Original Research Article

Effect of *Trichoderma harzianum* on Growth of Corn under Water Stress Condition

ABSTRACT:

Drought is the major abiotic stress which has been increased over the past decades affecting world's food security. One possibility to increase the drought tolerance is to use beneficial microorganisms as seed treatment. In this study the seed treatment with *Trichoderma harzianum* on the growth of Tomato (*Solanum lycopersicum* L.) under water stress condition has been tested. Parameters including drought tolerance capacity, shoot length, root length, plant height and chlorophyll content were recorded in all the treatments along with the untreated control. Application of *Trichoderma harzianum* during seed biopriming treatments in corn was found more effective in drought tolerance and resulted in improved vegetative growth performance in water stress condition. Among all the treatments *Trichoderma harzianum* seed treatment with water stress condition was found highly effective in reducing the water stress by increasing the cumulative mean water stress tolerance duration up to 6.54 days after the last irrigation followed by untreated seed with water stress condition with mean water stress tolerance of 5.36 days. Maximum cumulative mean shoot growth root growth and chlorophyll content 164 cm, 40 cm and 39.63 SPAD units respectively were recorded with *Trichoderma harzianum* seed treatment with water stress condition followed by *Trichoderma harzianum* seed treatment with regular irrigation having 163.25 cm, 38.25 cm and 37.29 SPAD units respectively. The seed treatment with *Trichoderma harzianum.* is comparatively easy, cost effective and ecofriendly practice for the farmers and good alternatives for chemical pesticides and fertilizers it also safe for environment.

Keywords: Seed treatment, *Trichoderma herzianum*, Drought tolerance, growth parameters, Corn.

INTRODUCTION:

Drought is one of the major abiotic stresses which has been increased over past decades affecting word's food security. It results in devastation of agricultural crops and causing considerable losses in yield (de Vries *et al*. 2012). Soil moisture is the most restricting element in dry cultivating and dry land farming situations. Plants absorb water to normal function like nutrient absorption, transpiration, metabolic activities leading to growth, development and yield. By 2050, water consumption for agricultural practices will increase by 60% to support a growing world population (Boretti and Rosa, 2019). Concurrently, drought is becoming a dominant environmental stressor for crop production globally, limiting food security with precarious economic and sociological impacts (Trenberth *et al*., 2014). Due to climate change, water deficit is spreading to regions where drought was infrequent in the past (Somerville and Briscoe, 2001). Globally, the reduction in yield due to drought is likely to exceed the combined loss of all other possible causes of yield decline (Blum, 1996; Foolad, 2007). Therefore, there is a need to identify solutions to mitigate water deficit stress and its impact on food security.

Corn (*Zea mays* L.) the third largest plant-based food source in the world after rice and wheat. Archaeological and molecular data indicates that modern maize was domesticated from annual teosinte (*Zea mays ssp*. parviglumis) in southern Mexico 9,000 years ago. It is one of the most versatile emerging crops having wider adaptability. Globally, maize is known as queen of cereals because of its highest genetic yield potential. In the new millennium, it is an alternate crop to rice and wheat. About 35% production is consumed by human, 25% poultry and cattle feed, 15% food processing.

Naturally occurring beneficial soil microorganisms, including members of the genus *Trichoderma* (Family – Hypocreaceae), are studied as accessible and sustainable tools for enhancing plant growth and mitigating biotic and abiotic stressors in both research and commercial production settings (Van Wees et al., 2008). As such, specific commercial *Trichoderma* isolates are widely used as effective biocontrol agents, biofertilizers, and phyto-stimulators (Harman, 2011), but evidence of contributions to abiotic stress enhancement in crop species is sparse. Several recent studies suggest that *Trichoderma* inoculation of water-stressed plants enhanced growth by improving root biomass, enhancing water holding capacity, and mobilizing nutrients (Mastouri et al., 2010; Harman, 2011; Bakhshandeh et al., 2020). Further, *Trichoderma* inoculated plants exhibit delayed wilting, enhanced leaf chlorophyll content, and greater net photosynthesis levels under water deficit stress conditions (Bae et al., 2009; Shukla et al., 2012). Thus, keeping this in the view present study is aimed for “enhancement of drought tolerance in Corn (*Zea mays* L.) by seed treatment of *Trichoderma harzianum.* With water stress tolerance effect of *Trichoderma harzianum* on plant growth promoting activities like increase in shoot length, root length and chlorophyll content in plant will be studied as these are indirectly responsible for drought stress in corn plant.

