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EDITED BY

Jingsong Li,
Hebei Academy of Agriculture and
Forestry Sciences (HAAFS), China

REVIEWED BY

Bruno Sousa,
Universidade do Porto GreenUPorto
Centro de Investigação em Produção
Agroalimentar Sustentável, Portugal
Arezo Paravar,
University of Hormozgan, Iran

*CORRESPONDENCE

Mansour Taghvaei
✉ taghvaei@shirazu.ac.ir
Beata Dedicova
✉ beata.dedicova@slu.se

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Arbuscular mycorrhizal fungi as a promising strategy to enhance root architecture in durum wheat (*Triticum durum* Desf.) cultivars under salinity stress

Neda Zavarshani¹, Mansour Taghvaei^{2,3*}, Mehdi Zarei^{1,4}
and Beata Dedicova^{5*}

¹Department of Soil Science, School of Agriculture, Shiraz University, Shiraz, Iran, ²Department of Plant
Production and Genetics, School of Agriculture, Shiraz University, Shiraz, Iran, ³Pioneer
Biotechnologists of Green Science (PBGs), Knowledge-Based Company, Shiraz University, Shiraz, Iran,

⁴Department of Agriculture and Natural Resources, Higher Education Center of Eghlid, Eghlid, Iran,

⁵Department of Plant Breeding, SLU Alnarp, Swedish University of Agricultural Sciences (SLU),
Alnarp, Sweden

Abiotic stress from salinity poses a significant challenge for crop plants, significantly impairing their growth. This study included three experiments: the first focused on evaluating the germination parameters of four durum wheat varieties under various salinity levels. The treatments involved four varieties (Shabrang, Taban, and lines D-98-8 and D-98-10) tested with NaCl solutions at nine salinity levels (0, 2, 4, 6, 8, 12, 16, 18, and 20 dS/m). The second experiment assessed seed cell membrane permeability to differentiate between salt-sensitive and salt-tolerant cultivars. The third examined the role of arbuscular mycorrhizal fungi in enhancing salt tolerance in both cultivars. The same four durum wheat varieties were used in the second experiment. The third experiment involved two durum wheat varieties (the salt-tolerant Shabrang and the salt-sensitive D-98-8), nine NaCl salinity levels, and inoculation with the fungus *Funneliformis mosseae*. Results from the first experiment showed that salinity stress reduced germination parameters across all cultivars, with a greater decline in the D-98-8 line. Additionally, the seed electrical conductivity test from the second experiment indicated that salinity tolerance in durum wheat was linked to lower cell membrane permeability. The Shabrang cultivar demonstrated the lowest permeability and leakage, while D-98-8 exhibited the highest. Therefore, both experiments identified Shabrang as salinity-tolerant and D-98-8 as salinity-sensitive. The third experiment showed that introducing mycorrhizal fungi increased root phosphate levels and improved root structure under saline conditions, with more pronounced effects in the salinity-tolerant cultivar.

KEYWORDS

electrical conductivity test, germination test, root architecture, seeds, wheat

1 Introduction

Soil salinity is a crucial factor that restricts plant growth and crop yields, especially in arid and semi-arid areas (Maach et al., 2020). High salt levels threaten about one-third of the world's farmland, and this threat may grow due to climate change (Hussein and Abou-Baker, 2018; Yang et al., 2020). Soil salinity occurs from the buildup of different ions, such as potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), sodium (Na^+), sulfate (SO_4^{2-}), carbonate (CO_3^{2-}), bicarbonate (HCO_3^-), and nitrate (NO_3^-), with Na and Cl being the most common ions and osmolytes (Ismail et al., 2014). Plants are vulnerable to salinity at every stage, from germination to seed production (Jeevan Kumar et al., 2015; Sorty et al., 2016). Among these stages, germination is especially vital for crop survival (Gong et al., 2018). High salt levels inhibit root growth by disrupting plant physiological and metabolic functions, thereby significantly reducing germination rates (Munns et al., 2006). Therefore, cultivating crops with advantageous root traits is often viewed as a way to increase yields on water-limited, saline soils (Rahnama et al., 2011). Exploring the impact of genetic diversity on root growth traits can enhance seedling establishment in dry, salty soils by selecting desirable cultivars (Richards, 2008). However, knowledge about genotypic variation in root growth traits remains limited (Pastuszak et al., 2022). Research indicates that salinity can affect canola seed germination through osmotic stress, depending on NaCl levels, resulting in slower germination and longer germination times (Bouzidi et al., 2021). Root system architecture (RSA), or the spatial arrangement of roots in the soil, directly influences water and nutrient uptake and is vital for how plants adapt to abiotic stresses such as salinity (Zhu et al., 2025).

