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Multifaceted role of *Trichoderma harzianum* isolates in mitigating drought stress and promoting adaptive responses in barley cultivars

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This study investigated the effects of seed biopriming with two *Trichoderma harzianum* isolates, wild type (TW) and gamma-ray mutant (TM), on drought tolerance in the two barley cultivars, Goharan and Nosrat. Barley seeds primed with Trichoderma and subsequently exposed to drought stress induced by polyethylene glycol (PEG 6000) at concentrations of 0%, 5% and 10%. Drought stress significantly reduced growth (20–45% decrease in fresh and dry weight), with Nosrat showing a greater reduction than Goharan. Biopriming with TW and TM significantly improved plant growth (fresh and dry biomass by 4–56%), particularly with TM, and alleviated drought-induced oxidative stress (reduced H_2O_2 and MDA levels) by enhancing antioxidant defense systems (increased superoxide dismutase, peroxidase, and polyphenol oxidase activity) and non-enzymatic antioxidant content (total phenolics, flavonoids, and anthocyanins). TW and TM priming also improved osmotic regulation (increased protein and soluble sugars) and increased IAA content and the activity of key respiratory enzymes (aconitase, succinate dehydrogenase, malate dehydrogenase, and fumarase). These findings highlight the multifaceted role of biopriming with *T. harzianum* isolates in enhancing adaptive responses of barley to drought stress. Seed biopriming with TW and TM significantly improved barley cultivar resilience under drought conditions, suggesting their potential as effective drought tolerance strategies for crops.

Keywords Barley, Drought tolerance, *Trichoderma*, Physiological responses, Respiratory enzymes

Drought stress, driven by rising temperatures and altered precipitation patterns, poses a substantial threat to agriculture, with projections indicating that 50% of cultivable areas will face drought by 2050[1–3]. Drought stress impairs plant physiological and biochemical processes, including photosynthesis, respiration, growth, cell division and expansion, nutrient absorption and transport, and phytohormone metabolism and signaling, while inducing reactive oxygen species (ROS) that cause oxidative stress and membrane damage[4–6].

Plants have developed multiple mechanisms to react to stress, maintaining necessary physiological functions for survival and adaptation. Identifying physiological traits involved in drought tolerance is crucial for developing stress-resistant crop varieties, using approaches such as comparing cultivars with varying drought tolerance and leveraging symbiotic microorganisms to induce stress adaptation pathways.

Seed bio-priming is an innovative and environmentally friendly strategy that involves the application of beneficial microbial agents, such as plant-associated fungi, to enhance seed vigor and improve stress tolerance[7]. By modulating key physiological and biochemical processes, this technique strengthens plant resilience under adverse conditions. Therefore, comparing primed and non-primed plants provides valuable insights into the mechanisms underlying stress resistance.

Trichoderma spp. enhances crop growth through various mechanisms such as nutrient acquisition, production of organic acids and siderophores, generation of antimicrobial metabolites, and synthesis of stress-responsive phytohormones[8]. The effectiveness of *Trichoderma* in assisting various crops, such as rice[9,10] wheat[11] maize[12] and barley[13] in coping with drought stress has been widely studied. These beneficial fungi improve drought

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tolerance through multiple physiological mechanisms, including enhancing antioxidant enzyme activity, reducing oxidative damage caused by reactive oxygen species (ROS), and improving osmotic adjustment via the accumulation of compatible solutes such as proline and soluble sugars[14,15]. Moreover, *Trichoderma* modulates key phytohormones like abscisic acid (ABA) and indole-3-acetic acid (IAA), facilitating better water-use efficiency and root architecture modification, ultimately improving plant growth and biomass under drought conditions[16].

Interestingly, researchers have developed novel Trichoderma mutants to enhance their effectiveness in supporting plants against stress. In previous research, the superior efficiency of a gamma-ray-induced *Trichoderma* mutant in mitigating salt stress in common bean[17] and as a biocontrol agent against *Fusarium oxysporum* f. sp. *Lycopersici* (Fol-101) in tomato plants were demonstrated[18]. This gamma-ray-induced mutant is characterized by distinct sporulation and colonization rates, which differentiate it from the wild-type strain[19].

While *Trichoderma* spp. have been widely studied for their role in enhancing plant stress tolerance, few studies have compared the efficacy of wild-type and gamma-ray-induced mutant strains in mitigating drought stress. Moreover, the genotype-specific responses of barley cultivars to *Trichoderma* biopriming and the underlying physiological and biochemical mechanisms, such as auxin regulation and respiratory enzyme activity, remain underexplored.

This study is the first to compare the effectiveness of *T. harzianum* wild-type (TW) and its gamma-ray-induced mutant (TM) in enhancing drought resistance in two barley cultivars, Nosrat (drought-susceptible) and Goharan (drought-tolerant), while also evaluating their physiological responses to drought stress. Through this approach, we seek to identify the key mechanisms underlying the effects of *Trichoderma* on the physiological properties of barley plants in response to drought stress.

To address these knowledge gaps, this study investigates the effects of seed biopriming with *Trichoderma harzianum* wild-type (TW) and gamma-ray-induced mutant (TM) strains on drought tolerance in two barley cultivars, *Nosrat* (drought-susceptible) and *Goharan* (drought-tolerant). We hypothesize that both TW and TM will enhance drought tolerance in barley by improving antioxidant and respiratory enzyme activity, osmotic adjustment, auxin production, and photosynthetic efficiency, with TM conferring greater benefits, particularly in the drought-susceptible *Nosrat* cultivar. Our objectives were to evaluate the physiological and biochemical responses of these cultivars under drought stress and to elucidate the mechanisms underlying *Trichoderma*-mediated stress tolerance.

Results

Growth attributes

Drought stress significantly reduced shoot length (from 9.5 to 25%) in both barley cultivars compared to controls (Fig. 1). TW and TM priming effectively mitigated this reduction, promoting shoot growth even under drought conditions. Root length responses to drought stress differed between cultivars; Nosrat displayed a decrease (18–23%), while Goharan showed an increase (16%). Notably, TW and TM treatments further enhanced root length in both cultivars under drought. 1 Drought stress significantly decreased these parameters in both cultivars, with Goharan generally showing a more pronounced reduction in shoot fresh weight. Both TW and TM treatments significantly increased shoot and root fresh/dry weight in drought-stressed plants of both cultivars.

Drought stress caused a reduction in IAA content in both shoots and roots of the Nosrat cultivar (Fig. 2). Conversely, drought stress in the Goharan cultivar displayed contrasting effects, decreasing shoot IAA content but increasing it in roots compared to the controls (Fig. 2). Seed biopriming with both TW and TM treatments resulted in a significant increase in IAA content of shoots and roots in both cultivars compared with the non-primed controls. The positive effect of TM on IAA content was greater than that of TW in both the cultivars (Fig. 2).

Photosynthesis pigments

Drought stress had contrasting effects on the chlorophyll precursors and pigments in the two barley cultivars (Table 1). Nosrat cultivar exhibited significant reductions in chlorophyll precursors (PPIX, MgPP, and Pchlide) and chlorophyll content (Chl a, Chl b, and Chl T) under drought conditions. Conversely, the Goharan cultivar displayed an increase in these pigments under drought stress conditions. Both TW and TM biopriming effectively mitigated the drought-induced decline in chlorophyll precursors and pigments in the Nosrat cultivar. In both cultivars, TW and TM seed biopriming significantly increased PPIX, MgPP, Pchlide, Chl a, Chl b, and Chl T contents in drought-stressed plants compared to non-primed drought-stressed plants.

Interestingly, both drought stress and Trichoderma priming (TW and TM) significantly reduced the carotenoid content in both cultivars. However, drought-stressed plants primed with TW or TM exhibited a significant increase in carotenoid content compared to drought-stressed plants (Table 1).

Soluble sugar, protein and amino acid content

Drought stress affected soluble sugar content differently in the two barley cultivars (Fig. 3). The Nosrat cultivar exhibited a decrease in shoot soluble sugar content, whereas the Goharan cultivar showed an increase under drought. Interestingly, TW and TM biopriming treatments significantly increased soluble sugar content in the shoots of both cultivars, even under drought conditions. In the roots, drought stress significantly reduced the soluble sugar content in both cultivars compared to the control. Both TW and TM treatments effectively enhanced the root soluble sugar content in drought-stressed plants of both the cultivars.

3 Both cultivars displayed a significant decrease in shoot and root protein contents under drought stress (Fig. 3). In contrast, TW and TM biopriming treatments significantly augmented the protein content in drought-stressed control plants of both cultivars.

The free amino acid content showed a different trend. Compared with control conditions, drought stress significantly increased the free amino acid content in the shoots and roots of both cultivars (Fig. 3). TW and TM