2 MATERIAL AND METHOD

This pot experiment was carried out in greenhouse during 2023-2024 at Umergaon, District Valsad, Gujarat on corn (*Zea mays L*.). The variety- P3546 of Pioneer seeds has selected for carrying this experiment.

2.1 Sources of *Trichoderma harzianum*, and Soil

Trichoderma harzianum (WP)-61, trade name Neemoderma-H of Shri Ram Solvent Extract Extractions (P) Ltd. having *Trichoderma harzianum* content 1% w/w (CFU Count 2x10^6/gm. Min) plus Carboxy methyl cellulose 1.0% w/w and Talc Powder 98.0% w/w. used for *Trichoderma harzianum* seed treatment. Surface soil (0-15 cm) collected from farmer’s field and mix thoroughly after air dried and pass through 2-mm sieve. Well decomposed FYM is mixed in collected surface soil (10:1 ratio) and autoclaved at 15 PSI pressure and 121ºC temperature for 30 minutes to sterilize. Autoclaved soil is filled in 12.5-inch plastic pots at the rate of 6.3 kg autoclaved soil in each pot.

2.2 Seed treatment with *Trichoderma harzianum* and sowing

Seeds of corn are surface sterilized with 1% sodium hypochlorite solution for 3 min, then rinsed with sterilized water and dried. The dried seeds of corn are primed by hydrating thin film of water and taken in the petri plates which were treated with the 1g *Trichoderma harzianum* per 30 seeds (Fig 1). Seeds soaked in distilled water were served as control. Seed treatments are given as per treatment details (Table 1). A single *Trichoderma harzianum* treated seed of corn sown in each pot. Pots are Irrigated uniformly and arranged in Greenhouse (Fig 2.). The greenhouse temperature is maintained 31ºC, and humidity at 55 to 60% throughout the crop lifecycle. Regular irrigation is applied to each pot for 15 days 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 15 days after sowing.

Table 1. Treatment details of seed treatment

Treatment	Treatment Details
T1	*T. harzianum* + Irrigated
T2	*T. harzianum* + Water Stress
T3	Untreated seed + Irrigated
T4	Untreated seed + Water Stress (Control)

Fig.1 Seed treatment with *Trichoderma harzianum*

Fig. 2 Overview of experiment in Greenhouse

2.3 Drought Stress Maintaining in Plants

Regular watering is given to all plants including control at the rate of 500 ml water per day from sowing day till corn plant achieve fifteen days maturity without any stress. Plant population at the rate of one corn plant per pot is maintained. After fifteen days sowing when all seeds germinated properly and corn plants well established, then the regular watering is continued in irrigated treatments and watering was stopped for subsequent days until the plants attaining physiological wilting stage due to drought exposure and water is provided to the plants of water stress treatments including control only after the appearance of wilting and leaf rolling symptoms due to physiological stress by providing one liter of water with the help of measuring cylinder. This watering method is carried out after 15 DAS till the end of the experiment. Suitable control (Untreated seed +Water stress growing condition) is used as reference for drawing conclusion while comparing the effect of. *Trichoderma harzianum*. Including drought tolerance capacity other parameters like root length, shoot length and chlorophyll content observations are recorded.

2.4 Drought Tolerance Capacity

Drought tolerance capacity was assessed by counting the maximum mean number of days withstand by plants after 15DAS between the last irrigation and till the plants showed the appearance of physiological wilting till end of the experiment.

2.5 Root and Shoot Length

At harvesting stage, the root and shoot length from each pot in each replication were measured with the help of measuring scale.

