma harzianum* restored the chlorophyll pigment content which is increased by 13.88% than control in mustard seedlings under salt stress.

4. CONCLUSION

The results of the experiments suggest that seed biopriming with drought tolerant isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* increased the ability of tomato to grow successfully under drought stress conditions. Among the isolates used in this study the isolate IRRI 1 was considered best in providing drought tolerance to tomato plant. The most important and consistent response, which enabled plants to tolerate drought was increased root length which helps the plant to complete its life cycle with less water consumption than the untreated plants. The bioinoculants can able to alter the eco-physiological conditions of the plant which result in the rapid adaptation of plants, allowing them to establish and survive in high-stress habitats. Increased root, shoot growth and chlorophyll contents indicated that both photosynthesis levels as well as efficiency were increased in the presence of *Trichoderma harzianum* and *Pseudomonas fluorescens* isolates helping the plants to tolerate drought stress conditions. This research merits attention and could additionally open the avenue for the use of *Trichoderma* and Pseudomonas inoculation through seed biopriming in the plants for enhanced drought tolerance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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