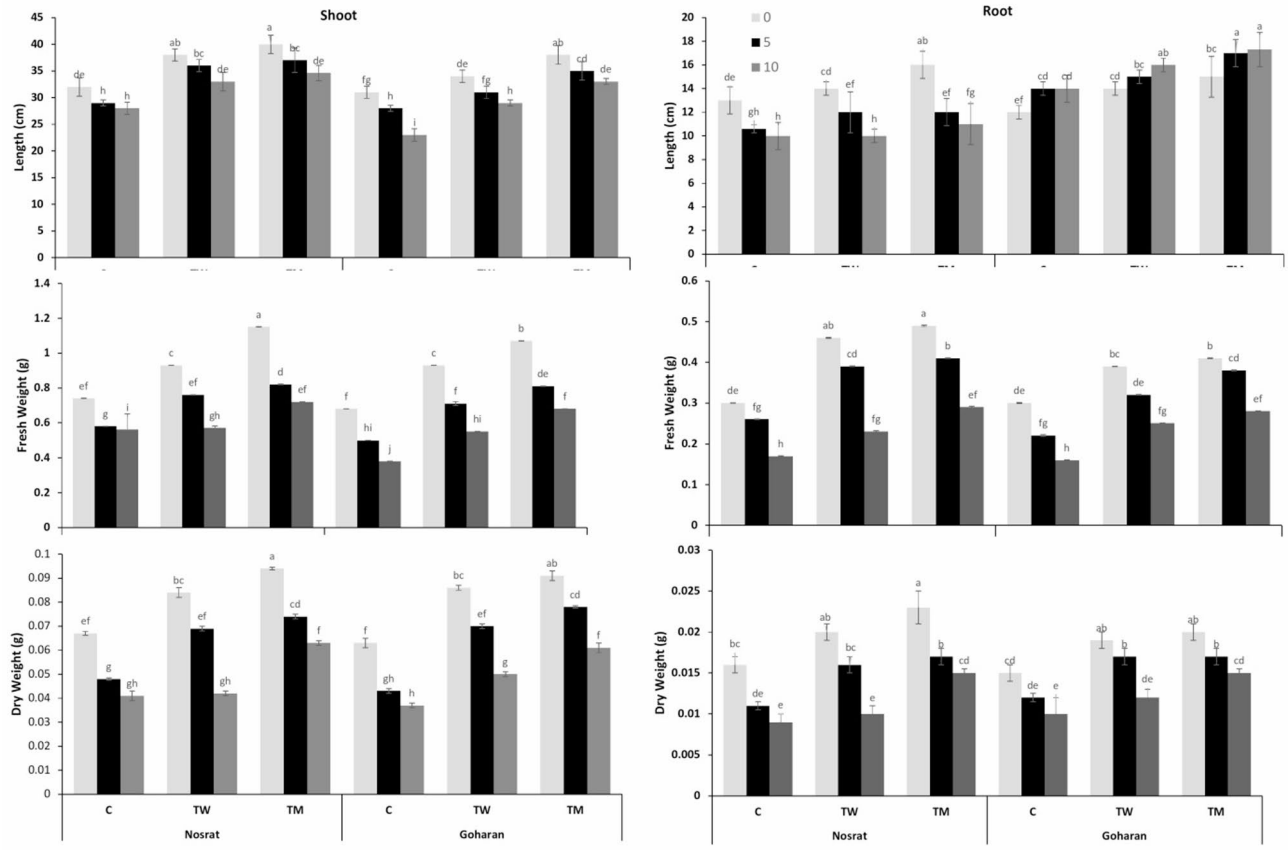

Fig. 1. Shoot and root fresh and dry weights (mg) and lengths (cm) of barley plants influenced by seed biopriming with two genotypes of *Trichoderma harzianum* (TW and TM) under drought stress. Columns indicate mean ± SE based on at least 9 plants of three replicates. Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.

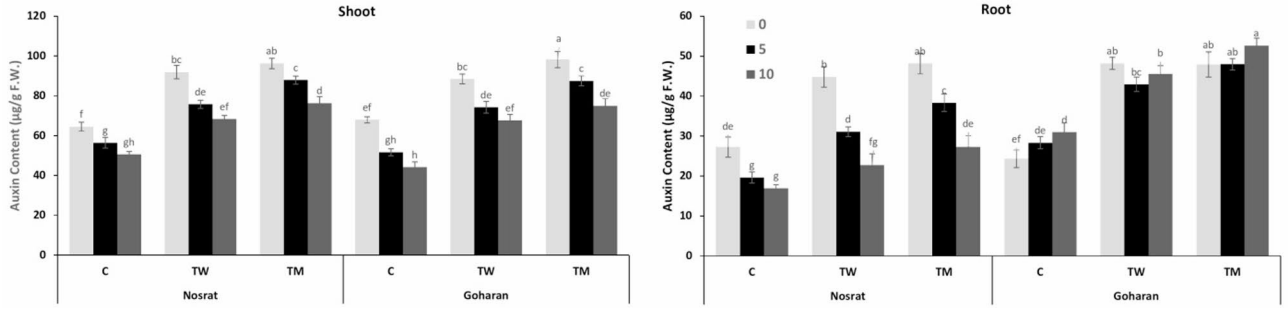

Fig. 2. IAA content in shoots and roots of Nosrat and Goharan cultivars under control and different level of PEG (0, 5 and 10% PEG in 1/2 hogland) following seed biopriming with two genotypes of *Trichoderma harzianum* (TW, TM). Data represent mean values ± standard error (SE) from nine biological replicates.

treatments effectively increased the free amino acid content in both shoots and roots under drought conditions in both cultivars.

H_2O_2 and MDA content

Drought stress enhanced H_2O_2 level in shoot of both cultivars and additional effect of drought stress on H_2O_2 level in Nosrat cultivar was higher than Goharan cultivar (Fig. 4). In shoot, TW and TM priming decreased H_2O_2 level in both cultivars grown under drought conditions. In root of both cultivars, H_2O_2 level significantly increased under drought stress compared to control plants. Oppositely, TW and TM caused significant decrease in H_2O_2 level of root in both cultivars under stressed conditions. MDA is the final product of lipid peroxidation in plant cells. In our work, MDA content was significantly augmented in shoot and root of both cultivars under

PEG level		0			5			10		
Trichoderma		C	TW	TM	C	TW	TM	C	TW	TM
PPIX	N	23.88 ± 1.86 g	35.3 ± 2.18 e	40.37 ± 2.27 bc	20.64 ± 1.9 h	30.58 ± 0.55 f	38.51 ± 0.68 cd	19.06 ± 2.59 h	38.51 ± 0.68 cd	31.12 ± 1.1 f
	G	26.42 ± 2.15 g	43.37 ± 0.81 b	39.42 ± 1.52 cd	34.99 ± 2.6 e	43.37 ± 0.81 b	39.42 ± 1.52 cd	36.1 ± 1.1 de	48.14 ± 2.16 a	42.91 ± 3.16 b
MgPP	N	15.35 ± 0.5 i	16.75 ± 0.34 h	21.7 ± 1.86 d	12.79 ± 0.48 j	14.75 ± 0.65 i	19.61 ± 0.45 e	10.82 ± 0.39 k	13.05 ± 0.22 j	17.67 ± 0.59 gh
	G	18.19 ± 0.27 fg	20.68 ± 0.49 de	19.4 ± 0.42 ef	19.75 ± 0.62 e	23.68 ± 0.59 c	21.79 ± 0.28 d	23.56 ± 0.52 c	29.14 ± 0.82 a	25.96 ± 1.56 b
Pchlide	N	8.76 ± 0.45 f	13.69 ± 0.57 d	15.33 ± 0.91 c	6.32 ± 0.47 g	9.52 ± 0.62 f	11.37 ± 0.35 e	5.28 ± 0.59 h	8.7 ± 0.52 f	10.71 ± 0.69 e
	G	9.31 ± 0.48 f	15.25 ± 0.7 c	12.93 ± 0.72 d	11.18 ± 0.44 e	17.46 ± 0.46 a	16.36 ± 0.55 b	12.93 ± 0.72 d	17.24 ± 0.56 ab	15.36 ± 0.45 c
Chl a	N	29.49 ± 0.85 gh	34.79 ± 1.16 cd	37.63 ± 1.3ab	26.28 ± 1.05 i	29.68 ± 0.64 gh	32.04 ± 1.68 ef	23.2 ± 0.18 j	28.17 ± 1.47hi	28.91 ± 2.12 hi
	G	31.44 ± 1.26 fg	37.04 ± 2.49 bc	34.14 ± 1.77de	34.77 ± 3.05 cd	39.23 ± 1.1 ab	36.89 ± 0.66 bc	38.39 ± 0.35 ab	40.30 ± 1.71 a	37.87 ± 1.33 ab
Chl b	N	13.77 ± 1.62 ij	21.0 ± 0.14 cd	19.96 ± 0.57 ef	10.03 ± 1.6 k	15.3 ± 1.14 hi	16.62 ± 1.97 gh	6.45 ± 1.0 l	11.59 ± 0.71 jk	13.85 ± 1.39 ij
	G	14.66 ± 2.16 hi	19.97 ± 1.78 ef	17.36 ± 0.64 fg	17.92 ± 0.99 fg	24.04 ± 1.49 ab	21.77 ± 1.94 cd	20.78 ± 2.83 de	25.86 ± 1.6 a	23.1 ± 2.92 bc
Chl T	N	43.28 ± 2.37 fg	55.42 ± 2.35 cd	57.72 ± 1.54 bc	36.41 ± 2.46 i	44.74 ± 1.65 fg	48.17 ± 0.46 ef	29.98 ± 2.01 j	38.3 ± 1.95 hi	42.47 ± 3.69 gh
	G	45.49 ± 2.28 fg	57.22 ± 0.7 bc	51.25 ± 0.72 de	52.14 ± 2.66 de	62.33 ± 0.57 ab	58.25 ± 1.88 bc	58.72 ± 2.55 bc	63.47 ± 7.46 a	61.25 ± 4.06 ab
Car	N	4.57 ± 0.29 cd	3.44 ± 0.19 f	3.83 ± 0.12 ef	1.8 ± 0.16 ij	2.11 ± 0.13 hi	2.72 ± 0.17 g	1.25 ± 0.09 k	1.93 ± 0.27ij	1.91 ± 0.21 ij
	G	4.66 ± 0.21 bc	3.5 ± 0.41 f	3.44 ± 0.23 f	2.35 ± 0.23 h	4.38 ± 0.29 cd	4.19 ± 0.07 de	1.55 ± 0.22 jk	4.99 ± 0.15 a	4.92 ± 0.1 ab

Table 1. The content of protoporphyrin IX (PPIX), Mg-protoporphyrin IX (Mg-PPIX), and protochlorophyllide (Pchlide), chlorophylls (Chl), carotenoids (Car) in shoot samples of barley plants influenced by seed biopriming with two genotypes of *Trichoderma harzianum* (TW and TM) under different level of drought stress. Columns indicate mean ± se based on three replicates. Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test. C=Control, N = Nosrat cultivar, G= Goharan.

