



Enhancing Salinity Tolerance in Triticale *via* Synergistic Effect of Plant Growth Promoting Bacteria and Arbuscular Mycorrhizal Fungi

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ABSTRACT

Background: Soil salinity is a major abiotic stress factor that severely limits crop productivity worldwide threatening the sustainability of modern agriculture. This study investigated the combined application of plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) in mitigating salt stress in triticale (*×Triticosecale*).

Methods: Three salinity levels (0, 100 and 200 mM NaCl) and nine microbial treatments including two PGPB strains (KF58B *Brevibacterium frigoritolerans* and KF63C *Paenibacillus xylanilyticus*) and two AMF species (*Glomus etunicatum* and *Funneliformis mosseae*) were tested using under controlled conditions. Glutathione peroxidase (GSH), catalase (CAT) and malondialdehyde (MDA) alterations were investigated to observe plant stress responses.

Result: Salinity had a significant inhibitory effect on plant growth. Average seedling fresh weight decreased from 0.759 g (control) to 0.393 g under 200 mM NaCl, while root fresh weight dropped from 0.687 g to 0.167 g. Root length declined by 48.2%, from 29.81 cm to 15.44 cm. Enzymatic activities and stress indicators showed notable variations. According to salinity × treatment interaction, the highest CAT (32.77 ng mL⁻¹) and GSH (5.12 ng mL⁻¹) activity were observed in the KF63C + *F. mosseae* and KF58B + *F. mosseae* under severe salinity, respectively. MDA reached a peak of 4.81 ng mL⁻¹ under 200 mM NaCl conditions, but, it reduced by 74.6% with KF58B + *G. etunicatum* combination. Although no statistically significant differences were detected in morphological characteristics at the seedling stage, enzymatic observations indicated stress mitigation in plants. These findings supported the potential of co-inoculation with PGPB (*Brevibacterium frigoritolerans* and *Paenibacillus xylanilyticus*) and AMF (*G. etunicatum* and *F. mosseae*) on improving salt stress tolerance in triticale during early seedling stage. In particular, combination of *B. frigoritolerans* and *F. mosseae* is recommended to mitigation of salinity stress in triticale during early seedling stage.

Key words: ACC deaminase, Antioxidant response, Bio-priming, Microbiology, Plant stress, Stress tolerance.

INTRODUCTION

Triticale is cultivated worldwide primarily for grain and forage production and more recently, for bioenergy purposes. A total of 341,000 tons of triticale were produced on 109,605 hectares in Türkiye in 2024. Additionally, the average yield per hectare was calculated to be 3,110 kg (TÜİK, 2025). Improving grain yield and quality per unit area in triticale cultivation is of significant importance for both regional and national agriculture.

Salinity is one of the critical environmental stress factors that negatively affect agricultural productivity. According to the global report on soil resources, salinity adversely impacts approximately 60 million hectares, or about 20% of irrigated arable land worldwide. Furthermore, it is projected that salinity-affected areas may expand to 50% of total arable land by 2050 (Butcher *et al.*, 2016). Improper irrigation practices and changing environmental conditions have been shown to increase Na⁺ and Cl⁻ ion concentrations in soils (Wei *et al.*, 2021). Moreover, salt stress leads to major adverse effects in plants, including ion toxicity, osmotic stress, reduced water uptake, enhanced oxidative stress and overall inhibition of growth and development (Monisha *et al.*, 2025).

One of the mechanisms through which arbuscular mycorrhizal fungi (AMF) promote plant growth is by facilitating nutrient uptake, especially phosphorus (Atakli *et al.*, 2022). This symbiosis is also essential for the acquisition of other

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elements such as nitrogen, sulfur and zinc (Hodge and Storer, 2015). AMF have been found to reduce or prevent nutrient loss from soil through the production of the glomalin protein, a glycoprotein that significantly improves soil aggregation and prevents nutrient loss *via* denitrification

(He *et al.*, 2020). In general, AMF can mitigate damage caused by soil-borne plant pathogens or parasites, enhance plant resistance or tolerance depending on fungal species and provide protection against phytopathogenic organisms (Song *et al.*, 2015).

