Effects of *Trichoderma viride* on Growth, SOD Activity and Fusarium Disease Severity in Three Wheat Genetic Backgrounds under Drought and Salinity Stress

Hussain Sabah Swaiedy[1], Azam Nikbakht Dehkordi[2*], Ali Akbar Maghsoudi Moud[3]

1- Department of Plant Genetics and Production Engineering, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. ORCID: https://orcid.org/0009-0008-5460-5669
2- Research and Technology Institute of Plant Production (RTIPP), Afzalipour Research Institute affiliated with Shahid Bahonar University of Kerman, Kerman, PO Box 7616914111, Iran. ORCID: https://orcid.org/0000-0001-9521-2206
3- Department of Plant Genetics and Production Engineering, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran. ORCID: https://orcid.org/0000-0002-2435-2110

***Corresponding author: anikbakht@uk.ac.ir**

Abstract

Drought, salinity, and soil-borne fungi can constrain wheat establishment at the seedling stage. This greenhouse study examined whether *Trichoderma viride* improves wheat performance under separately imposed drought and salinity and limits damage associated with *Fusarium* infection. A four-factor factorial experiment was conducted in a completely randomized design with three replicate pots per treatment combination. The factors were three wheat genetic backgrounds, three pathogen treatments (a non-inoculated control, *Fusarium oxysporum* and *F. graminearum*), *T. viride* application (absence or presence), and three abiotic environments (a non-stressed control, salinity induced by 100 mM NaCl and drought maintained at 50% field capacity). Shoot dry weight, superoxide dismutase (SOD) activity, and disease severity were measured. Drought imposes a greater limitation on shoot dry weight than salinity, with reductions of 36.6% and 25.3%, respectively. Across the tested environments, *T. viride* increases shoot dry weight by approximately 40% and raises SOD activity by 76.2% under salinity and 77.8% under drought. In *Fusarium*-inoculated plants, *T. viride* reduces disease severity by 46.2% for *F. oxysporum* and 69.2% for *F. graminearum*. Shoot dry weight, SOD activity, and disease severity also differ significantly among the three wheat materials. Because each ploidy level was represented by a single material, these differences are interpreted as responses of the tested genetic backgrounds rather than independent ploidy effects. Under the conditions of this experiment, *T. viride* improved shoot growth and antioxidant activity while reducing *Fusarium* disease across the pathogen and abiotic environments examined.

Keywords: Drought; *Fusarium*; Salinity; SOD; *Trichoderma Viride*.

Introduction

Wheat (*Triticum spp*.) is one of the world's most important cereal crops and plays a central role in global food security. The establishment of healthy wheat seedlings depends on their ability to maintain root function and biomass accumulation under both abiotic and biotic stresses. In dryland and salt-affected production systems, drought and soil salinity restrict water uptake, whereas soil-borne Fusarium species damage root tissues and interfere with water and nutrient uptake. Exposure to these stresses during early growth may intensify physiological stress, limit seedling development, and reduce overall seedling performance. Although drought and salinity both limit plant water availability, they affect wheat growth through partly different mechanisms. Drought decreases soil water availability and limits water uptake, cell expansion, stomatal conductance and photosynthetic carbon assimilation. Salinity also causes an initial reduction in water uptake, but prolonged exposure may additionally result in ion toxicity and nutrient imbalance. Comparing these stresses separately can provide a clearer understanding of their effects on wheat seedling growth and physiological responses (Van Zelm *et al*., 2020; Oljira *et al*., 2020; Ramezani Etedali & Koohi, 2026).

The seedling stage is also highly susceptible to infection by soil-borne *Fusarium* species. *Fusarium* can damage root tissues, impair root function, reduce seedling vigor and biomass accumulation, and increase disease severity. Under drought or salinity, root damage caused by the pathogen may further limit water uptake and increase physiological stress in infected seedlings. These conditions may also disturb the balance between reactive oxygen species production and the antioxidant defense system. Superoxide

dismutase is a key antioxidant enzyme involved in the detoxification of superoxide radicals. Therefore, the combined evaluation of seedling dry weight, SOD activity, and disease severity can provide complementary information on plant growth, antioxidant response, and disease development, rather than relying only on visible disease symptoms (Boamah *et al*., 2021; Saadaoui *et al*., 2023).

Wheat genotypes differing in ploidy level and genetic background can vary markedly in their responses to drought, salinity, and Fusarium head blight. Tetraploid wheat has shown greater yield, biomass, and water-use efficiency than diploid wheat under moderate drought, although these differences diminish under severe water limitation (Gui et al., 2021). Salinity tolerance also varies among ancestral, neglected, and domesticated wheat genotypes, reflecting differences in growth, ion regulation, antioxidant activity, and stress-responsive gene expression (Ahmadi et al., 2020). Similarly, Fusarium head blight resistance is strongly influenced by genetic background; durum wheat has fewer characterized resistance sources than hexaploid wheat, and resistance is generally controlled by multiple minor-effect loci (Haile et al., 2019; Ruan et al., 2020). However, it remains unclear whether the effects of *T. viride* are consistent across wheat backgrounds or depend on specific host–Fusarium species–abiotic stress combinations.

Trichoderma species are widely studied as biological control agents and plant biostimulants. Their beneficial effects may result from competition with pathogens, mycoparasitism, production of antifungal compounds, root colonization, improved nutrient uptake, regulation of plant hormones and activation of plant defense responses. However, the effectiveness of *Trichoderma* depends on the fungal strain, host genotype, pathogen species and environmental conditions. Therefore, its effects need to be evaluated under specific host–pathogen–environment combinations rather than assumed to be similar across experimental systems (Pedrero-Méndez et al., 2025).

Recent studies have provided evidence that *Trichoderma* can improve wheat growth and stress responses. Oljira *et al*. (2020) reported that selected *Trichoderma* isolates improved photosynthesis, water-use efficiency and biomass production in two wheat cultivars under salinity. Boamah *et al*. (2021) showed that *T. longibrachiatum* TG1 increased wheat seedling growth and antioxidant activity and reduced disease caused by *F. pseudograminearum* under different NaCl levels. Under water-deficit conditions, Pedrero-Méndez *et al*. (2025) and Illescas *et al*. (2022) found that the protective effects of *Trichoderma* were strain-dependent and were associated with changes in plant growth, antioxidant enzyme activity, phytohormone levels and yield-related traits. Other studies have focused mainly on the biological control of *Fusarium* diseases. Saadaoui *et al*. (2023) evaluated several *Trichoderma* isolates for their effects on wheat seedling growth and the control of *Fusarium* seedling blight, whereas more recent studies have examined the mechanisms by which selected *Trichoderma* strains suppress *F. pseudograminearum* or *F. graminearum* in wheat (Boamah *et al*., 2025; Pedrero-Méndez *et al*., 2025).