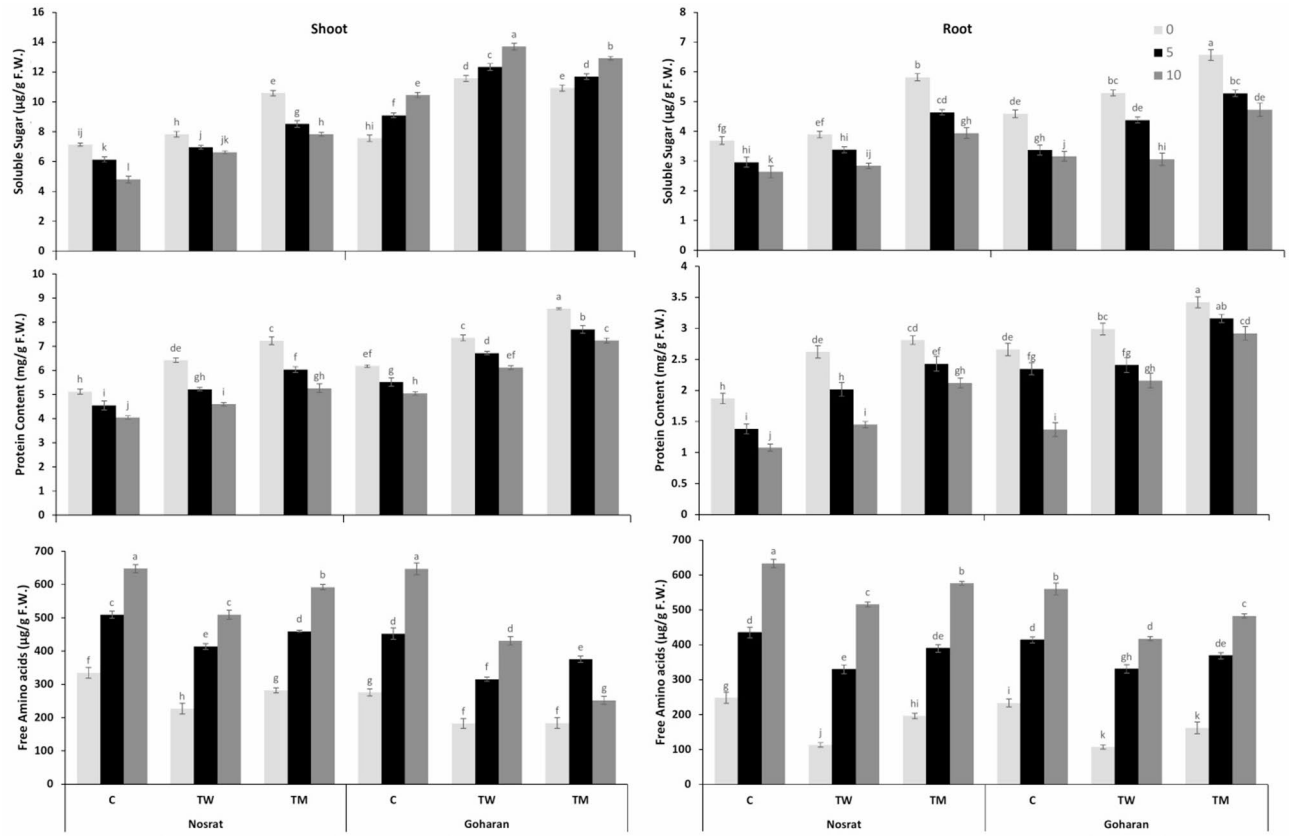

Fig. 3. Total soluble sugars, protein and free amino acids content in root and shoot samples of barley plants subjected to seed biopriming with two genotypes of *Trichoderma harzianum* (TW and TM) under three different levels of drought stress: control (0% PEG 6000), moderate drought stress (5% PEG 6000), and severe drought stress (10% PEG 6000). Columns represent mean ± SE based on three replicates. Means denoted by different letters indicate a significant difference at $p \leq 0.05$, determined by Duncan's multiple range test.

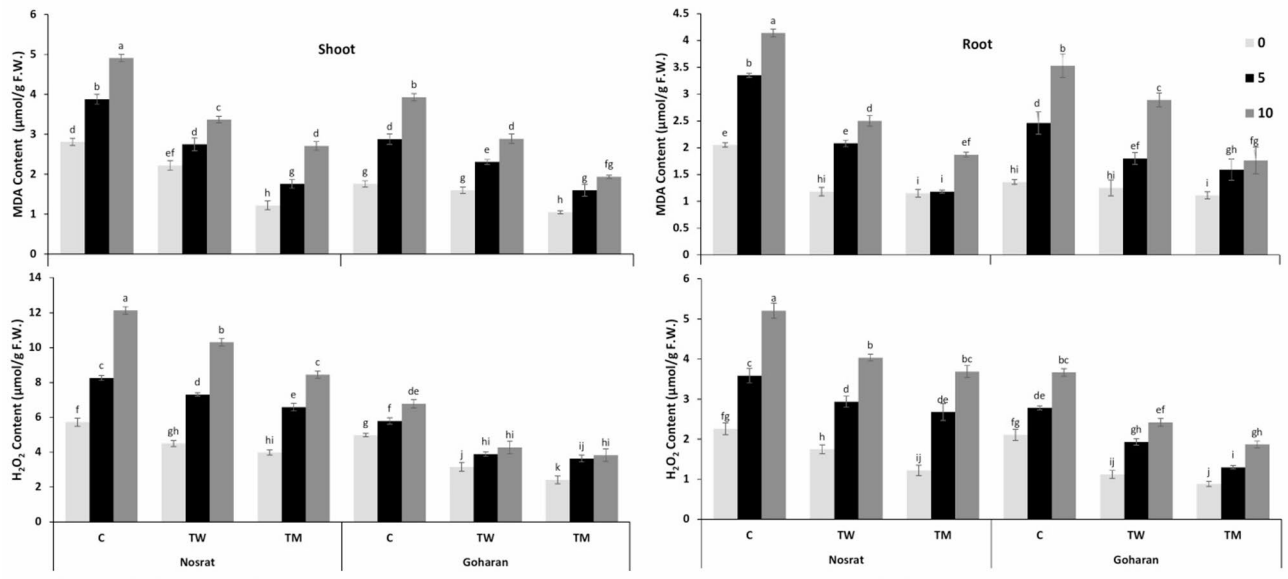

Fig. 4. H_2O_2 and MDA content in root and shoot samples of barley plants subjected to seed biopriming with *Trichoderma harzianum* (TW and TM) under three different levels of drought stress: control (0% PEG 6000), moderate drought stress (5% PEG 6000), and severe drought stress (10% PEG 6000). Columns represent mean ± SE based on three replicates. Means denoted by different letters indicate a significant difference at $p \leq 0.05$, determined by Duncan's multiple range test.

drought conditions, and this effect was more remarkable in Nosrat cultivar than Goharan cultivar. In shoot and root of both cultivars, MDA content significantly declined with TW and TM in drought-treated plants. So, decreasing trend on MDA content by TM was dominant than TW (Fig. 4).

Antioxidant enzymes

Drought stress caused a reduction in SOD activity in the shoots of Nosrat cultivar. However, in Goharan cultivar, the highest drought level (10% PEG) led to a significant increase in shoot SOD activity compared to control plants (Fig. 5). Root SOD activity in Nosrat remained unchanged under drought, while both drought levels increased SOD activity in the roots of Goharan cultivar. Seed biopriming with both TW and TM elevated SOD activity in shoots and roots of both cultivars under control and drought conditions. The positive effect of TW and TM on SOD activity was more pronounced in Goharan compared to Nosrat.

Drought stress resulted in an increasing trend of POX activity in both shoots and roots of both cultivars compared to controls (Fig. 5). Seed biopriming with TW and TM further induced POX activity in shoots and roots of both cultivars under drought conditions. The effect of TM was more dominant than TW. Exposure to drought stress caused a significant increase in PPO activity in both shoots and roots of both cultivars. Application of TW and TM further augmented PPO activity in shoots and roots of drought-stressed Nosrat and Goharan cultivars (Fig. 5).

Non-enzymatic antioxidants

Phenolics content in shoot and root decreased in Nosrat cultivar and increased in Goharan cultivar under drought stress (Fig. 6). In shoot and root of both cultivars, considerable increase in phenolics content occurred when plants primed with TW and TM before drought conditions. In shoot and root, there was no difference in flavonoids content of Nosrat cultivar between drought-stressed and well-watered plants. In Goharan cultivar, two levels of drought increased flavonoids content in shoot, but high level of drought enhanced this content in root (Fig. 6). TM application induced flavonoids content in shoot of Nosrat cultivar, while TW and TM caused non-significant change in root under drought stress. In contrast to Nosrat cultivar, TW and TM enhanced flavonoids content in shoot and root of Goharan cultivar under stress conditions. In shoot and root, drought treatment caused a considerable reduction in anthocyanin content in Nosrat cultivar when compared to the control plants; while in drought-exposed Goharan cultivar anthocyanin content increased in shoot and root. TW and TM enhanced anthocyanin content in shoot and root of Nosrat and Goharan cultivar under drought conditions (Fig. 6).

Respiratory enzymes

Drought stress decreased fumarase activity in shoot and root of Nosrat cultivar as compared to normal conditions (Fig. 7). Treatment with 10% PEG induced fumarase activity in shoot, while two concentrations of PEG increased this activity in root of Goharan cultivar. In drought conditions, TW and TM priming augmented fumarase activity in shoot and root of both cultivars. Drought stress reduced SDH activity in shoot, but 10% PEG increased SDH activity in root of Nosrat. In stressed-Goharan cultivar, SDH activity induced in shoot and root as