Beneficial microorganisms are gaining prominence with soil bacteria being the most viable alternative (Akçura and Çakmakçı, 2023). In addition to their direct nutritional contributions to plants, these bacteria can protect plants from multiple environmental stresses through ACC deaminase production and protect against diseases by producing siderophores. AMF can enhance salinity tolerance and mediate plant stress responses (Dastogeer *et al.*, 2020). Evelin *et al.* (2019) indicated that AMF colonization in roots supports plants to boost salinity stress tolerance. These characteristics make AMF a promising, eco-friendly strategy within sustainable agricultural systems.

The central hypothesis of this study is that the combined application of ACC deaminase-active PGPB and AMF strains will synergistically enhance the antioxidant responses of triticale under salinity stress, thereby improving the plant's physiological tolerance to adverse conditions. Accordingly, the aim of the research is to determine the effects of different PGPB and AMF combinations on the growth performance and molecular defense mechanisms of triticale under salt stress, with a particular focus on alterations in key agronomical and biochemical indicators including CAT, GSH and MDA. The originality of the study lies in the first-time evaluation of two native PGPB isolates with high ACC deaminase and siderophore production capacity in combination with two AMF strains in triticale. Additionally, the systematic assessment of both synergistic and antagonistic interactions among these biological agents highlights another novel aspect of this work. Overall, the study presents an innovative approach that reveals the potential of microbial consortia in managing salinity stress.

MATERIALS AND METHODS

Experimental site and conditions

The study was conducted as a pot experiment at 2024 in the climate chamber of Siirt University, Siirt, Türkiye. The climate chamber was maintained at an average temperature of $24 \pm 2^\circ\text{C}$ with a photoperiod of 16 hours light and 8 hours dark. Light intensity was set at $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ and its distribution was ensured to be homogeneous.

Experimental material

Tatlicak triticale cultivar was used in the study. The biological materials consisted of two PGPB strains (*Brevibacterium*

frigritolerans: KF58B and *Paenibacillus xylanilyticus*: KF63C) and two AMF species (*Glomus etunicatum* and *Funneliformis mosseae*). Both strains have high ACC deaminase activity and siderophore production ability. Bacterial materials were colonized in a nutrient agar medium and transferred into nutrient broth. The density of the nutrient broth medium in which the bacteria were inoculated was adjusted to 10^8 colony forming unit (cfu) by spectrophotometric method (OD_{600}). Bio-priming process was implemented by the methods of Ceritoglu *et al.* (2024).

Experimental design and lay out

The study was designed with a total of 9 treatments under 3 different salinity levels (0, 100 and 200 mM NaCl). Details of the 9 experimental treatments are provided in Table 1.

The experiment was laid out according to completely randomized design with three replications. A 2:1 (v/v) mixture of sterilized field soil and peat was mixed and filled in two-liter pots. The experimental soil was characterized for physical and chemical properties. Texture was determined using the hydrometer method. The pH and EC were determined via 1:2.5 soil-to-water suspension. Lime content was calculated with calcimeter method that was modified by Elfaki *et al.* (2015). Organic matter was determined by the method of the Walkley-Black wet oxidation procedure. Macro- and micronutrients were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) by protocol of Kacar (2009). The soil was classified as loam and exhibited an slightly alkaline pH. There was no salinity, however, it has low organic matter. The soil contained a moderate amount of lime. Available phosphorus were low, whereas potassium was sufficient. Calcium and magnesium contents were at moderate. Manganese, copper and iron were sufficient, however, zinc content was low (Table 2).

Table 1: Experimental treatments including plant growth promoting bacteria and arbuscular mycorrhizal fungi.

