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Comparative Study of *Trichoderma* BHU-1 Mediated Drought Alleviation of Susceptible and Tolerant Rice Cultivars

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Abstract

The study investigated *Trichoderma*-mediated morphological and biochemical responses in drought-susceptible and tolerant rice cultivars, namely IR-64 and DRR-44, under drought-stressed and normal conditions. Various morphological and biochemical parameters were recorded 30, 60, and 90 days after transplanting. The shoot length was insignificant, while the root length was significant in drought-susceptible DRR-44 compared to non-stressed plants. The number of roots was also significant in *Trichoderma* BHU-1 treated plants of both cultivars. Proline content was more substantial in drought susceptible cultivars than tolerant and similarly, lignin, TPC, PAL, and PO activities were higher in *Trichoderma* BHU-1 treated drought-stressed plants than in normal ones. The result revealed that *Trichoderma* BHU-1 treatment modulates an increase in root length, shoot length, and the total number of tillers and roots under drought conditions. It also maintained the level of phenolics in plants by upregulating the pathway thereby helping the plant to sustain drought.

Keywords: Drought Tolerance, Oxidative Stress, Peroxidase, Rhizospheric Microorganisms

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INTRODUCTION

Rice (*Oryza sativa*) forms a principal constituent of the diet for more than half of the global population from the Poaceae (Gramineae) family, originating about 130 million years ago. It is also a means of fodder, such as husk and straw, for animals in many countries.[1] It has been documented to be simultaneously domesticated in Southern China and North-Eastern India around 8000 years ago.[2] India comes first in the area under cultivation and second in rice production. Among Indian states, West Bengal, Punjab, and Uttar Pradesh are the highest producers, with Punjab also having the highest productivity. Rice cultivation depends heavily on irrigation facilities; hence, it is carried out primarily in well-puddled lands. Various abiotic stresses hamper rice production potential, and drought is the most threatening problem. It has progressed severely in many areas worldwide.[3] Rainfall uncertainty, groundwater depletion, and increased soil salinity have only increased the stress magnitude. Rice is invulnerable to drought at the reproductive growth stage, where even moderate stress can cause extreme yield decline.[4] Drought can cause yield loss of 15-50% in rice, based on the period and magnitude of stress.[5] Thus, one of the objectives of rice development programs has been to increase the ability to survive under long-day drought conditions.

Trichoderma spp. is a cosmopolitan fungus inhabiting many soil types and possesses root-colonizing ability in various plants in different ecosystems. It is a symbiotic avirulent microbe that can induce defence mechanisms and growth in host plants under substandard conditions.[6] For many years, the genus has been used as a biocontrol agent, but over the current period, it has become common as a plant growth promoter.[7] Various *Trichoderma* spp. are reported to improve the growth of plants under drought conditions in crops like wheat,[8] tomato,[9] *Arabidopsis*,[10] and rice.[11] The primary underlying mechanisms in imparting drought tolerance are root modulation, phytohormone stimulation,[12] siderophore production,[13] phosphate solubilization, upregulation of defence-related proteins,[14] and enhanced plant antioxidant system.[15] Root colonization of rice plants by *Trichoderma* spp. also changes net photosynthesis, stomatal conductance, and leaf greenness to alleviate drought stress.[13] The present investigation aims at the comparative analyses of a drought-tolerant *Trichoderma* spp., BHU-1 isolate on two rice cultivars, IR-64 (drought susceptible) and DRR-44 (drought tolerant).

MATERIALS AND METHODS

Biological material

Seeds of rice cultivars IR-64 and DRR-44 were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. The pure culture of *Trichoderma* spp. was obtained by isolating the microbe from the agricultural soil samples collected from drought-stressed regions of Uttar Pradesh, India, namely Banda, Bundelkhand (25.4530°N, 78.6098°E), Mirzapur (25.1337°N, 82.5644°E), and Chandauli (25.1794°N, 83.2934°E). Rhizospheric soil samples from agricultural fields of these regions were collected and stored in aseptic polybags. Single spores purified cultures of *Trichoderma* spp. were isolated using the method developed by Elad *et al*.,[16] and were preserved at 4 °C during the investigation period.