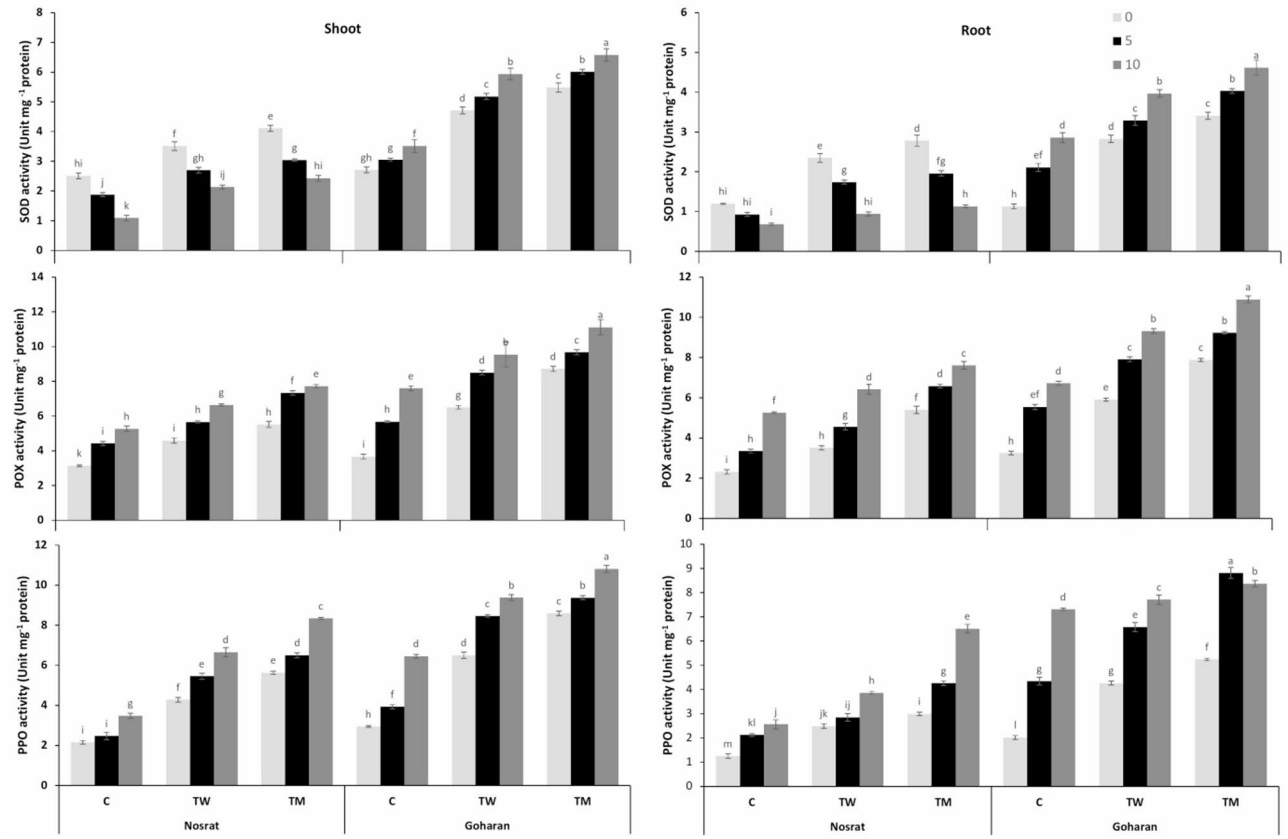

Fig. 5. Changes in activity of antioxidant enzymes (SOD, POX, and PPO) in root and shoot samples of barley plants influenced by seed biopriming with two genotypes of *Trichoderma harzianum* (TW and TM) under drought stress. Data are presented as mean ± SE (standard error) of three biological replicates. Different letters within a treatment (root or shoot) and enzyme indicate significant differences ($p \leq 0.05$) according to Duncan's multiple range test.

compared to unstressed plants (Fig. 7). SDH activity of shoot and root boosted in Nosrat and Goharan cultivar exposed to drought stress by TW and TM. Aconitase activity induced in shoot and root of both cultivars; also TW and TM augmented aconitase activity in stressed plants. In both cultivars, MDH activity decreased in shoot and root under drought stress (Fig. 78). TW and TM induced MDH activity in shoot of both cultivars under stress; impact of TM was more in Nosrat cultivar, but effect of TW was higher in Goharan cultivar. Priming with TW and TM increased MDH activity in root of both cultivars and positive effect of TM was upper than TW.

PCA analyses

Based on correlation analyses based on Pearson's coefficient, DW exhibited positive correlations with chlorophyll, PPIX, MgPP, Pchlide, SOD, POX, PPO, soluble sugar, protein, total phenol, flavonoid, anthocyanin, SDH, fumarase, MDH and auxin but showed negative correlation with H_2O_2, MDA in shoot (Fig. 8). In root, DW exhibited positive correlation with SOD, soluble sugar, protein, total phenol, SDH, fumarase, MDH and auxin and showed negative correlation with MDA and H_2O_2. In shoot, MDA and H_2O_2 negatively correlated with antioxidant systems includes enzymatic (SOD, POX and PPO) and non-enzymatic (total phenol, flavonoid and anthocyanin). In root, MDA and H_2O_2 displayed positive correlations to each other and showed negative correlation with total phenol, SOD, POX, PPO and anthocyanin.

Discussion

Drought stress represents a significant threat to global agriculture due to its disruptive effects on plant growth[20] photosynthesis[21] respiration[22] and hormonal balance[23]. These effects are often mediated by oxidative stress[24]. In this regard, seed bio-priming with beneficial microbes, such as *T. harzianum*, presents a promising strategy to enhance plant resilience under water-deficit conditions[25].

Our results align with reported drought-induced reductions in total biomass[26] and root elongation[27] while also revealing genotype-specific morphological strategies. Comparative growth analysis under PEG-induced drought stress showed that Nosrat experienced relatively smaller reductions in shoot fresh weight (FW), dry weight (DW), and length under both 5% and 10% PEG, suggesting a stronger tendency to preserve aerial biomass under water deficit. In contrast, Goharan exhibited a pronounced root-centered adjustment, with a 16% increase in root length under 10% PEG, strongly correlated with elevated root auxin levels ($r = 0.92$, $p < 0.01$)—a

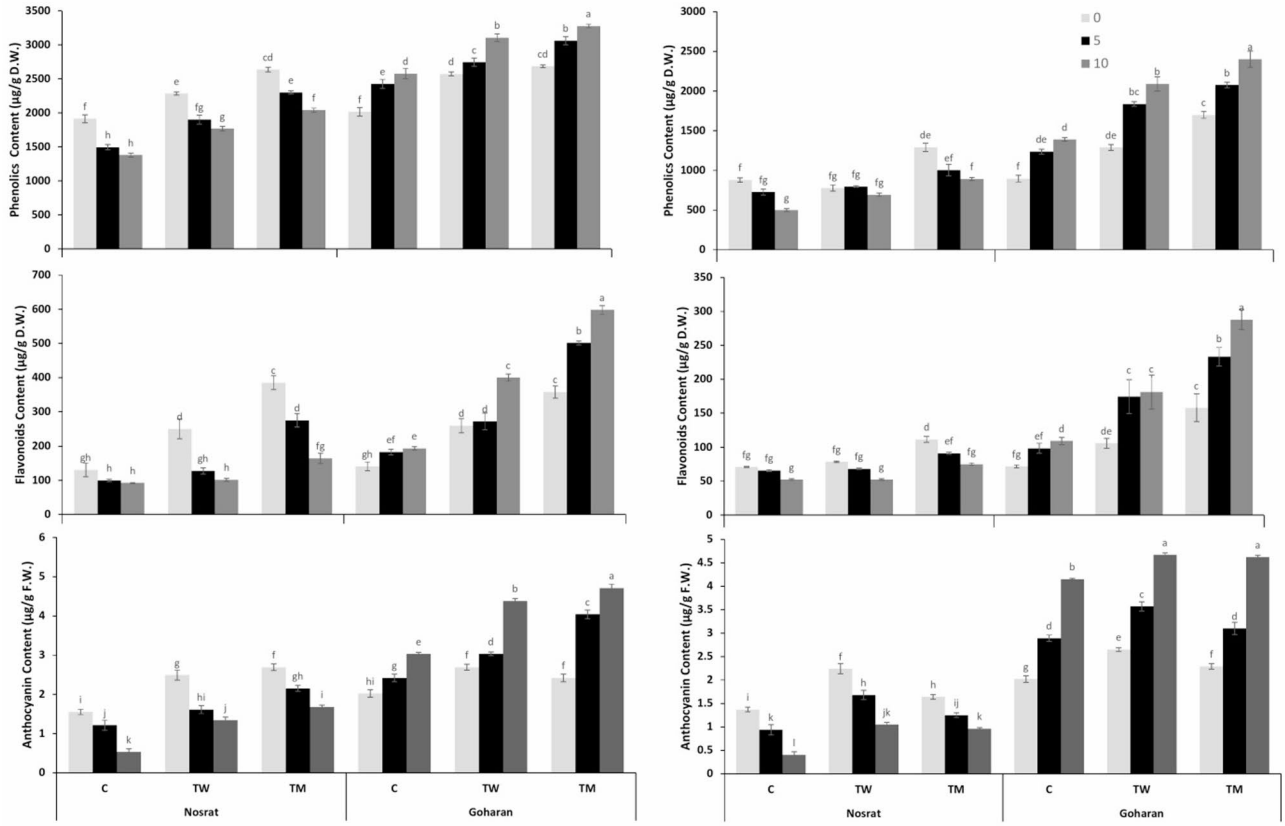

Fig. 6. Changes in phenolics, flavonoids, and anthocyanin content in root and shoot samples of barley plants influenced by seed biopriming with *Trichodermaharzianum* (TW and TM) under drought stress. Columns indicate mean ± SE based on three replicates. Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.

hormonal shift commonly linked to improved root development and drought adaptation. These patterns are consistent with previous findings that drought-tolerant genotypes often promote primary root growth to access deeper soil moisture[6,28].

Osmotic regulation in plants under drought stress relies on synthesis and accumulation of osmolytes such as soluble sugars, soluble protein and amino acid[29]. In our work, two barley cultivars used different compatible osmolytes to overcome the osmotic stress resulting from drought stress. Amino acids content increased in Nosrat cultivar, but soluble sugar and amino acids content enhanced in Goharan cultivar. Soluble sugars act in osmotic regulation, osmotic protection ROS detoxification against multiple stresses[30]. Increased content of soluble sugars can significantly decline the osmotic potential and thus performance a role in the osmoregulatory mechanisms under drought conditions[31].

Drought stress significantly alters plant metabolism, leading to the accumulation of reactive oxygen species (ROS), which disrupt cellular processes and cause damage to cellular components such as membranes, proteins, and DNA[32]. To mitigate these effects, plants rely on various defense mechanisms to maintain cellular homeostasis, including enzymatic and non-enzymatic systems that regulate oxidative stress[33]. Superoxide dismutases (SODs) catalyze the dismutation of superoxide anions, while peroxidases (POXs) and polyphenol oxidases (PPOs) detoxify hydrogen peroxide (H_2O_2) [34]. Phenolic compounds also contribute directly to ROS scavenging[35,36].

Elevated H_2O_2 and MDA levels (63–158% in Goharan, 37–100% in Nosrat) confirmed severe oxidative stress. Both cultivars increased POX/PPO activity, but only Goharan upregulated SOD, flavonoids and anthocyanins. However, the persistent MDA accumulation indicates these responses were insufficient for complete ROS neutralization. Further profiling of antioxidants (e.g., catalase, ascorbate) and free radicals could clarify whether this imbalance stems from inadequate capacity, delayed scavenging, or tissue-specific redox vulnerabilities. Interestingly, Nosrat maintained better biomass despite weaker non-enzymatic responses, suggesting alternative protective mechanisms.