Treatment's code	Treatment
T1	Control
T2	KF58B
T3	KF63C
T4	<i>Glomus etunicatum</i>
T5	<i>Funneliformis mosseae</i>
T6	KF58B × <i>Glomus etunicatum</i>
T7	KF58B × <i>Funneliformis mosseae</i>
T8	KF63C × <i>Glomus etunicatum</i>
T9	KF63C × <i>Funneliformis mosseae</i>

Table 2: Chemical characterization of experimental soil.

Sand	Silt %	Clay	T	pH	EC dS m^{-1}	Lime %	OM %	P	K	Ca	Mg	Zn	Mn	Fe	Cu
								-----kg da ⁻¹ -----				-----mg kg ⁻¹ -----			
38	32	30	L	7.5	0.21	8.05	1.65	3.24	65.8	2150	51.0	0.53	22.65	2.47	0.68

(T: Texture, L: Loam, SCL: Sandy clay loam, SIL: Silty loam).

The experimental soil was classified as loam in texture and exhibited an alkaline pH. It was non-saline and had a low organic matter content. The soil contained a moderate amount of lime. Available phosphorus levels were low, whereas potassium was found to be sufficient. Calcium and magnesium contents were at moderate levels. Manganese, copper and iron were present in adequate amounts; however, zinc content was found to be low (Table 2).

Pots were arranged at 80% of field capacity before sowing seeds and sustained through experiment. Seeds were treated for surface sterilization with 70% ethyl alcohol for 1 min and 10% sodium hypochlorite (NaOCl) for 5 min before seed priming process. Seeds were carefully washed with distilled water and then rinsed to remove surface water using sterile filter paper. Seeds were put in bacterial solutions for four hours and dried again (Ceritoglu *et al.*, 2024). After bio-priming process, eight uniform-sized seeds were carefully selected and sown in each pot. Recommended dose of AMF, i.e., 2 g per liter soil, was added into pots under seed bed (Ulukapı *et al.*, 2020). After germination, seedlings were thinned to five plants per pot. The experiment was concluded 40 days after thinning.

Data collection

Two plant samples were taken from each pot and transferred to a -86°C deep freezer for determination of glutathione peroxidase (GSH), catalase (CAT) and malondialdehyde (MDA). Leaf samples were crushed using liquid nitrogen in a mortar, centrifuged and obtained a homogenate by the methods of Akkemik *et al.* (2012). CAT, GSH and MDA concentrations were determined by the methods Lakhdar *et al.* (2011); Paglia and Valentine (1967) and Esterbauer and Cheeseman (1990), respectively. After harvest, plant height (PH), root length (RF), seedling fresh weight (SFW), root fresh weight (RFW), seedling dry weight (SDW) and root dry weight (RDW) was determined to observe morphological changes in the plants.

Statistical analysis

Two different factor (salinity stress and treatment) were used in the experiment that was laid out under controlled conditions. Normalization of data was controlled by Shapiro

and Wilk normalization test. Therefore, completely randomized design with factorial treatment were used in the experiment. The data were subjected to analysis of variance (ANOVA) based on completely randomized design. Mean comparisons were grouped using Tukey's test via JMP software.

RESULTS AND DISCUSSION

Results

ANOVA results revealed significant differences ($p < 0.05$ and 0.01) in experimental observations. Salinity stress caused statistically significant differences in all measured characteristics where MDA and CAT showed significance at the 5%, while the others were significant at the 1% level. Experimental treatments had no significant differences in growth attributes and biomass, however, caused significant differences ($p < 0.01$) in CAT and GSH, while it was at the 5% in MDA. Similarly, interaction between salinity and treatment ($S \times T$) caused significant differences in antioxidant response observations in which statistical differences was at the 1% in CAT and GSH, but it was 5% in MDA (Table 3).