Mitigating the challenges of salt stress on plants requires exploring and implementing innovative, sustainable, and cost-effective approaches. This is crucial because traditional breeding and genetic engineering are resource-intensive and time-consuming (Singh et al., 2024). Therefore, selecting cultivars tolerant of salinity in saline soils can be an effective and economical strategy for ensuring crop survival under salt stress (Niu et al., 2019). Additionally, soil microorganisms, especially endophytic fungi such as AMF, show significant promise in promoting plant growth under extreme salt conditions. The symbiosis between AMF and terrestrial plants, which account for 80% of plant species, has alleviated salt stress in various plants, including barley (Masrahi et al., 2023) and pistachio (Afshar and Abbaspour, 2023). AMF effectively enhances nutrient and water uptake beyond the root zone (Singh et al., 2024). The higher concentrations of macronutrients (P, K, Ca) and micronutrients (Fe, Zn, Mn) in the roots and leaves of mycorrhizal plants, compared to non-mycorrhizal plants, support the idea that mycorrhizal roots, via fungal hyphae, can explore a larger soil volume and therefore have a greater capacity to acquire nutrients than plants without fungi (Di Martino et al., 2018). Arbuscular mycorrhizal fungi (AMF) are known to regulate root system architecture, increase root branching, and boost overall uptake, thereby improving plant establishment and stress tolerance. Studies have shown that AMF inoculation modifies root system architecture by increasing lateral root formation and overall root development in host plants, potentially enhancing their ability

to acquire water and nutrients and cope with stress conditions (Zhu et al., 2025).

The symbiotic relationship between AM fungi and an environmentally friendly approach to enhancing the tolerance of agricultural products to salinity stress. Few studies on durum wheat have primarily focused on assessing salt tolerance, the effects of salinity on morphology, physiology, biochemistry, and various strategies employed to mitigate salt stress in this plant. Notably, to date, no study has specifically investigated the impact of applying the fungus *Funneliformis mosseae* on root architecture in durum wheat under salinity stress. The symbiotic relationship between AM fungi and plants can be a practical and beneficial support for both species.

Therefore, this study first introduced two durum wheat cultivars, one tolerant and one sensitive to salinity, and then assessed the effects of salinity on early root architectural traits in durum wheat. Additionally, the study sought to determine whether inoculation with *Funneliformis mosseae* can improve root development and salinity tolerance during early seedling growth.

2 Materials and methods

2.1 Experiment 1: screening of cultivars for salt tolerance through germination test

The effects of salinity stress on seed germination parameters and seedling growth were assessed for four durum wheat genotypes (varieties Shabrang and Taban and lines D-98-8 and D-98-10) in a laboratory setting at the Biology and Biotechnology Laboratory of the Soil Science Department at Shiraz University of Agriculture. Seeds were sourced from the Darab County Agricultural Research Station, which is part of the Fars Province Agricultural Research Organization. The first experiment's treatments used a factorial design, randomized complete block design, with four replications. The treatments included durum wheat (varieties Shabrang and Taban, lines D-98-8 and D-98-10) and NaCl salinity at nine levels (0, 2, 4, 6, 8, 12, 16, 18, and 20 dS/m). Before testing, seeds were rinsed with sterile distilled water. Twenty-five seeds were placed on a single layer of moist filter paper in 9-cm plastic Petri dishes under controlled laboratory light conditions. They were incubated at 25 °C under light for 9 days, with germinated seeds counted daily throughout. Seeds were considered germinated when the radicals reached at least 2 mm in length. To determine seedling dry weight, samples were dried in an oven at 70 °C for 24 hours and weighed with a scale accurate to 0.0001 grams. Seedling lengths were measured with a ruler precise to one millimeter. These traits were calculated using the relevant formulas.