Principal component analysis (PCA) identified SDH, MDH, flavonoids, and chlorophylls as major discriminators between cultivars. Under drought stress (10% PEG), Nosrat selectively upregulated aconitase (ACO) activity in both roots and leaves, while other TCA cycle enzymes (SDH, MDH, FUM) were downregulated. This focused activation coincided with a substantial accumulation of free amino acids (93% in leaves and 155% in roots), suggesting a metabolic rerouting toward 2-oxoglutarate production via isocitrate, a key node linking carbon and nitrogen metabolism[37,38]. 2-oxoglutarate serves as a precursor for glutamate and downstream amino acid synthesis, including GABA, which typically accumulates under abiotic stress[39]. Despite this, Nosrat

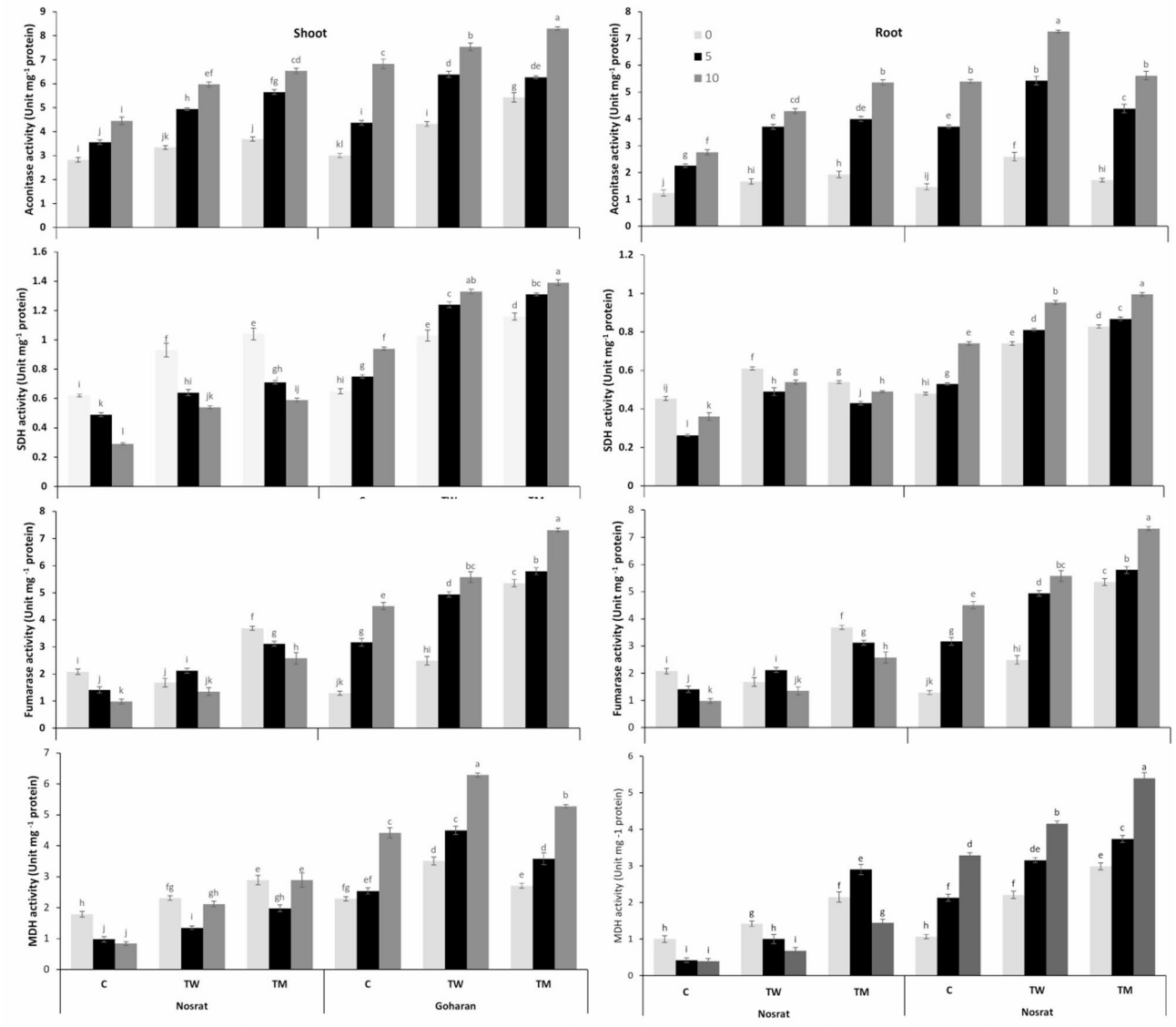

Fig. 7. Changes in activity of respiratory enzymes in root and shoot samples of barley plants influenced by seed biopriming with Trichoderma (TW and TM) under drought stress. Columns indicate mean ± SE based on three biological replicates. Means with different letters indicate a significant difference at $p \leq 0.05$ using Duncan multiple range test.SDH=succinate dehydrogenase, MDH=malate dehydrogenase, C=Control

exhibited a reduction in flavonoid content (29% in leaves and 26% in roots), implying that its stress response favored nitrogen-related metabolic pathways over antioxidant biosynthesis[40,41] .

In contrast, Goharan showed a coordinated upregulation of all analyzed TCA cycle enzymes and concurrently accumulated both free amino acids (134% in leaves and 139% in roots) and flavonoids (37% and 51% in leaves and roots, respectively). This pattern reflects a broader metabolic activation under drought, involving both energy production and secondary metabolism, likely supported by enhanced mitochondrial flux and 2-oxoglutarate availability for diverse biosynthetic routes including flavonoid and stress-related pathways[42,43].

Notably, while most studies report a drought-induced decline in chlorophyll content due to oxidative damage and impaired pigment biosynthesis[44] our results revealed contrasting genotype-specific responses. Under 10% PEG-induced drought stress, Nosrat showed a marked decrease in chlorophyll a and b, indicating a loss of photosynthetic capacity. In contrast, Goharan exhibited increased levels of both chlorophyll a (22%) and b (41%), suggesting an adaptive mechanism for maintaining or even enhancing photosynthetic function under stress. These contrasting trends align with previous findings that highlight the role of genetic background in modulating chlorophyll stability and stress resilience[45–47].

To further explore the mechanisms underlying drought tolerance, we examined the role of microbial priming using two strains of *T. harzianum*: a wild-type (TW) and a gamma-irradiated mutant (TM). These strains are known to influence plant growth and stress adaptation and their effects, however, can be genotype-dependent. The gamma-irradiated mutant (TM) strain demonstrated superior efficacy in mitigating drought impacts,

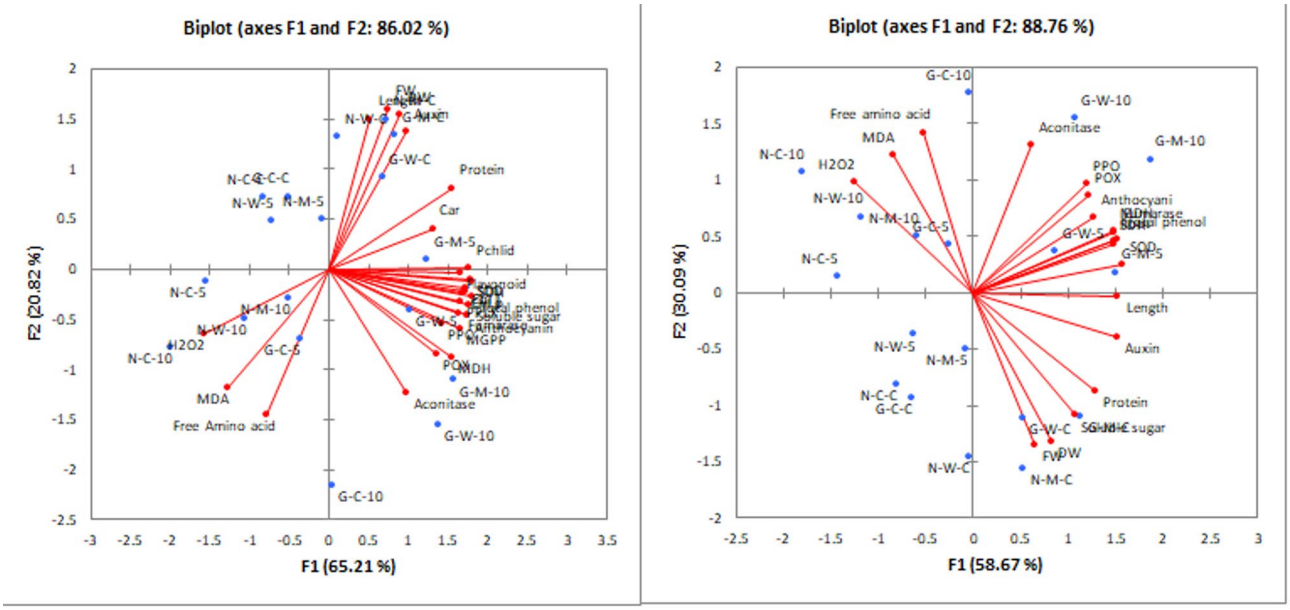

Fig. 8. Principal Component Analysis (PCA) of all assayed parameters of shoot and root of barely plants influenced by seed biopriming with two genotypes of *Trichoderma harzianum* (TW and TM) under three level of drought stress (0, 5 and 10% PEG).

particularly in root systems. TM treatment restored 52.7–77.5% of shoot biomass and 72.5–77.3% of root biomass lost to drought stress, outperforming the wild-type strain. In Nosrat, TM's ability to enhance auxin levels in roots (61–70% increase under stress) correlated strongly with improved root architecture, suggesting auxin-mediated pathways as a key mechanism for drought resilience in this genotype. This aligns with established research showing that auxin plays a pivotal role in root development and drought response[48] . Notably, *Trichoderma* may produce indole-3-acetic acid (IAA) and indole-3-acetaldehyde (IAAld), which activate auxin signaling pathways and promote root elongation, as demonstrated in Arabidopsis[49].

Auxin content and growth responses to *Trichoderma* priming also revealed genotype-specific effects. TM increased auxin content in both roots and leaves of Nosrat (+61% and +51%, respectively), coinciding with improved root and shoot growth. However, TM failed to stimulate auxin accumulation in Goharan and was associated with reduced root and shoot elongation. These results suggest that *Trichoderma*-induced auxin pathways may be more responsive or inducible in Nosrat, whereas Goharan's drought responses may rely more on auxin-independent mechanisms.

However, it should be noted that critical physiological parameters such as relative water content (RWC) and water use efficiency (WUE) were not measured in this study, limiting our ability to fully evaluate the hydraulic mechanisms underlying the observed improvements in drought tolerance.

TW and TM pretreatment by increasing protein and soluble sugars content helped to osmotic regulation in two cultivars under drought stress conditions. The accumulation of osmolytes contributions in osmotic regulation to sustain membrane structural integrity and cell hydration in response to drought stress[50]. *T. longibrachiatum* treatment increased the accumulation of soluble protein and soluble sugars under drought stress, which may play important functions in controlling the osmotic equilibrium of *Pinus massoniana* to decline water loss[51]. *Trichoderma* may be able to provide tolerance to the treated seedlings by accumulating the substances capable of creating osmotic regulation as well as energy storage[52,53].