Despite increasing evidence on the beneficial effects of Trichoderma in wheat, several important gaps remain unresolved. Previous studies have mainly evaluated single abiotic stresses, individual Fusarium species, or limited wheat genotypes, making it difficult to determine whether the protective effects of Trichoderma are consistent across different host genetic backgrounds and stress conditions. In particular, the combined influence of wheat genetic background, Fusarium species, and separately imposed drought and salinity stresses on Trichoderma-mediated improvements in seedling growth, antioxidant activity, and disease suppression remains insufficiently characterized. Addressing this gap is essential for understanding whether Trichoderma-based strategies can provide reliable protection under diverse stress scenarios.

Accordingly, this study evaluated the effects of *T. viride* on wheat seedling dry weight, SOD activity, and disease severity across three wheat genetic backgrounds, three pathogen treatments consisting of a non-inoculated control, *F. oxysporum*, and *F. graminearum*, and three separately imposed abiotic environments consisting of a non-stressed control, salinity, and drought. This factorial design allowed the effects of *T. viride* to be compared across different combinations of host background, pathogen treatment, and abiotic environment. We hypothesized that *T. viride* would increase seedling dry weight and SOD activity and reduce disease severity, but that the magnitude of these effects would vary among wheat genetic backgrounds, pathogen treatments, and abiotic environments.

Materials and Methods

Experimental design and plant materials

A four-factor factorial experiment was conducted in a completely randomized design. The factors were wheat genetic background (three levels), pathogen treatment (three levels), *T. viride* application (two levels), and abiotic environment (three levels). Pathogen treatments comprised a non-inoculated control, *F. oxysporum*, and *F. graminearum*. The abiotic environments were a non-stressed control, salinity, and drought. Salinity and drought were imposed separately; no combined salinity-drought treatment was included. The 3 × 3 × 2 × 3 arrangement produced 54 treatment combinations. Each combination was represented by three separate pots, giving 162 experimental units in total. A pot constituted one biological replicate. Eight seeds were sown per pot, and the seedlings were thinned to five uniform plants seven days after emergence. Measurements from plants within a pot were pooled or averaged before analysis; individual seedlings were not treated as independent replicates.

The plant materials were hexaploid bread wheat, *Triticum aestivum L.* cv. Abughasib (2n = 6x = 42); tetraploid durum wheat, *Triticum durum Desf.* cv. Ommarrah (2n = 4x = 28); and a local diploid accession of *Triticum boeoticum Boiss.* (2n = 2x = 14) maintained at the Iraq National Cereals Research Center, Baghdad, Iraq. All seed lots were supplied by this center. Before sowing, seeds were screened visually for uniform size, apparent health, and the absence of visible damage or disease symptoms. Preliminary germination tests were performed, and only lots with germination greater than 95% were used.

The experiment was conducted in a greenhouse in Kufa, Najaf, Iraq. Daytime temperature ranged from 20 to 25 °C, nighttime temperature from 16 to 18 °C, and relative humidity from 60 to 70%. Plants received natural daylight with an approximate 12 h light/12 h dark photoperiod; no supplemental lighting was used. Photosynthetic photon flux density at canopy height was approximately 300-500 µmol m^{-2} s^{-1} at midday. Temperature and relative humidity were recorded daily throughout the experiment.

Polyethylene pots (3.5 L; 15 cm diameter; 20 cm height) were used. Each pot contained 2.5 kg of air-dried clay-loam soil and had drainage holes approximately 1 cm in diameter. The soil was collected from an agricultural field in Kufa, homogenized, and autoclaved at 121 °C for 2 h before use. Before treatment application, the soil had a pH of 7.2, an electrical conductivity of 1.8 dS m^{-1}, an organic matter content of 2%, and a gravimetric field capacity of approximately 23% (w/w). Every pot received the same mass of soil.

Preparation of fungal inoculum and biological treatments

Fusarium graminearum, *F. oxysporum*, and *T. viride* were cultured separately on potato dextrose agar (PDA) at 25 ± 2 °C. Conidia were collected from 7-day-old *Fusarium* cultures by adding sterile distilled water containing 0.01% Tween 20 to the colony surface and gently dislodging the fungal growth. The suspensions were passed through four layers of sterile gauze and adjusted to 1 × 10^6 conidia mL^{-1} with a hemocytometer. Wheat seeds were surface disinfected in 70% ethanol for 1 min and then in 1% sodium hypochlorite for 3-5 min. They were rinsed several times with sterile distilled water and air-dried under aseptic conditions. For the biological treatment, disinfected seeds were soaked for 24 h in a *T. viride* suspension containing 1 × 10^7 spores mL^{-1}. Seeds assigned to the untreated level were soaked for the same period in sterile distilled water. After aseptic drying, seeds in the pathogen treatments were immersed for 30 min in a conidial suspension (1 × 10^6 conidia mL^{-1}) of either *F. oxysporum* or *F. graminearum*. All *Fusarium*-inoculated treatments received both seed and soil inoculation. For soil inoculation, autoclaved wheat grains were inoculated with the corresponding *Fusarium* isolate and incubated for 10 days until fully colonized. The colonized grain inoculum was mixed thoroughly with the potting soil at 2-3% (w/w) before sowing. Seed and soil inoculation together constituted a single pathogen treatment and were not analyzed as separate factors. The two *Fusarium* species were applied separately, and no pot received both pathogens. Non-inoculated controls received sterile distilled water in place of the conidial suspension, and an equivalent amount of sterile, non-colonized wheat grain was added to the soil.

Salinity and drought treatments

The abiotic environments were a non-stressed control, salinity, and drought. Control pots were irrigated with distilled water and maintained at 100% field capacity. Salinity was imposed with 100 mM NaCl (5.84 g L^{-1}); the solution had an electrical conductivity of approximately 10.2 dS m^{-1}. Drought was imposed by maintaining soil moisture at 50% field capacity. Treatments began at the three- to four-leaf stage, 21 days after sowing, and continued until harvest at 42 days after sowing. The NaCl solution was

introduced gradually to avoid an abrupt osmotic shock. Drought pots were weighed daily, and only the amount of water needed to restore the assigned moisture level was added. Soil moisture was monitored daily, electrical conductivity weekly, and soil pH every two weeks.