The results indicated that PH, RL, SFW, RFW, SWD and RDW were observed at least under 200 mM NaCl conditions while they were the highest without NaCl treatment. Decreases in PH, RL, SFW, RFW, SWD and RDW under 200 mM NaCl conditions over control were calculated by 19.2%, 48.2%, 48.2%, 75.7%, 11.21% and 69.1%, respectively. The lowest CAT ($11.821 \text{ ng mL}^{-1}$), GSH (1.08 ng mL^{-1}) and MDA (1.10 ng mL^{-1}) were observed in control plants, whereas the highest CAT (14.68 ng mL^{-1}), GSH (3.22 ng mL^{-1}) and MDA (2.02 ng mL^{-1}) were determined in 200 mM NaCl exposed plants (Fig 1). CAT, GSH and MDA increased by 83.6%, 24.18% and 198.2% with 200 mM NaCl over control. Experimental treatments were not effective on these growth attributes in which PH, RL, SFW, RFW, SWD and RDW varied between 31.2-38.3 cm, 18.17-22.89 cm, 0.488-0.728 g, 0.292-476 g, 0.0686-0.1038 g and 0.0367-0.0560 g, respectively (Table 4).

The lowest GSH (1.25 ng mL^{-1}) was found in KF58B treated plants, while the highest GSH (2.86 ng mL^{-1}) was

Table 3: Analysis of variance results for experimental treatments under different salinity conditions.

Variation source	Salinity		Treatment		S×T	
	SM	TUKEY	SM	TUKEY	SM	TUKEY
Plant height	716.7	2.80**	264.9	6.50ns	522	13.6ns
Root length	3339.71	3.25**	208.47	7.56ns	493	15.83ns
Shoot fresh weight	1.82	0.12**	0.41	0.29ns	0.83	0.59ns
Root fresh weight	4.42	0.12**	0.22	0.26ns	0.66	0.59ns
Shoot dry weight	0.04	0.002**	0.007	0.04ns	0.01	0.09ns
Root dry weight	0.06	0.02**	0.002	0.04ns	0.007	0.08ns
Malondialdehyde	12.66	0.51*	16.99	1.16*	21.6	2.46*
Catalase	124.86	2.72*	929.22	6.33**	1095.19	13.22**
Glutathione	137.48	0.43**	22.75	1.00**	51.7	2.07**

(*: $p < 0.05$, **: $p < 0.01$, ns: No significant difference).

observed with KF58B+*F. mosseae*. According to S×T, the lowest GSH (0.37 ng mL^{-1}) was found in control plants under 100 mM NaCl, while the highest GSH (5.12 ng mL^{-1}) was found with KF58B+*G. etunicatum* under 200 mM NaCl conditions. The lowest CAT (9.531 mL^{-1}) was observed in KF58B+*G. etunicatum* treated plants, while the highest CAT ($20.398 \text{ ng mL}^{-1}$) was found with KF63C+*F. mosseae*. Overall, S×T interaction indicated that the lowest CAT ($6,983 \text{ ng mL}^{-1}$) was found with KF58B+*F. mosseae* under 200 mM NaCl, whereas the highest CAT ($32.770 \text{ ng mL}^{-1}$) was found in KF63C+*F. mosseae* treated plants under 200 mM NaCl conditions. The lowest MDA (1.07 ng mL^{-1}) was found in KF58B+*G. etunicatum* treated plants, while the highest one (2.76 ng mL^{-1}) was observed in control plants. According to S×T, the lowest MDA (0.78 ng mL^{-1}) was found in plants treated with KF58B+ *G. etunicatum* under 100 mM NaCl conditions and the highest MDA (4.81 ng mL^{-1}) was found in control plants under 200 mM NaCl conditions (Fig 2).