Several studies have reported that *Trichoderma* spp. can alleviate drought-induced oxidative stress by enhancing antioxidant defenses[16,54]. Zhang et al. [55] indicated that Trichoderma regulated the transcriptional level of ROS detoxifying enzymes genes in response to stress. Consistent with this, both TW and TM strains mitigated oxidative damage under PEG stress, though TM showed overall stronger effects. TM reduced H_2O_2 and MDA more effectively in Goharan leaves, and stimulated distinct antioxidant responses in each genotype: SOD and PPO were more strongly induced in Nosrat, while flavonoids and total phenolics were higher in Goharan. In roots, a similar pattern emerged, with TM enhancing POX and phenolics in Goharan, and SOD, flavonoids, and anthocyanins in Nosrat. These observations highlight the complexity of cultivar–microbe interactions and suggest that both genetic background and microbial strain influence the resulting stress mitigation strategy. Trichoderma treatment induced the *PAL* gene transcripts and PAL activity and trigged higher accumulation of the phenolic compounds helped plants to cope oxidative stress created due to drought conditions[52].

A major distinguishing feature of TM was its effect on respiratory metabolism. While previous studies have demonstrated transcriptional upregulation of TCA cycle genes in *Trichoderma*-colonized plants[56] our biochemical assays confirm TM's capacity to boost enzyme activity. TM substantially increased root MDH (+265%) and fumarase (+162%) in Nosrat and enhanced MDH (+243%) and SDH (+104%) in its leaves. In

Goharan, these increases were more moderate, suggesting that the more pronounced TCA upregulation in Nosrat may compensate for its inherently higher oxidative burden or lower baseline energy status under stress.

TM significantly increased chlorophyll a, b, and their biosynthetic precursors in Nosrat, enhancing photosynthetic capacity under drought stress. This is likely due to upregulation of chlorophyll biosynthesis genes, such as *CHLH* and *NADPH: protochlorophyllide oxidoreductase* (*POR*), triggered by Trichoderma-derived symbiont-associated molecular patterns (SAMPs)[18,53]. In contrast, Goharan showed modest chlorophyll changes but stronger carotenoid induction, supporting photoprotection via non-photochemical quenching and ROS scavenging[57,58]. These genotype-specific responses indicate Nosrat prioritizes chlorophyll accumulation, while Goharan relies on carotenoid-mediated photoprotection under prolonged stress.

The observed genotype-specific effects of the mutant strain (TM) under drought stress are in line with our previous findings on tomato under biotic stress, where TM pretreatment markedly enhanced plant biomass, photosynthetic pigment accumulation, and antioxidant enzyme activities in *Fusarium*-challenged plant[18]. Notably, TM induced higher activities of SOD, POX, and PPO, and significantly reduced H_2O_2 and MDA levels—confirming its strong capacity to mitigate oxidative damage. A similar trend was observed in the present study, where TM not only suppressed oxidative stress markers more effectively than the wild type, but also stimulated differential antioxidant and hormonal responses depending on genotype. This consistency across different crops and stress types highlights the robust potential of the TM strain in priming plant defense and metabolic adjustment under diverse environmental challenges.

In conclusion, this study demonstrates that *T. harzianum*, particularly its gamma-irradiated mutant (TM), significantly enhances drought resilience in barley through genotype-specific modulation of growth, antioxidant defense, and auxin-mediated root adaptation. The TM strain's ability to restore > 50% of lost biomass and stimulate root auxin levels highlights its potential as a bio-tool for sustainable agriculture. Our findings strongly support the initial hypothesis that *Trichoderma harzianum* and its gamma-ray-induced mutant promote drought tolerance through interconnected physiological and biochemical mechanisms, including hormonal signaling, ROS detoxification, and metabolic regulation. Future research should: (1) Investigate Trichoderma's impact on water relations (e.g., RWC, WUE, and aquaporin expression), (2) Validate these findings under field conditions with yield-based assessments, and (3) Explore molecular crosstalk between fungal-derived signals and host stress-response pathways (e.g., ABA-auxin interplay) to optimize strain-cultivar matching.

Conclusion

Drought stress disrupted normal metabolism and reduced growth in both cultivars. Induction of H_2O_2 and MDA showed that drought stress caused oxidative stress in both cultivars. TW and TM strengthened adaptative responses in both cultivars and improved stress tolerance and growth. TW and TM enhanced antioxidant systems included SOD, POX and PPO activity as enzymatic antioxidants and phenolic compounds as non-enzymatic antioxidant, osmotic substances, auxin content and respiration enzymes such as aconitase, fumarase, SDH and MDH activity and photosynthesis pigments in both cultivars. Thus, further full studies are desired to complete our understanding of how TW and TM control the physiochemical responses in barley plant.

Materials and methods

All the plant experiments were in compliance with relevant intuitional, nation, and international guidelines and legislation. All experimental protocols were approved by the Ethics Committee of Alzahra University (IR.ALZAHRA.REC.1402.010).

The seeds of two barley cultivars, Nosrat (drought-susceptible) and Goharan (drought-tolerant), were received from the Seed and Plant Certification and Registration Institute (SPCRI) of I.R. Iran, and used for bio-priming. The seeds were surface sterilized with a mixture of 5% hydrogen peroxide and 95% ethanol (1:1 v/v) for 3 min and then rinsed at least six times with sterile distilled water.

T. harzianum NAS107 isolate (TW, NCBI code: MW718882) and its derived mutant (TM, NAS107-M7) were provided by the Nuclear Agriculture Research Institute collection (NSTRI, AEOI). The gamma-ray-induced mutant was described with distinct sporulation and colonization rates[17]. The Trichoderma spore suspension, with a concentration of 1×10^8 spores/ml, was prepared on Trichoderma Complete Medium (TCM)[59]. The biological coating mixture (consisting of 5% v/v spore suspension of Trichoderma (TW or TM), 1% v/v Bayer seed coating dye™, and 2.5% carboxymethylcellulose (CMC)) was used for seed biopriming of barley with Rezaloo et al.[60] seed coating method. The control was prepared by coating the barley seeds with the same coating mixture without Trichoderma in TCM. In each treatment, 100 g of barley seeds were incubated with the coating mixture (biological and control) with shaking at 180 rpm for 15 min at room temperature.

Seven barley seeds were germinated in pot containing sterilized perlite for 48 h and then three healthy seedlings of the same size were remained in each pot. The pots were kept in a growth chamber at 25 °C, with relative humidity levels set at 50–55%. The photoperiod was set at 16 h, and the photosynthetic photon flux density ranged from 850 to 950 µmol/m^2/s. The pots were irrigated regularly at 48 h intervals with 1/2 Hoagland's solution for 14 days in the growth chamber. The pots were then moved to a greenhouse for the drought stress treatment. After 10 d in the new environment, drought stress was applied at three levels: 0%, 5%, and 10%. In this study, polyethylene glycol with a molecular weight of 6000 (PEG 6000) was used to create artificial environments to control water availability and to evaluate drought tolerance in a controlled setting. This study employed a randomized complete block design with three treatments: control (0% PEG 6000), moderate drought stress (5% PEG 6000), and severe drought stress (10% PEG 6000). Each treatment was replicated three times, resulting in 27 pots arranged across three blocks to account for spatial variability within the greenhouse. By adding 5% and 10% PEG to Hoagland's solution for 20 days and ensuring that the PEG solution did not accumulate in the pots,

the pots were treated only with 1/2 Hoagland's solution for washing after every three drying cycles. Finally, plant samples were harvested 34 days after planting, following the observation of drought effects on the plants.

For physiological and biochemical assays, plants were flash-frozen in liquid nitrogen, kept at −70 °C and used for subsequent assays. Samples from three plants per treatment per replicate were combined and used as one replicate ($N = 3$ plants ×3 treatments×3 replicates). Photosynthetic pigments were quantified in the leaves, and other biochemical analyses were performed on shoot and root samples.

Plant biomass, photosynthetic pigments and auxin assay

Biometrical data from nine plants were collected, including the mean values of shoot and root fresh and dry weights (mg) and lengths (cm).

The contents of chlorophyll, carotenoids, and chlorophyll precursors in the leaves were determined using the described method[61,62]. Briefly fresh leaves material (0.1 g) was extracted with 80% acetone by using a mortar and pestle. The absorbance of the solutions was measured at various wavelengths using a spectrophotometer. The concentration of photosynthetic pigments was expressed as mg g^{-1} fresh weight (FW).

Auxin production was quantified using the Salkowski reagent, following the method described by Luziatelli et al.[63]. Briefly, 1 ml of filter-sterilized (0.22 µm) supernatant was mixed with 2 ml of Salkowski reagent (0.5 M $FeCl_3$ in 35% v/v $HClO_4$) and incubated at room temperature in the dark for 20 min. The presence of indole-3-acetic acid (IAA) and other indole auxins was detected by measuring the development of a pink color at 535 nm using a Cary 50 UV–Vis spectrophotometer (CECIL CE 9000). A calibration curve was established using a series of IAA standard solutions of known concentrations.

Measurement of free amino acids and soluble sugar

Following the method described by Lee and Takahashi[6463]the free amino acid content was determined. Plant materials were homogenized in a 50 mM phosphate buffer (pH 6.8) and subsequently centrifuged at 14,000 ×g for 15 min. The detection of free amino acids involved extracting samples with 70% ethanol. A 1 ml aliquot of the extract was added to a reaction mixture comprising 0.5 ml acetate buffer, 0.5 ml ninhydrin solution, and 3 ml of 55% glycerol-water diluent. This mixture was then boiled at 100 °C for 30 min. After cooling to room temperature, the absorbance was measured spectrophotometrically at 570 nm, and the free amino acid content was quantified using glycine as a standard.

The method of Dubois et al.[6564] was used to evaluate the soluble sugar content. Fresh plant tissue (0.1 g) was homogenized in 3 ml of distilled water and subsequently centrifuged at 5000 rpm for 20 min. The resulting supernatant (500 µl) was combined with 450 µL distilled water, 500 µL 5% phenol, and 97% sulfuric acid (2.5 ml). After 30 min, the absorbance was measured at 485 nm.

Determination of oxidative indicators and antioxidant enzymes

The procedure described by Heath and Packer[6665] was used to determine MDA content. First, the leaf tissue (0.5 g) was homogenized in 0.1% trichloroacetic acid (TCA) and subsequently centrifuged at 13,000 rpm for 10 min. The resulting supernatant (0.5 ml) was combined with 1 ml of thiobarbituric acid (0.5%) in 20% TCA. The mixture was heated at 95 °C for 30 min, followed by centrifugation at 13,000 rpm for 15 min. Finally, the absorbance of the supernatant was measured at 532 nm and 600 nm.