Plant growth, disease severity and SOD activity

Seedlings were harvested 42 days after sowing. Shoots were separated from roots and dried at 70 °C to constant weight. The combined shoot dry mass of the five seedlings in each pot was divided by five and expressed as mean shoot dry weight per seedling. The pot mean constituted one observation in the statistical analysis. Disease severity was assessed only in *Fusarium*-inoculated treatments. Each seedling was rated on a 0-5 scale based on root discoloration, root rot, tissue necrosis, and seedling collapse. A score of 0 denoted a healthy seedling, whereas 5 denoted severe disease or complete collapse (Agrios, 2005). The five scores within a pot were averaged before analysis. For SOD determination, the youngest fully expanded leaves were collected from all five seedlings in each pot at 42 days after sowing between 09:00 and 10:00 h. Leaf material was pooled, and a 0.5 g fresh sample from each pot constituted one biological replicate. Samples were frozen immediately in liquid nitrogen and stored at -80 °C until extraction.

Frozen tissue was homogenized in 5 mL of ice-cold extraction buffer containing 50 mM potassium phosphate (pH 7.8), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1% (w/v) polyvinylpyrrolidone-40. The homogenate was centrifuged at 15,000 × g for 20 min at 4 °C, and the supernatant served as the crude enzyme extract. SOD activity was determined spectrophotometrically from inhibition of nitroblue tetrazolium photoreduction at 560 nm. Each biological sample was assayed in three technical replicates, and their mean was used in the statistical analysis. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% under the assay conditions (Beauchamp and Fridovich, 1971; Giannopolitis and Ries, 1977).

Statistical analysis

Pot-level observations were analyzed by four-way factorial analysis of variance for a completely randomized design. Wheat genetic background, pathogen treatment, *T. viride* application, and abiotic environment were fitted as fixed factors. Each pot supplied one independent observation.

When an analysis of variance detected a significant effect, means were separated by Duncan's multiple range test at $P \leq 0.05$. Disease severity was analyzed only for *Fusarium*-inoculated treatments because the non-inoculated controls showed no disease symptoms. Partial eta-squared values were calculated for selected effects, and Pearson correlations among shoot dry weight, SOD activity, and disease severity were calculated for *Fusarium*-inoculated treatments. All analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Analysis of variance and overall factorial response

Analysis of variance showed that shoot dry weight and SOD activity were significantly affected by wheat genetic background, pathogen treatment, *T. viride* application, and abiotic environment (Table 1). For shoot dry weight, the largest mean squares were associated with pathogen treatment, *T. viride*, and abiotic environment, indicating that biomass accumulation reflected disease pressure, biological treatment, and abiotic stress. Seedling performance under drought- and salinity-prone conditions was therefore associated with several components of the experimental system rather than with a single factor. The P/F × T, P/F × S and T × S interactions were significant for shoot dry weight and SOD activity, showing that pathogen treatment, biological treatment, and abiotic environment did not act independently. The response to *T. viride* differed according to *Fusarium* exposure and the environment in which the seedlings were grown. Comparable context-dependent responses have been reported in wheat-*Trichoderma* systems, in which effectiveness varies with the host genotype, pathogen pressure, inoculation route, and environmental conditions (Santoyo et al., 2024; Ascaso et al., 2025).

Disease severity was analyzed only in infected treatments. Wheat genetic background, *Fusarium* species, *T. viride* application, and the *Fusarium* species × *T. viride* interaction were significant, whereas the abiotic environment was not. Within the limits of the visual rating scale, disease severity was therefore associated mainly with the host-pathogen-biological treatment combination rather than with salinity or drought alone.

Table 1. Analysis of variance for shoot dry weight, SOD activity and disease severity.

Source of variation	df	Dry weight MS	SOD activity MS	df	Disease severity MS
Wheat genetic background (W)	2	18346.62**	371.74**	2	12.00**
Pathogen treatment / *Fusarium* species (P/F)	2	162529.26**	311.92**	1	6.75**
T. viride treatment (T)	1	153070.44**	2645.07**	1	168.75**
Abiotic environment (S)	2	98900.79**	597.08**	2	0.00 ns
W × P/F	4	141.99 ns	13.30**	2	0.00 ns
W × T	2	498.88**	13.40**	2	0.00 ns
W × S	4	364.32**	2.01*	4	0.00 ns
P/F × T	2	16825.34**	32.39**	1	6.75**
P/F × S	4	2059.61**	4.16**	2	0.00 ns
T × S	2	2751.02**	49.16**	2	0.00 ns
W × P/F × T	4	188.53 ns	2.54**	2	0.00 ns
W × P/F × S	8	23.73 ns	1.05 ns	4	0.00 ns
W × T × S	4	61.68 ns	0.74 ns	4	0.00 ns
P/F × T × S	4	314.89*	0.69 ns	2	0.00 ns
W × P/F × T × S	8	16.59 ns	0.74 ns	4	0.00 ns
Error	108	94.54	0.61	72	0.03

W = wheat genetic background; P = pathogen treatment; F = *Fusarium* species; T = *Trichoderma viride* application; S = abiotic environment. For shoot dry weight and SOD activity, pathogen treatment included the non-inoculated control, *F. oxysporum*, and *F. graminearum*. Disease severity was analyzed only in *Fusarium*-inoculated treatments. ** and * indicate significance at $P < 0.01$ and $P < 0.05$, respectively; ns, not significant.

Shoot dry-weight response to salinity, drought and *T. viride*.

Shoot dry weight decreased under both abiotic stresses. The mean declined from 228.98 mg in the non-stressed control to 171.39 mg under salinity and 145.34 mg under drought; drought therefore imposed the greater biomass limitation. This pattern is physiologically plausible because water deficit directly restricts water uptake, cell expansion, stomatal function, and carbon gain, whereas the effect of moderate salinity depends on both osmotic stress and the duration of ion accumulation. Salinity at 100 mM NaCl also reduced biomass, but the larger decrease under drought indicates that restricted soil water was the principal constraint on early shoot growth in this experiment (Munns and Tester, 2008; Silletti et al., 2021; Illescas et al., 2022; Ascaso et al., 2025).

Across abiotic environments, *T. viride* increased shoot dry weight from 151.17 to 212.64 mg, an overall increase of 40.7%. The proportional increases were similar in the control (40.5%), salinity (41.0%), and drought (40.6%) environments (Table 2; Fig 1). Although the significant T × S interaction indicates that the absolute response varied among environments, *T. viride* increased shoot dry weight at each level examined. This pattern is consistent with reports that *Trichoderma* can support plant growth under both favorable and stressful conditions (Woo et al., 2023; Contreras-Cornejo et al., 2024; Boamah et al., 2025).