As a result of the experiment, it was determined that increasing NaCl concentrations in the growth medium adversely affected plant development parameters such as seedling and root length, as well as fresh and dry weights associated with biomass accumulation. It is well known that salt stress has harmful effects on plants at all developmental stages, from germination to harvest (Foti *et al.*, 2018). The primary causes of salinity's inhibitory effects on germination include the accumulation of toxic ions (Na^+ and Cl^-) and restricted water uptake by seeds. Salinity blocks enzymatic processes that convert endosperm reserves into sugars, thereby preventing proper germination and seedling development (Dash and Panda, 2001). In addition, K^+ ions that required for osmoregulation, cell growth, membrane polarization, enzyme activity and neutralization of negative ions compete with Na^+ accumulation. Moreover, Na^+ and Cl^- enter the cells and cause damage to cell membranes and cytosolic metabolic

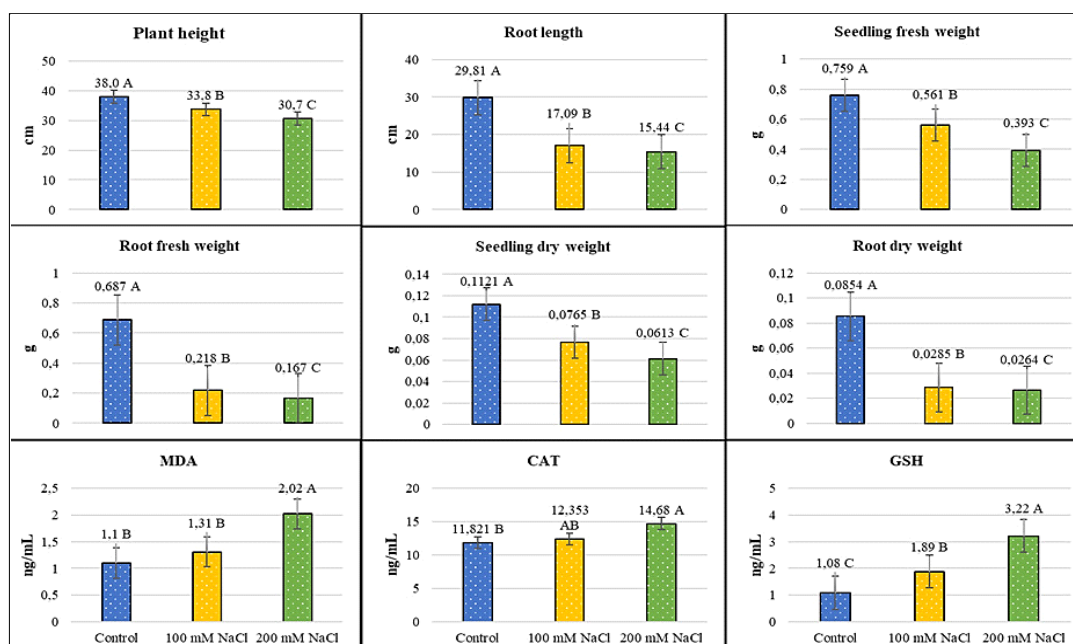


Fig 1: Alterations of growth attributes and antioxidant responses in triticale seedlings under different salinity conditions.

Table 4: Alterations in growth attributes with experimental treatments.

Treatments	PH	RL	SFW	RFW	SDW	RDW
Control	38.3	19.42	0.728	0.348	0.1038	0.0444
KF58B	35.0	22.89	0.532	0.411	0.0806	0.0560
KF63C	34.4	20.06	0.627	0.339	0.0812	0.0485
<i>G. etunicatum</i>	31.2	20.41	0.507	0.355	0.0762	0.0508
<i>F. mosseae</i>	34.3	22.82	0.613	0.347	0.0939	0.0456
KF58B + <i>G. etunicatum</i>	34.3	18.17	0.516	0.292	0.0797	0.0367
KF58B + <i>F. mosseae</i>	33.5	22.83	0.576	0.476	0.0832	0.0509
KF63C + <i>G. etunicatum</i>	33.0	20.67	0.488	0.332	0.0686	0.0474
KF63C + <i>F. mosseae</i>	33.3	19.79	0.547	0.317	0.0822	0.0409

(Treatments did not statistically affected the growth attributes. PH: Plant height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight).

processes (Zhu *et al.*, 2019). Consequently, increased salinity stress suppressed plant growth and limited dry matter accumulation. These findings are consistent with previous studies that have shown salinity stress restricts growth and biomass production in wheat (El Sabagh *et al.*, 2021), canola (Lone *et al.*, 2022), lentil (Ceritoglu *et al.*, 2023) and triticale (Alagoz *et al.*, 2023).