Germination percentage (Scott et al., 1984):

$$\text{Germination percentage} = \frac{\sum G}{N} \times 100$$

G = The number of germinated seeds per day, N = Total number of seeds

Germination rate (Maguire, 1962):

$$\text{Germination rate} = \frac{\sum Si}{Di}$$

Si = Number of germinated seeds per count, Di = The number of days

Seedling length vigor index (Powell, 2009):

$$\text{Seedling length vigor index} = (\text{SL} \times \text{NSP})$$

SL = Seedling length, NSP = Normal seedling percentage

-Seedling weight vigor index (Powell, 2009):

$$\text{Seedling weight vigor index} = (\text{SDW} \times \text{NSP})$$

SDW = Seedling dry weight, NSP = Normal seedling percentage

Reduction percent:

$$\text{Reduction percent} = \left(1 - \frac{N_x}{M_x}\right)$$

N_x = The amount of each trait after applying the salinity treatment, and M_c = The amount of each trait before applying the salinity treatment.

2.2 Experiment 2: seed electrical conductivity for salt tolerance screening

Durum wheat cultivars were evaluated for salt tolerance by measuring seed electrical conductivity (EC). The test took place at the Biology and Biotechnology Laboratory, Soil Science Department, Shiraz University of Agriculture. The experiment used a completely randomized design with three replications and a factorial arrangement. Treatments included four durum wheat genotypes: Shabrang, Taban, lines D-98-8, and D-98-10. For each genotype, 25 seeds were weighed and placed in 100-ml beakers with 50 ml of distilled water. The beakers were covered with aluminum foil and incubated at 25 °C for 24 hours. After incubation, the EC of the soaking solution was measured using an electrical conductivity meter. The EC of the distilled water was verified to be less than 0.3 μS cm⁻¹. This method indirectly evaluated seed vigor and membrane integrity, as higher EC values suggest greater solute leakage from seeds during imbibition, which typically correlates with lower seed vigor and germination potential.

Based on the results of Experiments 1 and 2, Shabrang and D-98-8 were identified as the salt-tolerant and salt-sensitive genotypes, respectively, and were therefore chosen for the greenhouse experiment.

2.3 Experiment 3: effects of arbuscular mycorrhizal fungi on root architecture and phosphate concentration

A greenhouse experiment was conducted in 2023 at the Department of Soil Science, Faculty of Agriculture, Shiraz University, to examine the effects of salinity and *Funneliformis mosseae* inoculation on two durum wheat genotypes with different salt tolerance levels (Shabrang as salt-tolerant and D-98-8 as salt-sensitive). The experiment was set up as a completely randomized factorial design with three replications. The factors included:

Salinity stress: nine levels (0, 2, 4, 6, 8, 12, 16, 18, and 20 dS/m)

Durum wheat genotypes: Shabrang (salt-tolerant) and D-98-8 (salt-sensitive)

Seed inoculation: with *Funneliformis mosseae*

This experiment aimed to assess the effects of arbuscular mycorrhizal fungi on root growth characteristics and root phosphate levels in selected salt-tolerant and salt-sensitive durum wheat cultivars under different salinity stress conditions. The *Funneliformis mosseae* inoculum was prepared at the Soil Biology and Biotechnology Laboratory within the Department of Soil Science at Shiraz University. The inoculum contained 12 spores per gram, mycelium, and 85% colonized corn (*Zea mays* L.) root fragments. The identity of the *Funneliformis mosseae* fungus grown in saline soils was confirmed through molecular and morphological analysis, as described in previous studies (Zarei et al., 2008, 2010).