Table 2. Environment-specific responses of shoot dry weight and SOD activity to *T. viride*.

Abiotic environment	Shoot DW without *T. viride*	Shoot DW with *T. viride*	DW increase (%)	SOD without *T. viride*	SOD with *T. viride*	SOD increase (%)
Control	190.44 b	267.51 a	40.5	12.96 c	23.12 a	78.5
Salinity	142.23 c	200.56 b	41.0	10.04 d	17.70 b	76.2
Drought	120.82 c	169.87 b	40.6	8.26 d	14.68 c	77.8

Within each response variable, means followed by different letters differ significantly among abiotic environment × *T. viride* combinations according to Duncan's multiple range test ($P \leq 0.05$).

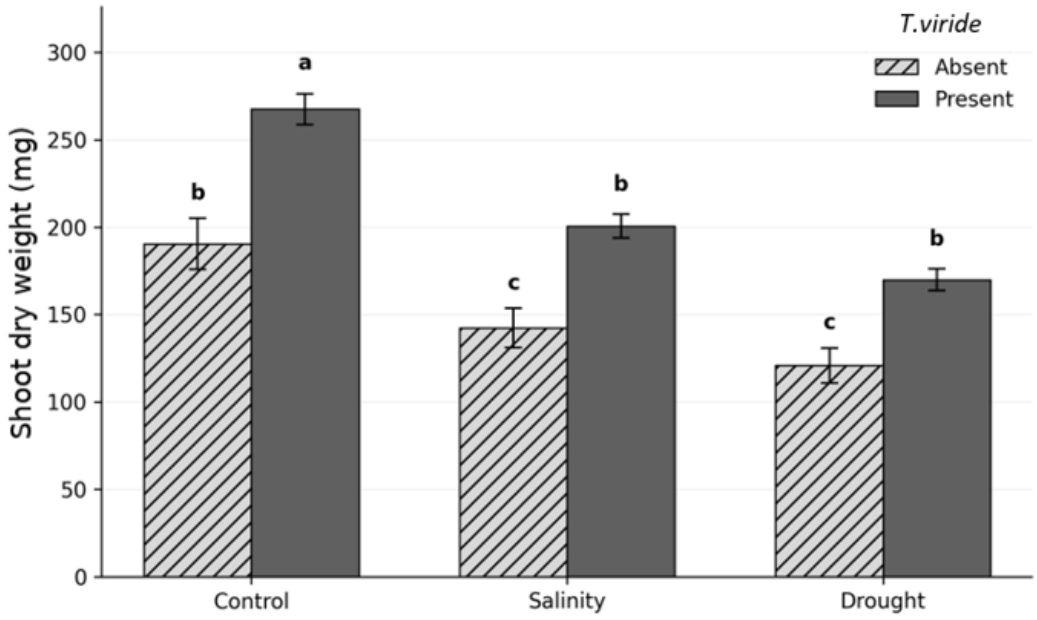

Fig 1. Mean shoot dry weight under control, salinity and drought environments in the absence and presence of *T. viride*. Bars represent means ± SE; different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

Pathogen-specific shoot biomass response

Pathogen inoculation markedly reduced shoot biomass. Mean shoot dry weight was highest in non-inoculated plants and substantially lower in both *Fusarium* treatments, with a slightly larger reduction under *F. oxysporum* than under *F. graminearum*. The pattern is consistent with early growth limitation associated with root-zone injury, impaired water uptake, and reduced seedling vigor. Similar reductions in wheat seedling growth under *Fusarium* pressure have been reported in recent biocontrol studies (Saadaoui et al., 2023; Stummer et al., 2024; Pedrero-Méndez et al., 2025).

The increase associated with *T. viride* was larger in pathogen-inoculated plants than in non-inoculated controls. *T. viride* increased shoot dry weight by 9.0% in the control, 72.9% under *F. oxysporum*, and 76.1% under *F. graminearum* (Table 3; Fig 2). Thus, its principal benefit in this experiment was expressed under disease pressure rather than as growth promotion in healthy plants alone. The response is compatible with several mechanisms reported for *Trichoderma-Fusarium* interactions, including competition, mycoparasitism, antifungal metabolites, volatile compounds, and induced host defenses (Özkale et al., 2023; Saadaoui et al., 2023; Stummer et al., 2024; Pedrero-Méndez et al., 2025).

Table 3. Pathogen-specific responses of shoot dry weight and disease severity to *T. viride*.

Pathogen treatment	Shoot DW without *T. viride*	Shoot DW with *T. viride*	DW increase (%)	Disease severity without *T. viride*	Disease severity with *T. viride*	Disease reduction (%)
Control	234.34 a	255.40 a	9.0	NA	NA	NA
F. oxysporum	105.84 c	182.97 b	72.9	4.33 a	2.33 b	46.2
F. graminearum	113.31 c	199.57 b	76.1	4.33 a	1.33 c	69.2

Within each response variable, means followed by different letters differ significantly among groups according to Duncan's multiple range test ($P \leq 0.05$). NA, not applicable because disease severity was not evaluated for non-inoculated controls.

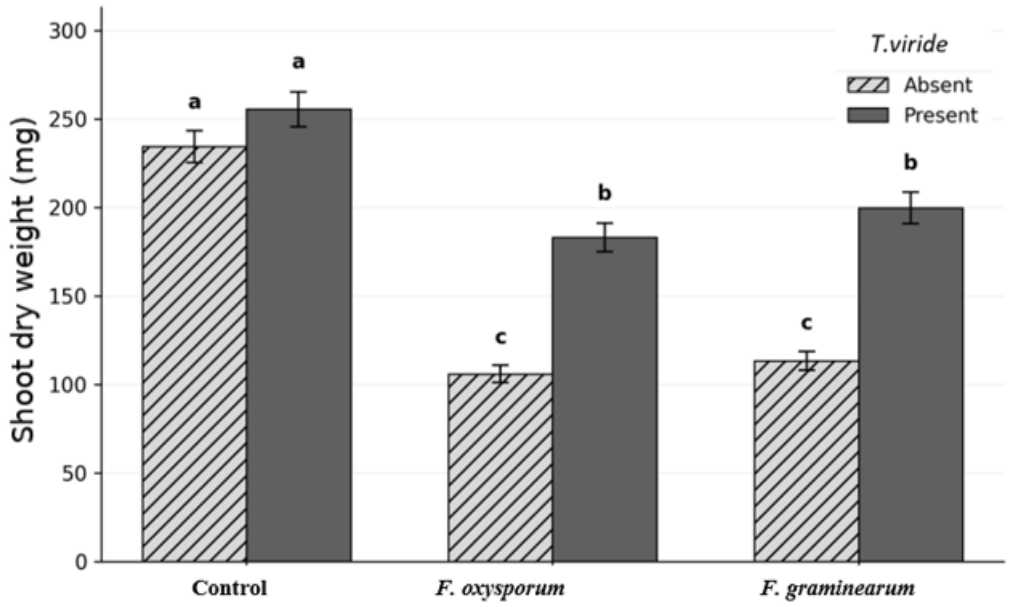

Fig 2. Mean shoot dry weight in non-inoculated, *F. oxysporum*-inoculated, and *F. graminearum*-inoculated wheat seedlings in the absence and presence of *T. viride*. Bars represent means ± SE; different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