On the other hand, it was observed that antioxidant enzyme activities such as CAT and GSH increased under salt stress along with higher MDA concentrations. Salinity tolerance in plants is closely related to the activities of antioxidant enzymes such as SOD, CAT, GPX, APX and GR, as well as non-enzymatic antioxidant compounds (Gupta *et al.*, 2005). Disruption of electron transport chains causes molecular oxygen to act as an electron acceptor, leading to excessive ROS production under stress conditions. Salt-induced osmotic stress alters plant metabolism and enzymatic activity, resulting in the overproduction of ROS (Menezes-Benavente *et al.*, 2004).

Reactive oxygen species such as 1O_2 , OH^\cdot , O_2^\cdot and H_2O_2 , can damage proteins, lipids, carbohydrates and DNA (Groß *et al.*, 2013). Within chloroplasts, photosystems I and II, ubiquinone and mitochondrial ETC complex III are key sites for ROS generation (Gill and Tuteja, 2010). For instance, excessive H_2O_2 accumulation leads to the

collapse of maize leaf veins due to leakage between neighboring cells under salt stress (Menezes-Benavente *et al.*, 2004). Plants respond to salt stress with multigenic mechanisms, including osmotic and ionic homeostasis and antioxidant defense systems involving cellular detoxification (Hasanuzzaman *et al.*, 2021).

PGPB and AMF-based treatments did not show significant effects on plant growth and biomass accumulation during the early seedling stage. It is known that the endosperm reserves of seeds play a major role in early seedling development (Açikbaş *et al.*, 2022). Furthermore, microbial materials generally act more slowly but systematically compared to chemical fertilizers. Thus, it is suggested that biological applications may not have made a noticeable contribution to morphological traits within the short time frame of early seedling development in this experiment. However, molecular analyses revealed significant differences caused by PGPB and AMF applications in triticale seedlings under salt stress. The resistance of PGPB to stress is based on the breakdown of ethylene-which rises sharply under stress-into α -ketobutyrate and ammonia *via* ACC deaminase activity (del Carmen Orozco-Mosqueda *et al.*, 2020). One of the mechanisms by which AMF promote plant growth is through enhanced nutrient uptake, especially phosphorus. This symbiosis also