In vitro selection of effective *Trichoderma* isolates against drought

The drought tolerance potential of *Trichoderma* isolates was measured using the protocol described by Aujla and Paulitz.[17] Potato dextrose agar (PDA), amended with 10% polyethene glycol (PEG), was used to study all the isolates' growth parameters for seven days. The mycelial growth of all isolates was evaluated after the incubation period, and those with the highest growth were selected for the pot experiment.

Experimental setup

The pot experiments were carried out in Kharif by placing them in a well-ventilated net house in a natural setting. The maximum and minimum temperatures during the total growth period of rice ranged from 31.0 °C to 41.0 °C and 15.8 °C to 33.5 °C, respectively. All experiments were conducted in a completely randomized block design with four replications. Seeds of rice cultivars

IR-64 and DRR-44 were sown in different pots filled with sterilized puddled soil for raising the seedlings. Four-week-old seedlings were treated with spore suspension of *Trichoderma* isolate BHU-1 @ 1.6×10^6 CFU/mL^{-1} (colony forming unit)[18] for one hour. The untreated seedlings of both cultivars served as control. The untreated and treated seedlings were transplanted in plastic pots filled with sterilized puddled soil and grown in a net house under the natural conditions described earlier. Plants were subjected to drought stress by withholding irrigation for five days and then re-irrigation on the sixth day.[19]

Evaluation of morphological and biochemical parameters

Various morphological parameters were recorded at different time intervals after the transplanting. The shoot length of the plants was measured employing a meter scale at 30, 60, and 90 days after transplanting (DAT). The root length and total number of roots were measured at 30 and 60 DAT. The total number of tillers and leaves was counted at 60 DAT, while the total number of panicles was counted at 90 DAT. The biochemical parameters, namely phenylalanine ammonia-lyase (PAL), proline content, total phenolic content (TPC), and peroxidase (PO), were estimated for all the samples at the time of panicle initiation, while chlorophyll and lignin content were estimated at 30 and 60 DAT. PAL activity in samples was estimated and expressed in terms of 1 m TCA g^{-1} fresh weight (FW).[20] Proline content was estimated by Bates *et al*.,[21] and TPC as per Zheng and Shetty.[22] PO enzyme activity of the samples was estimated according to Zia *et al*.[23] The chlorophyll and lignin content of the samples was estimated by Arnon[24] and Zhang *et al*.,[25] respectively.

Statistical analysis

All experimental data was analyzed with SPSS (v16.0). The differences among the treatments were calculated by applying one-way ANOVA.