SOD activity and antioxidant response

SOD activity was strongly affected by *T. viride* and the abiotic environment. Across all treatments, *T. viride* increased SOD activity from 10.42 to 18.50, corresponding to a 77.6% increase. The proportional increases were 78.5% in the control, 76.2% under salinity, and 77.8% under drought (Table 2; Fig 3). Because SOD is a primary enzymatic defense against superoxide radicals, the higher activity in treated seedlings indicates a stronger measured component of antioxidant defense alongside greater shoot biomass. This result does not establish the entire antioxidant mechanism, but it does show that the biological treatment was associated with improved physiological performance under stress (Boamah et al., 2021; Boamah et al., 2022; Ascaso et al., 2025).

The observed response is consistent with previous work in wheat. Boamah et al. (2021) reported higher SOD, POD, and CAT activities in *Trichoderma*-treated seedlings exposed to *Fusarium* and salinity. Boamah et al. (2022) further showed that *T. longibrachiatum* increased endogenous salicylic acid and antioxidant activity under salt stress. Zhang et al. (2019) linked *Trichoderma*-mediated salt tolerance to IAA production and ACC deaminase activity, while Illescas et al. (2022) and Ascaso et al. (2025) reported improved wheat responses to water deficit after *Trichoderma* treatment.

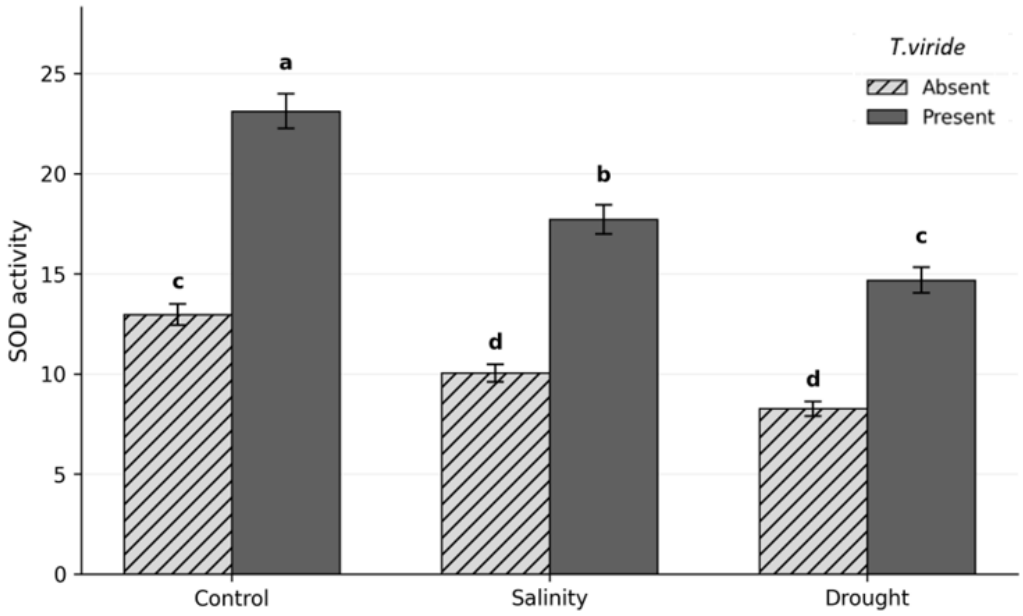

Fig 3. Mean SOD activity under control, salinity, and drought environments in the absence and presence of *T. viride*. Bars represent means ± SE; different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$).

Disease severity and biocontrol efficiency

Disease severity was evaluated only in *Fusarium*-inoculated plants. *T. viride* reduced the mean score from 4.33 to 1.83, an overall reduction of 57.7%. The response differed between pathogens; disease severity fell by 46.2% under *F. oxysporum* and by 69.2% under *F. graminearum* (Table 3; Fig 4). *T. viride* therefore suppressed disease caused by both fungi, with the larger reduction observed for *F. graminearum* in this dataset.

The magnitude of suppression agrees with recent work in wheat. Saadaoui et al. (2023) showed that *Trichoderma* seed treatment promoted seedling growth and reduced *Fusarium* seedling blight. Pedrero-Méndez et al. (2025) reported a reduction of more than 60% in the *Fusarium* head blight disease index after treatment with *T. asperellum* T25, with evidence for both direct and indirect activity. Özkale et al. (2023) also found that *T. atroviride* altered *F. graminearum* metabolism. These studies identify plausible mechanisms for the disease reduction observed here, although antagonism and host-mediated defense were not measured directly in the present experiment.

The abiotic environment did not significantly affect disease severity. This result does not imply that drought and salinity did not affect the plants; both altered shoot dry weight and SOD activity. Rather, under the conditions of this experiment, the ordinal disease score varied mainly with wheat genetic background, *Fusarium* species, and *T. viride* application and did not distinguish a separate effect of the abiotic environments.