2.6 Chlorophyll content

Chlorophyll content was measured with the help of portable chlorophyll meter SPAD (Soil Plant Analytical Development) chlorophyll meter (MinoltaTM). It was used to acquire rapid estimation of chlorophyll content in SPAD units (Lah *et al*. (2011). The measurement was done at morning 10 am. It helps to avoid droplets content on leaf surface.

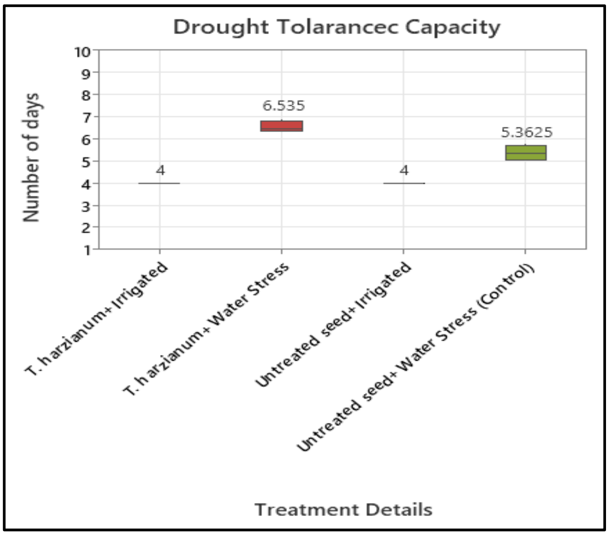
2.7 Statistical analysis

The data generated from the pot culture experiment was analyzed by completely randomized design. The data obtained was statistically analyzed and appropriately interpreted as per the methods described in “Statistical Methods for Agricultural Workers” by Panse and Sukhatme, (1985). Appropriate standard error (S.E.) and critical differences (C.D.) at 5% level were worked out as and when necessary and used for data interpretation. The data were statistically

3 Result and Discussion

3.1 Drought Tolerance Capacity

In the present study treatment T2 i.e. seed treatment of *Trichoderma harzianum* with water stress shows maximum drought tolerance capacity 6.53 days with the increase of 19.45% over control i.e. treatment T4 untreated seed with water stress condition. While the control plants were recorded with only 5.36 days drought tolerance capacity (Fig. 3) (Table. 2). Malinowski *et al.* (2000) and Mastouri *et al.* (2010) have been conducted similar studies on *Trichoderma harzianum* and the beneficial effects on drought stress tolerance on important crop such as tomato. Bashir et al. (2020) also conducted experiment and reported that priming seeds with *Trichoderma* spp. delays drought stress by 3–5 days.



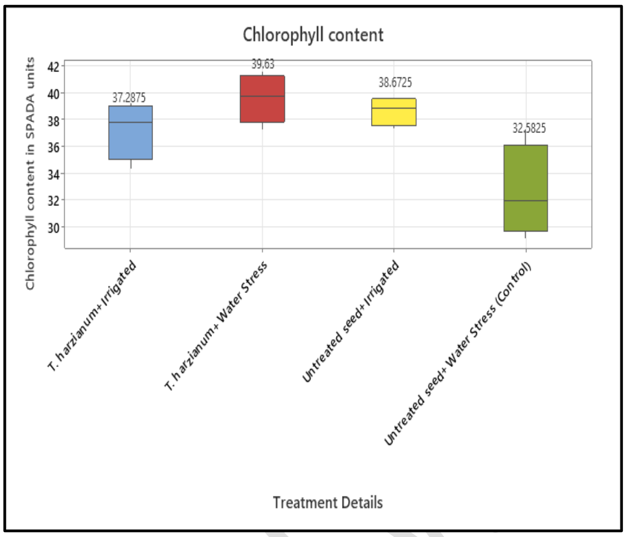


Fig 3. Effect of seed treatment with *Trichoderm harzianum* on drought tolerance capacity

Fig 4. Effect of seed treatment with *Trichoderma harzianum* on chlorophyll content