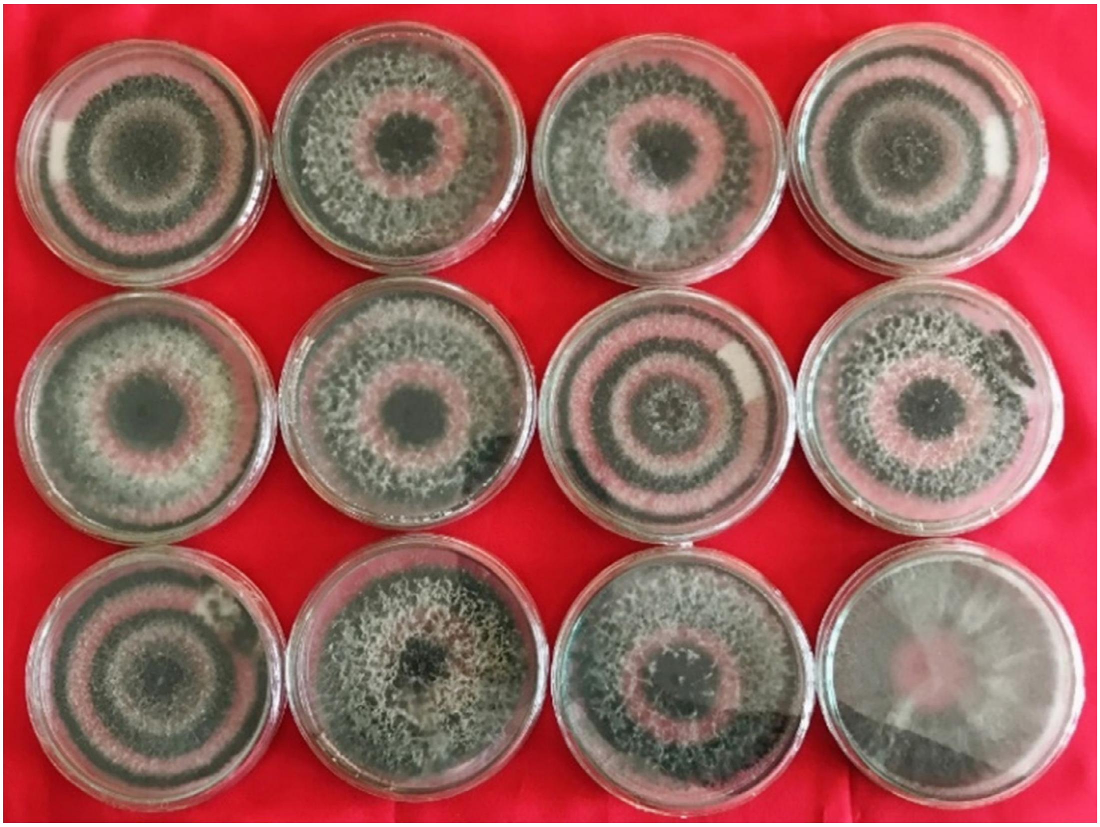

Figure 1. Growth of various isolates of *Trichoderma* spp. on potato dextrose agar (PDA) medium

RESULTS

Selection of potential isolates of *Trichoderma* spp. for drought tolerance

All the isolates of *Trichoderma* covered the 10 cm viable growth space in Petri plates within seven days of incubation on the PDA medium (Figure 1). However, restricted mycelial growth of all the isolates was observed on the PDA medium amended with 10% PEG. BHU-1 isolate exhibited better colony diameters than all the other tested isolates.

Morphological parameters

BHU-1 treated seedlings of both IR-64 and DRR-44 showed higher shoot lengths than untreated ones under irrigated conditions (Figure 2). There was, however, an insignificant difference in the shoot lengths of treated and untreated IR-64 seedlings under drought conditions. In contrast, BHU-1-treated seedlings of DRR-44 showed higher shoot lengths than the untreated ones under drought conditions (Figure 3a). The shoot length was significantly minimal for the untreated IR-64 seedlings under stress. The root lengths and the total number of roots were significantly higher in BHU-1 treated seedlings of both the cultivars under normal and drought conditions than the untreated ones. Among the cultivars, the seedlings of DRR-44 had higher root lengths (Figure 3b) and a higher total number of roots (Figure 3c) compared to the seedlings of IR-64 for each treatment, both at 30 and 60 DAT. The total number of tillers per hill and the total number of leaves per hill were both highest in BHU-1-treated IR-64 seedlings under normal conditions. BHU-1 treated seedlings of IR-64 and DRR-64 also had higher tillers (Figure 3d) and the number of leaves (Figure 3e) per hill compared to untreated ones under stress. Interestingly, the difference was insignificant in treated and untreated DRR-44 seedlings under drought conditions. The trend was similar for leaf chlorophyll content in both the cultivars at 30 and 60 DAT (Figure 3f).

Biochemical parameters

Proline was recorded significantly higher in BHU-1 treated seedlings of IR-64 and DRR-44 than in untreated ones under drought stress. BHU-1 treated seedlings of both cultivars also showed a higher accumulation of proline under stress than in normal conditions (Figure 4a). PAL activity was higher in BHU-1 treated seedlings of both cultivars under drought stress. There was, however, an insignificant difference in the PAL activity of BHU-1 treated IR-64 seedlings under normal and drought stress. In contrast, BHU-1-treated DRR-44 seedlings have significantly