The desired pots were first disinfected using sodium hypochlorite to set up the experiment. The durum wheat seeds were surface-sterilized by immersion in a 5% sodium hypochlorite solution for 3 minutes, followed by five rinses with distilled water. The culture medium for the experiment was prepared by mixing quartz sand (<1 mm) in a 1:4 (v/v) ratio. The mixture was then autoclaved at 120 °C. Five sterilized seeds were planted in each pot filled with autoclaved culture medium, with five pots per treatment. For the arbuscular mycorrhizal fungi inoculation treatment, 10 g of *Funneliformis mosseae* inoculum was placed at a depth of 2–3 cm beneath each seed in each 1 kg pot, and the seeds were then covered with an appropriate amount of culture medium. In the control treatment, the same amount of sterilized inoculum was added to the pots after autoclaving.

Plants were grown in a greenhouse under natural light conditions without supplementary artificial lighting, with a photoperiod of approximately 10–12 hours and day/night temperatures of 25/18 °C. Based on soil texture, the field capacity (FC) was estimated to be approximately 5–10% (w/w), consistent with typical field capacity values for sandy soils (~9% volumetric) (Brady, 2007). Therefore, the amount of water needed to reach FC was calculated to be between 50 and 100 ml per pot. Irrigation was performed manually, with soil moisture kept close to FC by regularly weighing the pots and adding water to compensate for evapotranspiration losses. Ten days after sowing, the plants were thinned to one per pot. To prevent the adverse effects of salinity on the newly emerged plants and support the establishment of mycorrhizal symbiosis, the salinity stress treatment was initiated 21 days after sowing and continued for two weeks. In the control treatment, plants were irrigated only with non-saline water. Each pot was watered once before applying the salt stress and twice afterward, using 100 ml of half-strength Hoagland's nutrient solution (pH 6.0 ± 0.1) with a reduced phosphate concentration, half that in the original solution. This low-phosphate nutrient solution enhanced the symbiotic relationship between the plants and the arbuscular mycorrhizal fungus (Hoagland and Arnon, 1938). These applications of the Hoagland nutrient solution were separate from routine irrigation events to maintain soil moisture near field capacity. Salinity stress was applied using NaCl solutions, and salinity levels were measured accurately with an electrical conductivity meter. After 60 days from the start of the experiment, the pots were placed in water-filled containers for 30 minutes to saturate the soil and facilitate root removal. The plants were then carefully removed from the pots, and the roots were

gently washed with water to remove soil (Fakhri, 2013). Root system architecture was assessed using a DELTA-T SCAN instrument (London, UK) with GiA Roots software (Galkovsky et al., 2012). Specifically, four parameters were measured: (i) average root width, representing the average thickness of the roots; (ii) network length, indicating the total length of root segments; (iii) network length distribution, reflecting the dispersion of root lengths; and (iv) the number of connected components, which is the count of discrete subnetworks within the root system. These traits provide a comprehensive assessment of root branching and connectivity under saline conditions and in response to mycorrhizal inoculation. Subsamples of root biomass were ground, ashed at 500°C for 10 hours, and analyzed for phosphate content using spectrophotometric methods (Murphy and Riley, 1962).

2.4 Root colonization (staining method)

To determine the extent of root colonization by arbuscular mycorrhizal fungi, 1-cm-long fresh root segments were stained using the method described by (Phillips and Hayman, 1970) with minor modifications. The stained root samples were then observed under a light microscope, and root colonization percentage was quantified using the grid-line-intersection method (Giovannetti and Mosse, 1980).

2.5 Statistical analysis

The collected data were analyzed using SAS, version 9.4. To evaluate statistical differences in the mean values of the measured traits, the Least Significant Difference (LSD) test was used when the experimental factors were significant. Additionally, Excel software was used to create graphical representations of the results. For Experiment 3, a three-way analysis of variance (ANOVA) was