3.2 Shoot and Root length

Drought reduces the plant growth drastically, and *Trichoderma harzianum* not only ameliorated the negative effects but also enhanced growth significantly. We recorded significant results of *Trichoderma harzianum* seed treatment, the maximum shoot length was recorded in the treatment T2 i.e. seed treatment of *Trichoderma harzianum* with water stress condition, which gave (164.00 cm)16.72 % increase over control followed by the T1 treatment plants i.e. seed treatment with *Trichoderma harzianum* with regular irrigation (163.25cm). 16.19% increase over control. The T3 treatment untreated seed with regular irrigation has (156.5cm) 11.38 % increase over control i.e. T4 treatment untreated seed with water stress condition (control has (140.50cm) shoot length (Table 2.) (Fig 6) similar experiment conducted by Contreras-Cornejo *et al.* (2016) and reported that in studies of maize, cotton, and cucumber treated with *Trichoderma*, seed germination and plant growth have been improved to varying degrees, manifested as improvements in seed vigor, germination rate, emergence rate, seedling root activity, plant height, and plant dry quality. Harman *et al.* (2004) also found that *Trichoderma* spp. are plant symbiont opportunistic avirulent organisms, able to colonize plant roots and to produce compounds that stimulate growth and plant defense mechanisms under suboptimal conditions.