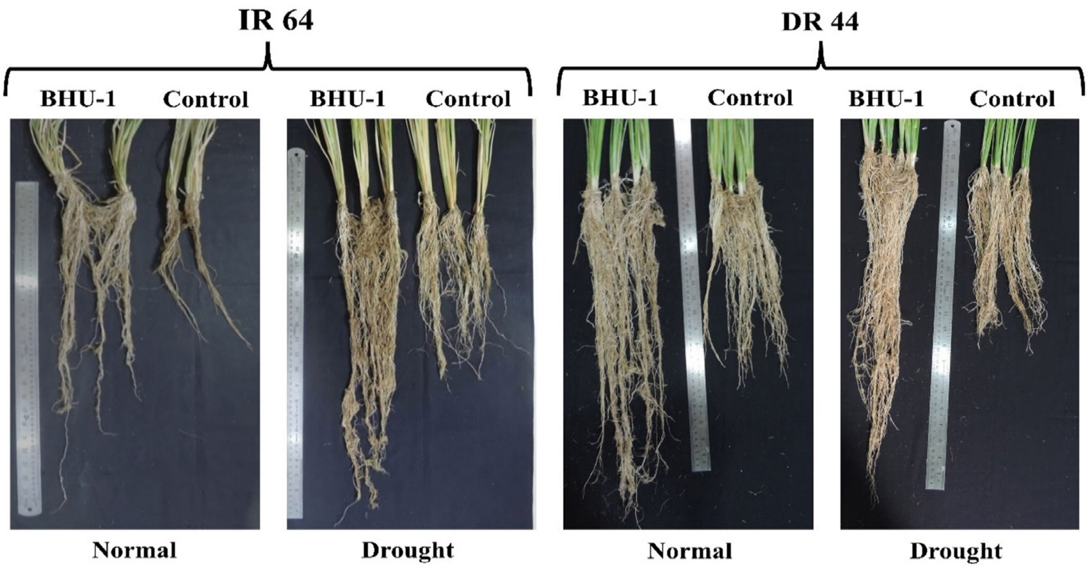

Figure 2. Root growth of IR-64 and DRR-44 cultivars treated with *Trichoderma* BHU-1 isolate and untreated plants under normal and drought conditions

lower PAL activity under normal conditions than drought stress (Figure 4b). The estimated TPC was also higher in BHU-1 treated seedlings of both cultivars under normal and drought conditions. Additionally, the BHU-1 treated seedlings of both cultivars have higher TPC under drought stress than normal ones (Figure 4c). Lignin was observed to be lower rice plants under drought stress in both the cultivars for BHU-1-treated and untreated seedlings. Among the cultivars, BHU-1-treated DRR-44 control plants had higher lignin than IR-64 control plants, while the opposite was for drought-stressed plants (Figure 4d). There was a higher peroxide (PO) activity in BHU-1-treated seedlings of both cultivars than untreated ones under both conditions. Higher activity was also observed in drought-stressed plants than normal (Figure 4e).

DISCUSSION

Potential of *Trichoderma* spp. for imparting drought tolerance

PEG is the most common salt used for studying the effect of water potential on fungal mycelial growth.[17,26] Various researchers have used the same *in vitro* culture method for the selection of novel *Trichoderma* isolates tolerant to drought[13,27,28] and different soil microbes.[29,30] Microbes from environmentally diverse sites are considered reservoirs of traits that have the potential to benefit a wide range of host plants to abiotic stresses.[30,31] Plants and microbes in mutualism have adapted themselves through the co-evolution of heritable genetic variation, which promotes colonization and