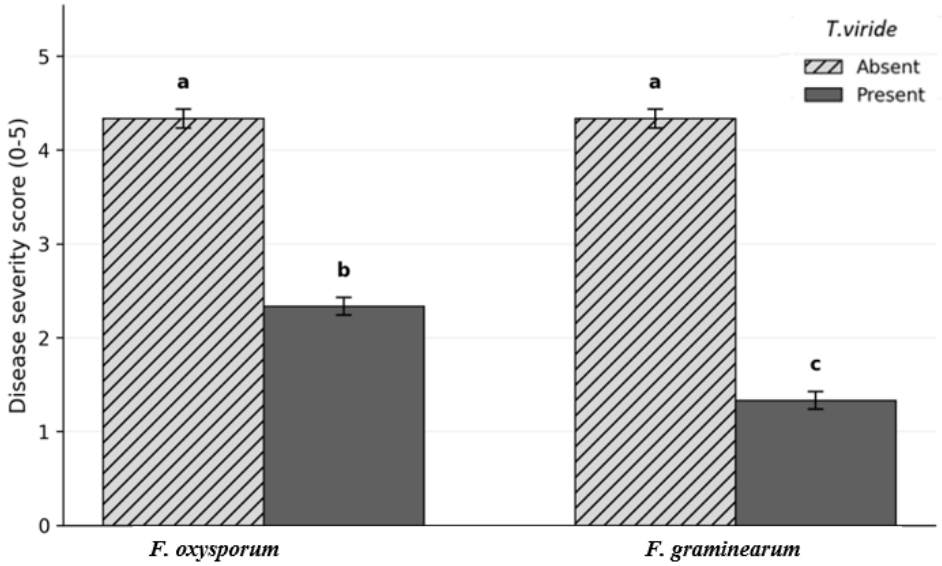

Fig 4. Mean disease severity in *F. oxysporum*- and *F. graminearum*-inoculated wheat seedlings in the absence and presence of *T. viride*. Bars represent means ± SE; different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

3.6. Effect size and relationships among response variables

Partial eta-squared values identified *T. viride* as a major source of variation, particularly for SOD activity and disease severity (Table 4). For shoot dry weight, pathogen treatment, abiotic environment, and *T. viride* each had large effect sizes, consistent with the combined influence of infection, abiotic stress, and biological treatment on growth. *T. viride* had the largest partial eta-squared value for SOD activity.

Among *Fusarium*-inoculated treatments, shoot dry weight was strongly and positively correlated with SOD activity ($r = 0.988$, $P \leq 0.01$), whereas disease severity was negatively correlated with both shoot dry weight ($r = -0.770$, $P \leq 0.01$) and SOD activity ($r = -0.783$, $P \leq 0.01$) (Table 5). Treatments with lower disease scores therefore tended to have greater shoot biomass and higher SOD activity. Because all three variables responded to the same experimental factors, these coefficients describe coordinated responses and should not be interpreted as evidence of direct causation.

Table 4. Partial eta-squared effect sizes for selected experimental factors.

Effect	Shoot dry weight partial eta²	SOD partial eta²	Disease severity partial eta²
Wheat background (W)	0.782	0.919	0.921
Pathogen treatment / *Fusarium* species (P/F)	0.970	0.905	0.767
T. viride treatment (T)	0.937	0.976	0.988
Abiotic environment (S)	0.951	0.948	0.000
P/F × T	0.767	0.497	0.767
T × S	0.350	0.600	0.000
P/F × T × S	0.110	0.040	0.000

Larger partial eta-squared values indicate a stronger effect relative to the associated error term within the fitted model; the values are not additive. Disease severity was calculated only for *Fusarium*-inoculated treatments.

Table 5. Pearson correlation matrix for shoot dry weight, SOD activity and disease severity in *Fusarium*-inoculated treatments. ** indicates significance at P ≤ 0.01.

Variable	Shoot dry weight	SOD activity	Disease severity
DW	1.000	0.988**	-0.770**
SOD	0.988**	1.000	-0.783**
DS	-0.770**	-0.783**	1.000

Integrated interpretation for drought- and salinity-stress management

Under the conditions of this experiment, *T. viride* performed two related functions. It reduced *Fusarium* disease severity and improved shoot dry weight and SOD activity in the non-stressed, saline, and drought environments. The increase in shoot biomass was greatest in pathogen-inoculated treatments, indicating that the benefit of *T. viride* was most evident under disease pressure.

For drought- and salinity-prone agriculture, the relevant finding is not complete removal of stress injury. Drought remained the strongest constraint on biomass, and salinity continued to reduce growth relative to the control. *T. viride* nevertheless improved seedling performance and SOD activity in each abiotic environment. Such partial protection is agronomically plausible because biological agents generally mitigate stress rather than eliminate its effects (Silletti et al., 2021; Illescas et al., 2022; Senizza et al., 2023; Santoyo et al., 2024; Contreras-Cornejo et al., 2024; Ascaso et al., 2025; Sorahinobar et al., 2025). The study extends previous work by placing two *Fusarium* species, three wheat genetic backgrounds and two separately imposed abiotic stresses within one factorial framework. This approach follows recommendations that microbial biostimulants be evaluated across host, pathogen and environmental contexts rather than under a single simplified condition (Bashyal et al., 2021; Contreras-Cornejo et al., 2024; Santoyo et al., 2024). It also allowed general growth promotion to be distinguished from pathogen-specific and environment-specific responses. The results suggest that *T. viride* merits further evaluation for wheat seedling establishment where soil-borne *Fusarium* infection coincides with either drought or salinity.

Discussion

Trichoderma viride improved wheat seedling performance under both abiotic and biotic stress, although the response represents partial buffering rather than complete removal of stress. Drought remained the strongest constraint on shoot biomass, and salinity continued to reduce shoot dry weight relative to the non-stressed control. Within each environment, however, *T. viride* increased shoot dry weight and SOD activity, indicating that treated seedlings maintained better growth and a stronger measured antioxidant response under drought, salinity, and *Fusarium* pressure.

The larger biomass reduction under drought emphasizes the need to evaluate biological stress-mitigation strategies in water-limited production systems. Early drought restricts the water supply required for cell expansion and shoot-root development, which can reduce biomass before seedlings establish a fully functional root system. Salinity also imposes osmotic stress, but the extent of biomass loss depends on salt concentration, exposure time, and genotype-specific ion regulation. Because this experiment did not include a combined salinity × drought treatment, the responses to the two stresses must be interpreted separately (Silletti et al., 2021; Illescas et al., 2022; Contreras-Cornejo et al., 2024; Hajebi et al., 2026). The similar proportional increase in shoot dry weight across the three abiotic environments is an important feature of the response to *T. viride*. Improvement under the non-stressed control alone would provide limited evidence for use in dryland systems; comparable proportional gains were also observed under salinity and drought. The experiment did not identify the mechanisms responsible, but improved root function, nutrient mobilization, hormonal regulation, defense priming and redox control have all been reported in *Trichoderma*-plant interactions (Woo et al., 2023; Santoyo et al., 2024; Akbari et al., 2024; Boamah et al., 2025).

The pathogen-specific pattern further clarifies the role of the treatment. The increase in shoot dry weight associated with *T. viride* was modest in non-inoculated plants but substantially larger under *F. oxysporum* and *F. graminearum*. Its main value in this experiment was therefore expressed under disease pressure. *Trichoderma* isolates can restrict *Fusarium* through direct antagonism, mycoparasitism, metabolite production, and activation of host defense pathways (Özkale et al., 2023; Saadaoui et al.,

2023; Stummer et al., 2024; Pedrero-Méndez et al., 2025). The present data are consistent with these mechanisms, although pathogen biomass and root colonization were not quantified.