The Velikova et al.[6766] method was used to determine the H_2O_2 content. Briefly, 0.5 g of plant tissue was homogenized in 0.1% TCA and then centrifuged at 12,000 rpm for 15 min. After centrifugation, 0.5 ml of the supernatant was combined with 0.5 ml of potassium phosphate buffer (pH 7.0) and 1 ml of potassium iodide (1 M). The absorbance of the mixture was measured at 390 nm wavelength.

To determine the different enzyme activities, plant material (0.5 g) was mixed thoroughly with 1 M Tris-HCl (pH 6.8) at 4 °C. The resulting homogenate was centrifuged at 13,000 rpm for 20 min at 4 °C. The supernatant obtained after centrifugation was stored at −70 °C and used for enzyme assays at a later stage. The protein content was determined using bovine serum albumin serving as the reference standard[6867].

The activity of superoxide dismutase (SOD) was evaluated using the method described by Giannopolitis and Ries[6968]. The reaction mixture consisted of potassium phosphate buffer (50 mM), 0.1 mM EDTA, 13 mM methionine, 75 µM NBT, 75 µM riboflavin, and 100 µl of the enzyme extract. The reaction mixture was exposed to light for 18 min and the absorbance was recorded at 560 nm.

The activity of proxidase (POX; EC 1.11.1.7) was measured according to Abeles and Biles's protocol[7069]. Reaction mixture consisted 0.1 ml benzidine (40 mM), 0.2 ml H_2O_2 (3%), 2 ml of 0.2 M acetate bufer (pH 4.8) and 50 µl of enzyme extract. Activity of this enzyme was recorded at 530 nm. POX activity was defined as 1 µM of benzidine oxidized per minute per mg protein [Unit mg^{-1} (protein)].

The activity of polyphenol oxidase (PPO) was assessed using the method described by Raymond et al.[7170]. In the reaction mixture, there was 2.5 ml of 200 mM potassium phosphate buffer (with a pH of 6.8), 0.2 ml of 20 mM pyrogallol, and 20 µl of enzyme extract. Enzyme activity was measured at a wavelength of 430 nm. PPO activity was defined as 1 µM of pyrogallol oxidized per minute per mg protein [Unit mg^{-1} (protein)].

Assay of phenolic compounds

Dry tissue (0.1 g) was homogenized in 5 ml of 80% methanol and centrifuged at 5000 rpm for 20 min. For the total phenol content measurement, 0.1 ml of the methanolic extract was mixed with 2.5 ml of 10% Folin-Ciocalteu reagent. The mixtures were neutralized by sodium bicarbonate 7% and the absorbance was recorded at 765 nm[7271].The mixtures were neutralized with 7% sodium bicarbonate, and the absorbance was recorded at 765 nm. The aluminum chloride method[7372] was used to measure flavonoid content. In this approach, 1 g of plant material was thoroughly mixed with 2 ml of 80% methanol. The methanolic extract (0.5 ml) was combined with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of

distilled water. The absorbance was measured at 415 nm after 30 min. Anthocyanin content was determined in 0.3% HCl in methanol at a temperature of 25 °C, utilizing the extinction coefficient (33 cm^2/mol) at 550 nm[7473].

Respiratory enzymes activity

Aconitase activity was measured following the method described by Murthy et al.[7574]. The reaction mixture consisted of 20 µl of enzyme extract, 220 µmol phosphate buffer (pH 7), and 30 µmol citrate. Enzyme activity was determined by monitoring the increase in absorbance at 240 nm in the presence of citrate.

Fumarase activity was assayed using the method of Murthy et al.[7574]. The reaction mixture (3 ml) contained 50 mM potassium phosphate buffer (pH 7.3), 50 mM L-malate, and 20 µl of enzyme extract. The reaction was initiated by the addition of malate and followed by measuring fumarate formation at 300 nm.

For the succinate dehydrogenase (SDH) activity assay, the reaction mixture contained 0.6 ml of 0.2 M phosphate buffer (pH 7.5), 0.2 ml of 0.2 M succinate, 0.3 ml of 0.01 M KCN, and 20 µl of enzyme extract. The enzyme activity was recorded at 410 nm. Malate dehydrogenase (MDH) activity was assayed spectrophotometrically by measuring the decrease in absorbance at 340 nm, following the oxidation of NADH by oxaloacetate as described by Murthy et al.[7574]. The reaction mixture contained 33 mM potassium phosphate buffer (pH 7.5), 0.33 mM NADH, and enzyme extract. The reaction was initiated by adding 0.5 µmol of oxaloacetate.

Statistical analysis

The collected data were analyzed using one-way ANOVA in SPSS (version 21). The means were compared using Duncan's test at a confidence level of 0.05. Principal component analysis (PCA) was employed to obtain a correlation matrix, which provided Pearson's correlation coefficients for the tested analytical parameters. PCA was performed using XLSTAT (2016) software. All the data produced or examined during this study have been incorporated into this published article.

Data availability

All data generated or analyzed during this study are included in this published article.

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References

1. Boken, V. K., Cracknell, A. P. & Heathcote, R. L. *Monitoring and Predicting Agricultural Drought: A Global Study* (Oxford University Press, Oxford, 2005).
2. Sallam, A., Alqudah, A. M., Dawood, M. F., Baenziger, P. S. & Börner, A. Drought stress tolerance in wheat and barley: Advances in physiology, breeding and genetics research. *Int. J. Mol. Sci.* **20**(13), 3137 (2019).
3. Sherwood, S. & Fu, Q. A drier future?. *Science* **343**(6172), 737–739 (2014).
4. Sah, J. K. et al. Enhancing physio biochemical traits and yield of common buckwheat *Fagopyrum esculentum* with rice husk biochar and nano iron oxide under water stress. *Sci. Rep.* **15**(1), 7859 (2025).
5. Litvin, A. G., van Iersel, M. W. & Malladi, A. Drought stress reduces stem elongation and alters gibberellin-related gene expression during vegetative growth of tomato. *J. Am. Soc. Hortic. Sci.* **141**(6), 591–597 (2016).
6. Wang, P. et al. Reactive oxygen species: Multidimensional regulators of plant adaptation to abiotic stress and development. *J. Integr. Plant Biol.* **66**(3), 330–367 (2024).
7. Chakraborti, S., Bera, K., Sadhukhan, S. & Dutta, P. Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. *Plant Stress* **3**, 100052 (2022).
8. Tyśkiewicz, R., Nowak, A., Ozimek, E. & Jaroszuk-Ściseł, J. Trichoderma: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *Int. J. Mol. Sci.* **23**(4), 2329. https://doi.org/10.1016/S0168-9452(99)00197-1 (2022).
9. Shukla, N., Awasthi, R. P., Rawat, L. & Kumar, J. Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. *Plant Physiol. Biochem.* **54**, 78–88 (2012).
10. Shah, M. H. & Khan, M. R. Effectiveness of local isolates of *Trichoderma* spp. in imparting drought tolerance in rice, *Oryza sativa*. *Sci. Rep.* **14**(1), 17672 (2024).
11. Afifi, H. H., Hassan, N. A., Abou-Hadid, A. F. & Madkor, M. A. The ability of selected *Trichoderma* spp. to improve wheat (*Triticum asetivum* L.) tolerance under heat, salinity, and drought stresses through seed treatments. *Plant Cell Biotechnol. Mol. Biol.* **22**(47–48), 61–74 (2021).
12. Gaikwad, N. & Verma, S. Effect of *Trichoderma harzianum* on growth of corn under water stress condition. *Int. J. Plant Soil Sci.* **36**(8), 447–454. https://doi.org/10.9734/ijpss/2024/v36i84874 (2024).
13. Tiwari, M., Devi, B., Sinha, S., Yadav, N. & Singh, P. Intergenerational priming by *Trichoderma* alleviates drought stress in barley. *Environ. Exp. Bot.* **223**, 105772 (2024).
14. Gupta, S. et al. Inoculation of barley with *Trichoderma harzianum* T-22 modifies lipids and metabolites to improve salt tolerance. *J. Exp. Bot.* **72**(20), 7229–7246 (2021).
15. Datta, D. et al. Alleviating drought stress in rice plant through intervention of *Trichoderma* spp. *J. Environ. Biol.* **44**(3), 373–379 (2023).
16. Illescas, M., Morán-Diez, M. E., Martínez de Alba, Á. E., Hermosa, R. & Monte, E. Effect of *Trichoderma asperellum* on wheat plants' biochemical and molecular responses, and yield under different water stress conditions. *Int. J. Mol. Sci.* **23**(12), 6782 (2022).
17. Rezaloo, Z., Shahbazi, S., Ali Loo, A. A. & Sarajuoghi, M. Impact of mutants of trichoderma species in modulating salinity stress of beans (*Phaseolus vulgaris* L. CV Khomein). *J. Nucl. Res. Appl.* **3**(4), 47–58 (2023).
18. Sorahinobar, M., Eslami, S., Shahbazi, S. & Najafi, J. A mutant *Trichoderma harzianum* improves tomato growth and defense against Fusarium wilt. *Eur. J. Plant Pathol.* **172**, 1–16 (2025).
19. Abbasi, S., Safaie, N., Shams-Bakhsh, M. & Shahbazi, S. Biocontrol activities of gamma induced mutants of *Trichoderma harzianum* against some soilborne fungal pathogens and their DNA fingerprinting. *Iran. J. Biotechnol.* **14**(4), 260 (2016).
20. Lisar, S. Y., Motafakkerazad, R., Hossain, M. M. & Rahman, I. M. Causes, effects and responses. *Water Stress* **25**, 33 (2012).
21. Qiao, M., Hong, C., Jiao, Y., Hou, S. & Gao, H. Impacts of drought on photosynthesis in major food crops and the related mechanisms of plant responses to drought. *Plants* **13**(13), 1808 (2024).
22. Yang, X. et al. Response mechanism of plants to drought stress. *Horticulturae* **7**(3), 50 (2021).