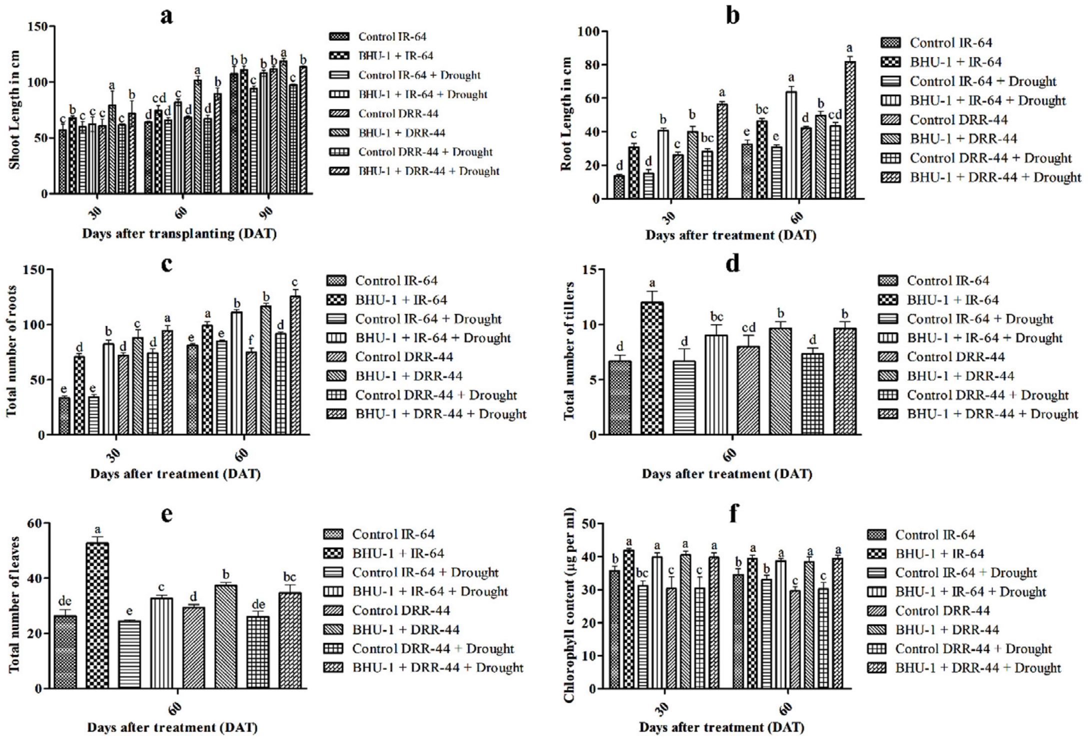

Figure 3. (a) Shoot length of different treatments at 30, 60 and 90 DAT; (b) root length of different treatments at 30 and 60 DAT; (c) total number of roots of different treatments at 30, 60 and 90 DAT; (d) total number of tillers of different treatments at 60 DAT; (e) total number of leaves of different treatments at 60 DAT; and (f) chlorophyll content of different treatments at 30 and 60 DAT. The letters on vertical bars indicate the significance level in the mean standard deviation according to DMRT at $p \leq 0.05$

abiotic stress responses.[32] *Trichoderma* spp. are "environmental generalists and opportunists" capable of rapid evolution for surviving new or stressful environmental conditions as a plant symbiont or a free-living microbe.[33] It is mainly attributed to the production of asexual conidiospores and chlamydospores, which can survive in harsh environmental conditions. Hence, it is very plausible that the BHU-1 isolate of *Trichoderma* spp. in the present investigation can potentially alleviate drought stress in rice as it evolved in the drier region of Uttar Pradesh.

Effects of drought on morphological parameters in the presence of *Trichoderma* spp.

Drought is a primary constraint in rice production, and developing drought-tolerant rice cultivars is very difficult since quantitative traits control them. Due to this stress, there are many morphological effects on rice plants, such as reduced plant height, rolling of leaves, leaf senescence, stomatal closure, low biomass, etc.[34] Moreover, the prolonged water deficit leads to wilting, oxidative damage, and lower photosynthesis and metabolic reactions. Many inherent tolerance mechanisms in rice plants help them overcome these stresses, such as stomatal closure adjustment and alteration of turgor pressure for hydraulic conductivity in leaves and roots. However, these adjustments are insufficient, and thus, the application of *Trichoderma* spp. is proposed for combating the stress as they can alter plant response for enhanced drought tolerance by several mechanisms.[9]

Many *Trichoderma* spp. have been identified as drought-escape microbes in crops by inducing higher relative water content and induction of lateral roots.[35-37] The most universal mode of action of *Trichoderma* spp. is promoting plant growth by enhancing the development of