The SOD response links the growth and physiological components of the study. Salinity, drought, and pathogen infection can increase reactive oxygen species and damage membranes, proteins, and photosynthetic structures. SOD catalyzes the dismutation of superoxide radicals and is an early component of enzymatic antioxidant defense. Its higher activity in *T. viride*-treated seedlings, together with greater shoot dry weight, is consistent with better physiological maintenance under stress. Similar activation of antioxidant defenses after *Trichoderma* treatment has been reported in wheat and other cereals exposed to salinity or drought (Boamah et al., 2021; Boamah et al., 2022; Senizza et al., 2023; Sorahinobar et al., 2025; Pakgohar et al., 2025).

The non-significant effect of abiotic environment on disease severity requires cautious interpretation. It does not show that drought and salinity were biologically unimportant, because both affected shoot dry weight and SOD activity. Instead, the visual disease score was determined mainly by *Fusarium* species, wheat genetic background, and *T. viride* application. An ordinal symptom scale may not capture the physiological effects of abiotic stress as clearly as growth and enzyme measurements.

Differences among the wheat materials must also be interpreted conservatively. The three backgrounds differed in shoot biomass, SOD activity and disease response, but the experiment does not isolate ploidy as the cause. Seed size, genotype, domestication history and root architecture may also contribute to the observed variation. The findings therefore describe the responses of the three tested wheat materials, not a general effect of chromosome number. Future experiments should include several genotypes within each ploidy level to separate ploidy-related patterns from genotype-specific adaptation.

From an applied perspective, *T. viride* is a candidate component of integrated seedling management in drought- and salinity-prone systems. It cannot replace drought-tolerant germplasm, irrigation management, or salinity control, but it may support establishment when *Fusarium* infection occurs together with either water deficit or salinity. Validation under field conditions remains necessary, particularly where soil moisture, salinity, and pathogen communities vary spatially and over time.

Conclusion

Under the tested greenhouse conditions, drought reduced wheat shoot dry weight more strongly than salinity. *Fusarium* infection imposed an additional limitation, whereas *T. viride* increased shoot dry weight and SOD activity in the non-stressed, saline, and drought environments. The biological treatment also reduced disease severity in *Fusarium*-inoculated seedlings, with greater suppression of *F. graminearum* than *F. oxysporum* in this dataset. The results support a measured conclusion that *T. viride* did not eliminate injury caused by drought or salinity, but it improved seedling performance within each abiotic environment. It may therefore be useful as one biological component of early wheat establishment in drought- and salinity-prone systems where *Fusarium* infection also occurs. Field validation should include a broader set of genotypes and direct measurements of root colonization, pathogen biomass, and additional physiological indicators of drought and salinity response.

Acknowledgment

The authors thank the Iraq National Cereals Research Center for providing the wheat seed material and the laboratory and greenhouse staff for technical assistance. Shahid Bahonar University of Kerman is also gratefully acknowledged.

References

Agrios, G. N. (2005). *Plant pathology* (5th ed.). Elsevier Academic Press.

Ahmadi, J., Pour-Aboughadareh, A., Fabriki Ourang, S., Khalili, P., & Poczai, P. (2020). Unraveling salinity stress responses in ancestral and neglected wheat species at early growth stage: A baseline for utilization in future wheat improvement programs. *Physiology and Molecular Biology of Plants, 26*(3), 537–549. https://doi.org/10.1007/s12298-020-00768-4

Akbari, S. I., Prismantoro, D., Permadi, N., Rossiana, N., Miranti, M., Mispan, M. S., Mohamed, Z., & Doni, F. (2024). Bioprospecting the roles of Trichoderma in alleviating plants' drought tolerance: Principles, mechanisms of action, and prospects. *Microbiological Research, 283*, 127665. https://doi.org/10.1016/j.micres.2024.127665

Ascaso, J., Mendoza-Salido, D., Pedrero-Méndez, A., Monte, E., Quijada, N. M., & Hermosa, R. (2025). Transcriptomic and biochemical analyses reveal wheat drought mitigation by Trichoderma simmonsii and reduced