23. Iqbal, S. et al. Phytohormones trigger drought tolerance in crop plants: Outlook and future perspectives. *Front. Plant Sci.* **12**, 799318 (2022).
24. Laxa, M., Liebthal, M., Telman, W., Chibani, K. & Dietz, K. J. The role of the plant antioxidant system in drought tolerance. *Antioxidants* **8**(4), 94 (2019).
25. Shukla, N., Awasthi, R. P., Rawat, L. & Kumar, J. Seed biopriming with drought tolerant isolates of *Trichoderma harzianum* promote growth and drought tolerance in *Triticum aestivum*. *Ann. Appl. Biol.* **166**(2), 171–182 (2015).
26. Wu, Y. et al. The critical role of biochar to mitigate the adverse impacts of drought and salinity stress in plants. *Front. Plant Sci.* **14**, 1163451 (2023).
27. Brunner, I., Herzog, C., Dawes, M. A., Arend, M. & Sperisen, C. How tree roots respond to drought. *Front. Plant Sci.* **6**, 547 (2015).
28. Sharp, R. E., Silk, W. K. & Hsiao, T. C. Growth of the maize primary root at low water potentials. *Plant Physiol.* **87**(1), 50–57 (1988).
29. Wang, P. et al. Reactive oxygen species: Multidimensional regulators of plant adaptation to abiotic stress and development. *J. Integr. Plant Biol.* **66**(3), 330–367 (2024).
30. Gurrieri, L., Merico, M., Trost, P., Forlani, G. & Sparla, F. Impact of drought on soluble sugars and free proline content in selected *Arabidopsis mutants*. *Biology* **9**(9), 367 (2020).
31. Traversari, S., Francini, A., Traversi, M. L., Sebastiani, L. & Vitagliano, C. Can sugar metabolism in the cambial region explain the water deficit tolerance in poplar?. *J. Exp. Bot.* **69**(16), 4083–4097 (2018).
32. Granda, E. & Camarero, J. J. Drought reduces growth and stimulates sugar accumulation: New evidence of environmentally driven non-structural carbohydrate use. *Tree Physiol.* **37**(7), 997–1000 (2017).
33. Kabiri, R., Nasibi, F. & Farahbakhsh, H. Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. *Plant Prot. Sci.* **50**(1), 43–51 (2014).
34. Hussain, S. et al. Oxidative stress and antioxidant defense in plants under drought conditions. In *Plant Abiotic Stress Tolerance* 207–219 (Springer, Cham, 2019).
35. Sharma, P., Jha, A. B., Dubey, R. S. & Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, 1–26 (2012).
36. Król, A., Amarowicz, R. & Weidner, S. Changes in the composition of phenolic compounds and antioxidant properties of grapevine roots and leaves (*Vitis vinifera* L.) under continuous long-term drought stress. *Acta Physiol. Plant.* **36**(6), 1491–1499 (2014).
37. Nasiri, M., Hassanpour, H., Sorahinobar, M. & Niknam, V. Impact of static magnetic field on the callogenesis, phytochemical production and antioxidant enzymes in *Anthemis gilanica*. *Russ. J. Plant Physiol.* **69**(4), 77 (2022).
38. Condori-Apfata, J. A. et al. Downregulation of the E2 subunit of 2-oxoglutarate dehydrogenase modulates plant growth by impacting carbon-nitrogen metabolism in *Arabidopsis thaliana*. *Plant Cell Physiol.* **62**(5), 798–814 (2021).
39. Zandalinas, S. I., Balfagón, D., Gómez-Cadenas, A. & Mittler, R. Plant responses to climate change: Metabolic changes under combined abiotic stresses. *J. Exp. Bot.* **73**(11), 3339–3354 (2022).
40. Kawai, Y., Ono, E. & Mizutani, M. Evolution and diversity of the 2-oxoglutarate-dependent dioxygenase superfamily in plants. *Plant J.* **78**(2), 328–343 (2014).
41. Shomali, A. et al. Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. *Plants* **11**(22), 3158 (2022).
42. Araújo, W. L., Martins, A. O., Fernie, A. R. & Tohge, T. 2-oxoglutarate: Linking TCA cycle function with amino acid, glucosinolate, flavonoid, alkaloid, and gibberellin biosynthesis. *Front. Plant Sci.* **5**, 552 (2014).
43. Monteoliva, M. I., Guzzo, M. C. & Posada, G. A. Breeding for drought tolerance by monitoring chlorophyll content. *Gene Technol.* **10**(1), 165 (2021).
44. Muhammad, I. et al. Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. *Front. Plant Sci.* **11**, 615942 (2021).
45. Rolando, J. L., Ramírez, D. A., Yactayo, W., Monneveux, P. & Quiroz, R. Leaf greenness as a drought tolerance related trait in potato (*Solanum tuberosum* L.). *Environ. Exp. Bot.* **110**, 27–35 (2015).
46. Rehman, S. U. et al. Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought. *Crop Pasture Sci.* **67**(7), 712–718 (2016).
47. Bae, H. et al. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. *J. Exp. Bot.* **60**(11), 3279–3295 (2009).
48. Saini, S., Sharma, I., Kaur, N. & Pati, P. K. Auxin: A master regulator in plant root development. *Plant Cell Rep.* **32**(6), 741–757 (2013).
49. Llanes, A. S., Andrade, A. M., Alemano, S. G. & Luna, M. V. Alterations of endogenous hormonal levels in plants under drought and salinity. *J. Plant Sci.* **7**(8), 1357–1371 (2016).
50. Yu, C., Jiang, X., Xu, H. & Ding, G. *Trichoderma longibrachiatum* inoculation improves drought resistance and growth of *Pinus massoniana* seedlings through regulating physiological responses and soil microbial community. *J. Fungi* **9**(7), 694 (2023).
51. Rasool, S., Ahmad, A., Siddiqi, T. O. & Ahmad, P. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol. Plant.* **35**(4), 1039–1050 (2013).
52. Zhang, S., Gan, Y. & Xu, B. Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front. Plant Sci.* **7**, 1405 (2016).
53. Singh, D. P. et al. Microbial inoculation in rice regulates antioxidative reactions and defense related genes to mitigate drought stress. *Sci. Rep.* **10**(1), 4818 (2020).
54. Harman, G. E., Doni, F., Khadka, R. B. & Uphoff, N. Endophytic strains of Trichoderma increase plants' photosynthetic capability. *J. Appl. Microbiol.* **130**, 14368. https://doi.org/10.1111/jam.14368 (2019).
55. Singh, B. N., Singh, A., Singh, G. S. & Dwivedi, P. Potential role of *Trichoderma asperellum* T42 strain in growth of pea plant for sustainable agriculture. *J. Pure Appl. Microbiol.* **9**(2), 1069–1074 (2015).
56. Zhang, S., Xu, B. & Gan, Y. Seed treatment with *Trichoderma longibrachiatum* T6 promotes wheat seedling growth under NaCl stress through activating the enzymatic and nonenzymatic antioxidant defense systems. *Int J Mol Sci* **20**, 3729 (2019).
57. Manganiello, G. et al. Modulation of tomato response to *Rhizoctonia solani* by *Trichoderma harzianum* and its secondary metabolite harzianic acid. *Front. Microbiol.* **9**, 1966 (2018).
58. Leonelli, L., Brooks, M. D. & Niyogi, K. K. Engineering the lutein epoxide cycle into *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci.* **114**(33), E7002–E7008 (2017).
59. Swapnil, P., Meena, M., Singh, S. K., Dhuldhaj, U. P. & Marwal, A. Vital roles of carotenoids in plants and humans to deteriorate stress with its structure, biosynthesis, metabolic engineering and functional aspects. *Curr. Plant Biol.* **26**, 100203 (2021).
60. Shahbazi, S., Askari, H. & Mojerlou, S. The impact of different physicochemical parameters of fermentation on extracellular cellulolytic enzyme production by *Trichoderma harzianum*. *J. Crop Prot.* **5**(3), 397–412 (2016).
61. Rezaloo, Z., Shahbazi, S. & Askari, H. Biopriming with *Trichoderma* on Germination and vegetative characteristics of sweet corn, sugar beet and wheat. *Iran. J. Seed Sci. Technol.* **8**(2), 199–210 (2020).
62. Lichtenthaler, H. K. & Wellburn, A. R. *Determinations of Total Carotenoids and Chlorophylls a and b of Leaf Extracts in Different Solvents* (Portland Press Ltd, London, 1983). https://doi.org/10.1042/bst0110591.
63. Luziatelli, F. et al. Metabolites secreted by a plant-growth-promoting Pantoea agglomerans strain improved rooting of *Pyrus communis* L. cv Dar Gazi cuttings. *Front. Microbiol.* **11**, 539359 (2020).
64. Lee, Y. P. & Takahashi, T. An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.* **14**(1), 71–77 (1966).

65. DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. & Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **28**(3), 350–356 (1956).
66. Heath, R. L. & Packer, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* **125**(1), 189–198. https://doi.org/10.1016/0003-9861(68)90654-1 (1968).
67. Velikova, V., Yordanov, I. & Edreva, A. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Sci.* **151**(1), 59–66 (2000).
68. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254. https://doi.org/10.1006/abio.1976.9999 (1976).
69. Giannopolitis, C. N. & Ries, S. K. Superoxide dismutases: II. Purification and quantitative relationship with water-soluble protein in seedlings. *Plant Physiol.* **59**(2), 315–318. https://doi.org/10.1104/pp.59.2.315 (1977).
70. Abeles, F. B. & Biles, C. L. Characterization of peroxidases in lignifying peach fruit endocarp. *Plant Physiol.* **95**(1), 269–273. https://doi.org/10.1104/pp.95.1.269 (1991).
71. Raymond, J., Rakariyatham, N. & Azanza, J. L. Purification and some properties of polyphenoloxidase from sunflower seeds. *Phytochem* **34**(4), 927–931. https://doi.org/10.1016/S0031-9422(00)90689-7 (1993).
72. Conde, E., Cadahia, E., Garcia-Vallejo, M. C. & Tomas-Barberan, F. Low molecular weight polyphenols in wood and bark of *Eucalyptus globulus*. *Wood Fiber Sci.* **27**, 379–383 (1995).
73. Chang, C. C., Yang, M. H., Wen, H. M. & Chern, J. C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* **10**(3), 3. https://doi.org/10.38212/2224-6614.2748 (2002).
74. Krizek, D. T., Kramer, G. F., Upadhyaya, A. & Mirecki, R. M. UV-B response of cucumber seedlings grown under metal halide and high-pressure sodium/deluxe lamps. *Physiol. Plant.* **88**(2), 350–358. https://doi.org/10.1111/j.1399-3054.1993.tb05509.x (1993).
75. Murthy, P. S., Sirsi, M. & Ramakrishnan, T. Tricarboxylic acid cycle and related enzymes in cell-free extracts of *Mycobacterium tuberculosis* H37Rv. *Biochem. J.* **84**, 263–269. https://doi.org/10.1042/bj0840263 (1962).

Author contributions

NY collected data and performed the analysis. MS, MR and SSH verified the analytical methods. MS and MR supervised the research. All authors discussed the results and contributed to the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

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