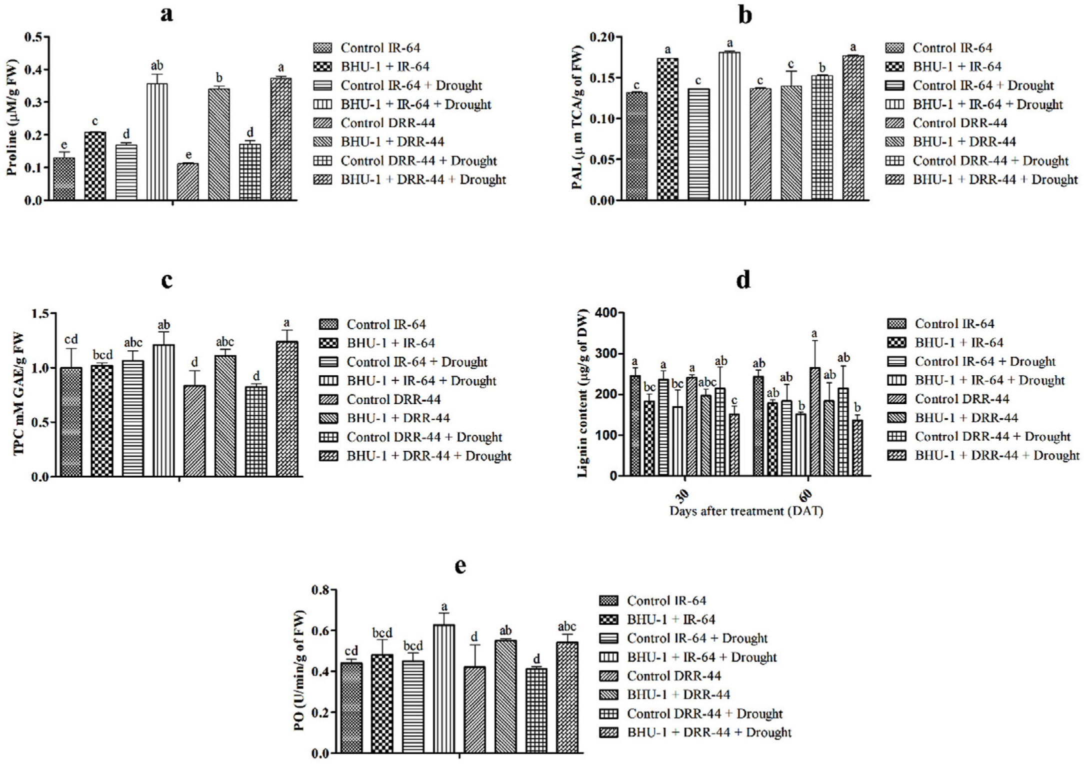

Figure 4. (a) Proline content of different treatments at panicle initiation; (b) PAL activity of different treatments at panicle initiation; (c) TPC of different treatments at panicle initiation; (d) lignin content of different treatments at 30 and 60 DAT; and (e) PO activity of different treatments at panicle initiation. The letters on vertical bars indicate the significance level in mean standard deviation according to DMRT at $p \leq 0.05$

root and shoot. The improved growth of roots serves the dual purpose of providing niches for the growth of fungi and increasing mineral and water uptake.[38] Plant root modifications by *Trichoderma* spp. is the most studied mechanism as it is hypothesized that thicker and deeper roots (Figure 4) might be an imminent mode of drought stress alleviation.[39] However, the trend is not the same every time since roots' response to water deficit is highly correlated to crop genotype and period and drought intensity.[40, 41] Additionally, with an increase in root length, there is increased water conductance owing to the increased area of root contact with soil moisture, which enhances plant productivity under water stress.[42]

Genus *Trichoderma* has been described to upregulate the production of several proteins related to photosynthesis and carbohydrate metabolisms in plants they interact with.[43,44] It directly reflects the prowess of *Trichoderma* spp. in inducing plant growth and energy metabolism. Simultaneously, the secondary metabolite concoction of *Trichoderma* spp. is shown to have several auxin-like metabolites possessing the ability to induce plant growth and development.[12,45] Concurrently, *Trichoderma* spp. also has mineral solubilizing ability, indirectly aiding plant growth promotion by increasing the nutrient uptake in plant-available forms.[46,47] Thus, the root colonization of *Trichoderma* spp. in plants undoubtedly promotes the development of roots and conjointly imparts several benefits.