demand for canonical plant stress responses. *Frontiers in Plant Science, 16*, 1716657. https://doi.org/10.3389/fpls.2025.1716657
Bashyal, B. M., Parmar, P., Zaidi, N. W., & Aggarwal, R. (2021). Molecular programming of drought-challenged Trichoderma harzianum-bioprimed rice (Oryza sativa L.). *Frontiers in Microbiology, 12*, 655165. https://doi.org/10.3389/fmicb.2021.655165
Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry, 44*, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8
Boamah, S., Zhang, S., Xu, B., Li, T., & Calderon-Urrea, A. (2021). Trichoderma longibrachiatum (TG1) enhances wheat seedlings' tolerance to salt stress and resistance to Fusarium pseudograminearum. *Frontiers in Plant Science, 12*, 741231. https://doi.org/10.3389/fpls.2021.741231
Boamah, S., Zhang, S., Xu, B., Li, T., Calderon-Urrea, A., & Tiika, R. J. (2022). Trichoderma longibrachiatum TG1 increases endogenous salicylic acid content and antioxidant activity in wheat seedlings under salinity stress. *PeerJ, 10*, e12923. https://doi.org/10.7717/peerj.12923
Boamah, S., Zhang, S., Xu, B., Zhu, N., & Li, E. (2025). Trichoderma longibrachiatum TG1 colonization and signal pathway in alleviating salinity and Fusarium pseudograminearum stress in wheat. *International Journal of Molecular Sciences, 26*(9), 4018. https://doi.org/10.3390/ijms26094018
Contreras-Cornejo, H. A., Schmoll, M., Esquivel-Ayala, B. A., González-Esquivel, C. E., Rocha-Ramirez, V., & Larsen, J. (2024). Abiotic plant stress mitigation by Trichoderma species. *Soil Ecology Letters, 6*(4), 240240. https://doi.org/10.1007/s42832-024-0240-8
Giannopolitis, C. N., & Ries, S. K. (1977). Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology, 59*, 309–314. https://doi.org/10.1104/pp.59.2.309
Gui, Y.-W., Sheteiwy, M. S., Zhu, S.-G., Batool, A., & Xiong, Y.-C. (2021). Differentiate effects of non-hydraulic and hydraulic root signaling on yield and water use efficiency in diploid and tetraploid wheat under drought stress. *Environmental and Experimental Botany, 181*, 104287. https://doi.org/10.1016/j.envexpbot.2020.104287
Haile, J. K., N'Diaye, A., Walkowiak, S., Nilsen, K. T., Clarke, J. M., Kutcher, H. R., Steiner, B., Buerstmayr, H., & Pozniak, C. J. (2019). Fusarium head blight in durum wheat: Recent status, breeding directions, and future research prospects. *Phytopathology, 109*(10), 1664–1675. https://doi.org/10.1094/PHYTO-03-19-0095-RVW
Hajebi, F., Moradi, N., Bazrafshan, O., & Sadeghi Lari, A. (2026). Effects of selected mineral and organic amendments at nano and conventional scales on the physical and hydraulic properties of saline soils. *Journal of Drought and Climate Change Research*, *4*(1), 41–60. https://doi.org/10.22077/jdcr.2025.9403.1144
Illescas, M., Moran-Diez, M. E., Martinez de Alba, A. E., Hermosa, R., & Monte, E. (2022). Effect of Trichoderma asperellum on wheat plants' biochemical and molecular responses, and yield under different water stress conditions. *International Journal of Molecular Sciences, 23*(12), 6782. https://doi.org/10.3390/ijms23126782
Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology, 59*, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911
Oljira, A. M., Hussain, T., Waghmode, T. R., Zhao, H., Sun, H., Liu, X., Wang, X., & Liu, B. (2020). Trichoderma enhances net photosynthesis, water use efficiency, and growth of wheat (Triticum aestivum L.) under salt stress. *Microorganisms, 8*(10), 1565. https://doi.org/10.3390/microorganisms8101565
Özkale, E., Yörük, E., Budak, M., & Korkmaz, E. M. (2023). Trichoderma atroviride suppresses Fusarium graminearum by altering primary and secondary metabolite biosynthesis profiling. *Plant Pathology, 72*, 1428–1441. https://doi.org/10.1111/ppa.13768
Pakgohar, N., Maghsoudi Moud, A. A., Farahbakhsh, H., & Pasandipour, A. (2025). Effects of brassinosteroid and melatonin on antioxidant enzymes and grain yield of quinoa (*Chenopodium quinoa* Willd.) under drought stress. *Journal of Drought and Climate Change Research*. Advance online publication. https://doi.org/10.22077/jdcr.2025.9942.1163
Pedrero-Méndez, A., Cesarini, M., Mendoza-Salido, D., Petrucci, A., Sarrocco, S., Monte, E., & Hermosa, R. (2025). Trichoderma strain-dependent direct and indirect biocontrol of Fusarium head blight caused by Fusarium graminearum in wheat. *Microbiological Research, 296*, 128153. https://doi.org/10.1016/j.micres.2025.128153
Ramezani Etedali, H., & Koohi, S. (2026). Evaluation of climate change effects on heat stress imposed on strategic agricultural products in Qazvin Province. *Journal of Drought and Climate Change Research*, *4*(1), 81–104. https://doi.org/10.22077/jdcr.2025.9739.1157
Ruan, Y., Zhang, W., Knox, R. E., Berraies, S., Campbell, H. L., Ragupathy, R., Boyle, K., Polley, B., Henriquez, M. A., Burt, A., Kumar, S., Cuthbert, R. D., Fobert, P. R., Buerstmayr, H., & DePauw, R. M. (2020). Characterization of the genetic architecture for Fusarium head blight resistance in durum wheat: The complex association of resistance, flowering time, and height genes. *Frontiers in Plant Science, 11*, 592064. https://doi.org/10.3389/fpls.2020.592064
Saadaoui, M., Faize, M., Bonhomme, L., Benyoussef, N. O., Kharrat, M., Chaar, H., Label, P., & Venisse, J.-S. (2023). Assessment of Tunisian Trichoderma isolates on wheat seed germination, seedling growth, and Fusarium seedling blight suppression. *Microorganisms, 11*(6), 1512. https://doi.org/10.3390/microorganisms11061512

Santoyo, G., Orozco-Mosqueda, M. C., Afridi, M. S., Mitra, D., Valencia-Cantero, E., & Macias-Rodriguez, L. (2024). Trichoderma and Bacillus multifunctional allies for plant growth and health in saline soils: Recent advances and future challenges. *Frontiers in Microbiology, 15*, 1423980. https://doi.org/10.3389/fmicb.2024.1423980

Senizza, B., Araniti, F., Lewin, S., Wende, S., Kolb, S., & Lucini, L. (2023). Trichoderma spp.-mediated mitigation of heat, drought, and their combination on the Arabidopsis thaliana holobiont: A metabolomics and metabarcoding approach. *Frontiers in Plant Science, 14*, 1190304. https://doi.org/10.3389/fpls.2023.1190304

Silletti, S., Di Stasio, E., Van Oosten, M. J., Ventorino, V., Pepe, O., Napolitano, M., Marra, R., Woo, S. L., Cirillo, V., & Maggio, A. (2021). Biostimulant activity of Azotobacter chroococcum and Trichoderma harzianum in durum wheat under water and nitrogen deficiency. *Agronomy, 11*(2), 380. https://doi.org/10.3390/agronomy11020380

Sorahinobar, M., Yusefieh, N., Rezayian, M., & Shahbazi, S. (2025). Multifaceted role of Trichoderma harzianum isolates in mitigating drought stress and promoting adaptive responses in barley cultivars. *Scientific Reports, 15*, 26552. https://doi.org/10.1038/s41598-025-08922-2

Stummer, B. E., Zhang, X., Moghaddam, M. M., Yang, H., & Harvey, P. R. (2024). Wheat rhizosphere dynamics of Trichoderma gamsii A5MH and suppression of a Pythium root rot–Fusarium crown rot disease complex over two consecutive cropping seasons. *Journal of Applied Microbiology, 135*(4), lxae069. https://doi.org/10.1093/jambio/lxae069

Van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annual Review of Plant Biology, 71*, 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

Woo, S. L., Hermosa, R., Lorito, M., & Monte, E. (2023). Trichoderma: A multipurpose, plant-beneficial microorganism for eco-sustainable agriculture. *Nature Reviews Microbiology, 21*, 312–326. https://doi.org/10.1038/s41579-022-00819-5

Zhang, S., Gan, Y., & Xu, B. (2019). Mechanisms of the IAA and ACC-deaminase producing strain of Trichoderma longibrachiatum T6 in enhancing wheat seedling tolerance to NaCl stress. *BMC Plant Biology, 19*, 22. https://doi.org/10.1186/s12870-018-1618-5