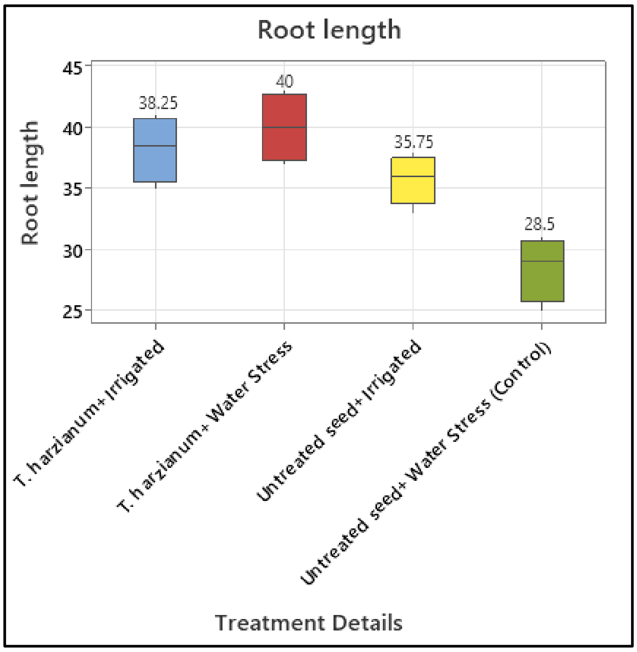


Fig 5. Effect of seed treatment of *Trichoderma harzianum* on root length

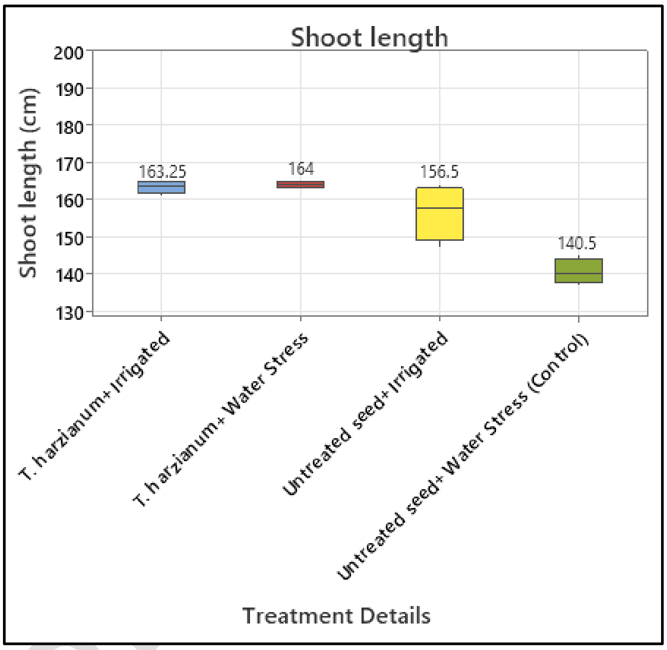


Fig 6. Effect of seed treatment of *Trichoderma harzianum* on shoot length

It also observed that water stress reduced the root length, however the significant improvement in root length was achieved in the T2 treatment i.e. seed treatment of *Trichoderma harzianum* with water stress condition(40.00cm) which increase the root length by 40.35 % over control followed by the T1 treatment i.e. seed treatment with *Trichoderma harzianum* with regular irrigation (38.25cm) with 34.21% increase over control. The T3 treatment untreated seed with regular irrigation enhanced the root length by 35.43% (28.50cm) over control. Where as in T4 untreated seed with water stress (control) was recorded with only 28.50cm root length (Table 2) (Fig 5). Similar results recorded by Bjorkman *et al.* (1998) and Cai *et al.* (2013) in maize plants, *Trichoderma* inoculation affected root system architecture, which was related to increased yield of plants. Reported effects include enhanced root biomass production and increased root hair development.

Fig 7. Effect of seed treatment with *Trichoderma harzianum* **on root length of corn**

Fig 8. Effect of seed treatment of *Trichoderma* harzianum **on shoot length of corn**

3.3 Chlorophyll content

Impact of drought was observed that water stress reduced chlorophyll content. The maximum chlorophyll content is recorded in the plants of treatment T2 i.e. seed treatment of *Trichoderma harzianum* with water stress condition which gave 21.63% (39.63SPADA units) increase over control. The remaining treatments T1 seed treatment with *Trichoderma harzianum* with regular irrigation and treatment T3 untreated seed with regular irrigation were recorded with 14.45% (37.28 PADA units) and 12.55% (36.67 PADA units) respectively which is higher than control. The treatment T4 i.e, untreated seed with water stress (control) were only 32.58 SPADA units. Estévez-Geffriaud *et al.* (2020) reported similar results in corn that *Trichoderma* spp. play a crucial role in increasing the resistance of plants to various stresses by increasing photosynthetic efficiency, stimulating root growth, improving water and nutrient absorption. Shukla *et al.* (2012) also observed that *Trichoderma harzianum* inoculated plants exhibit delayed wilting, increased stomatal conductance, enhanced leaf chlorophyll content, and greater net photosynthesis levels under water deficit stress conditions.

Table 2. Effect of Trichoderma harzianum on drought tolerance capacity, plant shoot and root length and Chlorophyll content.

Trt. #	Treatment Details	Drought tolerant capacity	Shoot Length	Root Length	Chlorophyll content
T1	*T. harzianum* +Irrigated	4.00	163.25	38.25	37.28
T2	*T. harzianum* +Water Stress	6.57	164.00	40.00	39.63
T3	Untreated seed +Irrigated	4.00	156.50	35.75	38.67
T4	Untreated seed +Water Stress (Control)	5.50	140.50	28.50	32.58
	S.Em.±	0.07	2.14	1.37	1.24
	C.D. at 5 %	0.20	6.84	4.37	3.96
	C.V. %	3.74	2.74	7.67	6.68

4 Conclusion

From the aforementioned results in present study, It could be revealed that water stress affects the growth of corn plant. However, seed treatment with *Trichoderma harzianum* increased the ability of corn to grow successfully under water stress condition. The most important and consistent response, which enabled plants to tolerate drought was increased root growth which helps the plant to complete its life cycle with less water consumption than the untreated plants. The *Trichoderma harzianum* seed treatment in corn can able to alter the eco-physiological conditions of the plant which outcome in the quick adaption of plants permitting them to establish and survive in water stress habitats. *Trichoderma harzianum* proved to be useful in mitigating the negative effect of water stress by increased root, shoot growth and chlorophyll contents indicated that both photosynthesis levels as well as efficiency were increased in plant to tolerate water stress condition.

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