The levels of proline increase in leaves during abiotic stresses,[19] and the same was observed in the present investigation. However, the increase was comparatively higher in *Trichoderma*-treated seedlings than the untreated ones under drought conditions. Since metabolic processes are protected by proline during the stresses, elevated proline levels become imperative in stabilizing crucial cellular structures.[48] Thus, it is evident that BHU-1 isolate increases the proline accumulation to combat the stress in both cultivars. Additionally, proline also has an essential role in scavenging reactive oxygen species (ROS), maintaining protein and membrane structure, and improving energy generation,[49,50] thus increasing growth in *Trichoderma*-treated plants.

Trichoderma-inoculation enhances PAL activity, and TPC in rice leaves in the present investigation as microbial inoculation is proven to influence the accumulation of polyphenolics and activation of PAL enzyme activity.[51,52] As PAL is a defence-related enzyme and polyphenols are antioxidants, a higher PAL and TPC in leaves are deemed to enhance the tolerance of rice under drought conditions. The phenylpropanoid pathway gets activated during stresses, where PAL is a crucial enzyme that aids in plant structural and physiological protection.[53] TPC accumulation has been concluded to increase in plant cells under different stresses,[54,55] aligning with the results of our present investigation. Priming of plants with *Trichoderma* spp. also increases TPC accumulation under stressed conditions.[56] Thus, it is evident that *Trichoderma* spp. stimulate the accumulation of PAL and TPC during the stressed environments, thereby imparting tolerance. A simultaneous growth improvement in *Trichoderma*-treated rice plants under drought conditions results from oxidative stress protection by higher PAL and TPC.[13] PAL and TPC accumulations are directly related to antioxidative activity and protecting the plants by scavenging ROS and stimulating cell wall formation.[49,50]

The present investigation also shows a positive influence of *Trichoderma* BHU-1 isolate on PAL and TPC, significantly increasing stressed plants compared to the control. The increase could result from the upregulation in defence-related enzyme production in plants stimulated by *Trichoderma* spp. through root colonization.[39,57] Chitinases, peroxidases, hydroperoxide lyase, and β-1,3-glucanase lipoxygenase are the major plant enzymes associated with it. There can also be an accumulation of antimicrobial molecules like phytoalexins resulting from modulated plant metabolism, which impart tolerance against stresses. Lignin deposition of plant tissues strengthens their tolerance to stress as the level of lignification modulates the hydraulic conductivity of vascular tissues during drought.[58] It also serves as plants' mechanical support, decreasing evaporation and water leakage from plant cells.[59] *Trichoderma* spp. are concluded to trigger the production of detoxification proteins in response to ROS production in plants under abiotic stresses. These detoxification proteins serve as ROS scavengers and shield the cells from oxidative damage.[13] The ROS scavenging ability is also evident by lower lipid peroxide concentration in *Trichoderma*-treated plants than the untreated ones.[15] All these results confirm the role of *Trichoderma* spp. in stress alleviation by managing the oxidative damage of ROS.

CONCLUSION

The results revealed that the selected isolates could tolerate *in vitro* drought stress up to 1% polyethene glycol. Seeds and seedlings treatment with *Trichoderma* isolate BHU-1 showed a significant increase in dry weight, root length, flag leaf length, and the total number of tillers under drought-challenged conditions. Moreover, Trichoderma's application efficiently responded to various antioxidative enzymes like PO. It also maintained the level of phenolics in plants by increasing the activity of PAL and TPC, thereby helping the plant sustain itself during abiotic stress. An optimum level of TPC was also observed in leaf extracts, which lower the stress level in plants; similarly, an increased level of lignin was also observed in the roots of rice plants under drought-challenged conditions. Thus, the application of a native drought-tolerant *Trichoderma* isolate is a potential method of alleviating abiotic stresses through the modulation of both morphological and biochemical parameters of plants.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

HBS conceptualized and visualized the study. RY and RNY performed fieldwork. NWZ, PB and HBS applied methodology. PB and SKY performed statistical analyses. RNY wrote the original draft. MMR wrote the manuscript. SKY and MMR reviewed and edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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