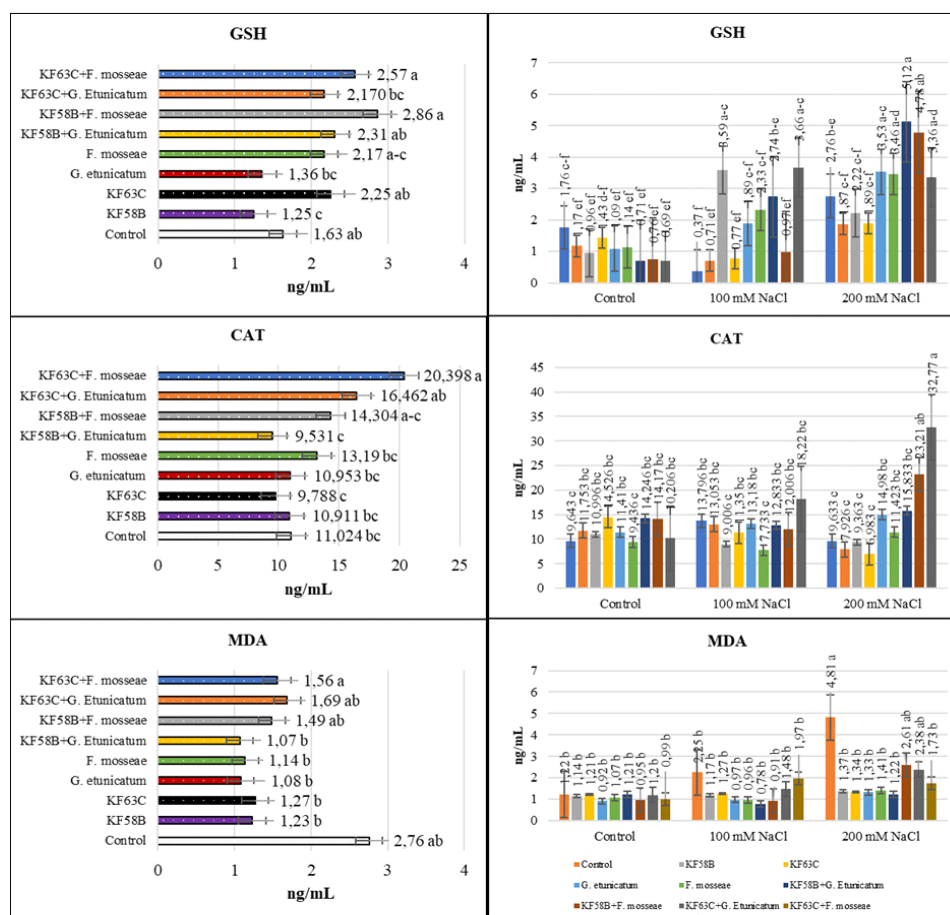


Fig 2: Alterations of antioxidant responses depending on experimental treatments in triticale plants.

facilitates the acquisition of nitrogen, sulfur and zinc (Hodge and Storer, 2015). AMF have also been shown to reduce or prevent nutrient loss from soil through the production of glomalin, a glycoprotein that significantly improves soil aggregation and prevents nutrient loss via denitrification (He *et al.*, 2020). Additionally, AMF can reduce the harmful effects of soil-borne pathogens and parasites and enhance plant resistance (Song *et al.*, 2015).

Regarding the effects of PGPB and AMF on plant growth, co-inoculation has often been suggested as a better strategy than single inoculation due to enhanced synergistic effects (Nacoon *et al.*, 2020). Bacteria have been found to improve AMF germination by disrupting fungal cell envelopes or releasing volatile compounds (Turrini *et al.*, 2018). *Bacillus thuringiensis* inoculation in wheat resulted in increases of 38.8% in proline content and 21.4% in soluble sugar content (Huang *et al.*, 2022). Moreira *et al.* (2022) reported that co-application of PGPB with AMF (*Rhizoglyphus irregularis*) resulted in synergistic effect on stress responses in maize under saline conditions. Sabah *et al.* (2025) reported that PGPB treatment enhanced AMF root colonization compared to AMF-only treatments. Yadav *et al.* (2022) indicated that *Bacillus* sp. and arbuscular mycorrhizal fungi consortia enhance wheat nutrient and yield in the second year field trial in which maximum AMF colonization occurred with PGPB+AMF co-inoculation.

CONCLUSION

While PGPB and AMF applications did not produce significant morphological effects during early seedling development in triticale, they led to notable differences at the molecular level under salt stress. This suggests that these treatments may be effective in stress management during later developmental stages. Another important finding of this study was the emergence of both synergistic and antagonistic interactions among different PGPB and AMF strains. Specifically, bio-priming with *Paenibacillus xylanilyticus* and soil application of *F. mosseae* resulted in higher CAT and GSH activation in response to salt stress. Additionally, MDA was reduced by 74.6% in treatments combining *Brevibacterium frigoritolerans* and *G. etunicatum* compared to the control under high saline (200 mM) conditions. This outcome is believed to be associated with better utilization of soil moisture and nutrients, as well as the reduction of ethylene synthesis through the ACC deaminase mechanism. It is recommended that dual relationships of PGPB and AMF should be deeply investigated via molecular tests under control and field conditions.

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Disclaimers

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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