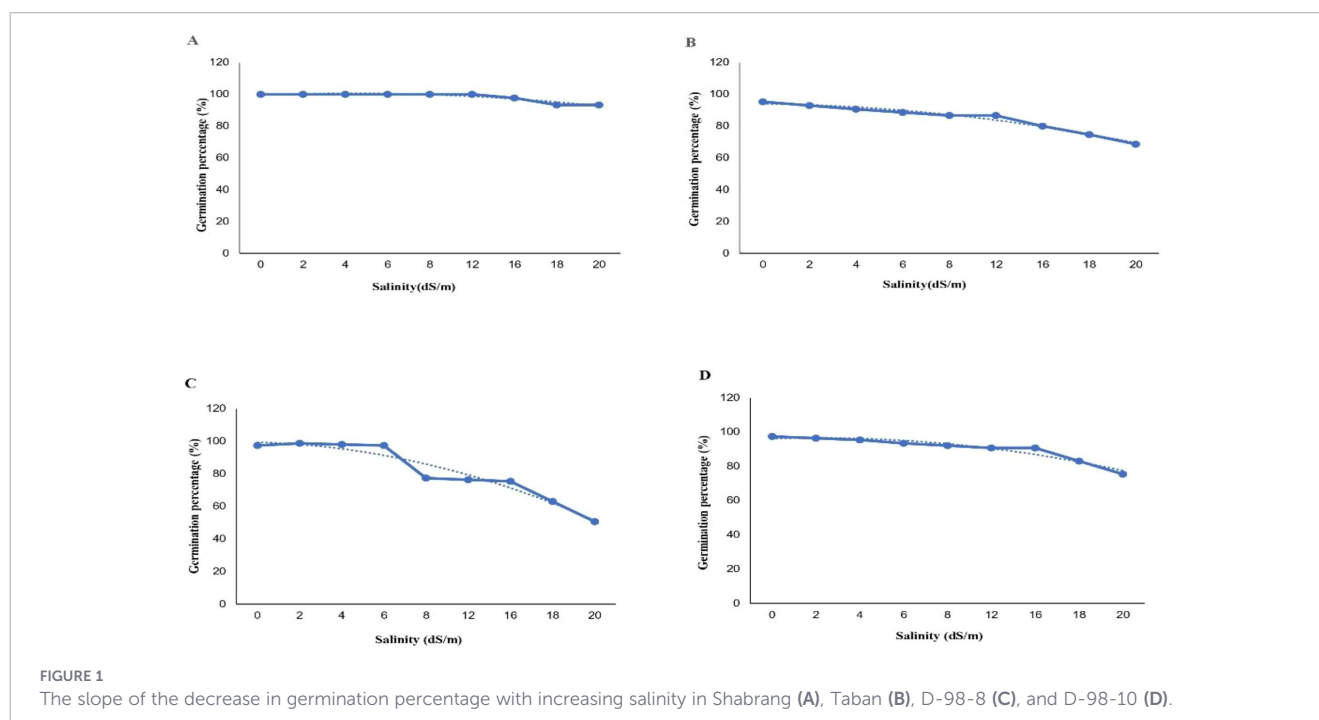
conducted considering genotype (G), salinity level (S), and AMF inoculation (F) as fixed factors. The significance of main effects and all interactions (G, S, F, G×S, G×F, S×F, and G×S×F) was determined at $p < 0.05$ and $p < 0.01$. The corresponding significance levels are shown in Supplementary Table 3.

3 Results

In this study, we evaluated the effects of salinity stress on the germination parameters of four durum wheat cultivars. Using seed germination traits and electrical conductivity tests, we identified one salt-tolerant and one salt-sensitive genotype. Afterwards, we examined the impact of arbuscular mycorrhizal fungal inoculation on the root architecture of both salt-tolerant and salt-sensitive durum wheat cultivars under NaCl-induced salinity stress.

3.1 Experiment 1: Screening of cultivars for salt tolerance using the germination test

Based on the mean comparison results, the Shabrang cultivar showed the highest germination percentage (100%) under the control (non-saline) treatment. In contrast, the lowest germination percentage (50.6%) was observed for the D-98-8 line at 20 dS/m salinity (Figures 1A, C). The Shabrang cultivar showed the smallest reduction in germination percentage across different salinity levels (6.7%), while the D-98-8 line showed the most significant decrease (47.9%). This suggests that the Shabrang cultivar is more tolerant of salinity than the D-98-8 line. The Shabrang cultivar had the lowest slope (0.233) for the decline in germination percentage with increasing salinity, whereas the D-98-8 line showed the highest slope (0.66), indicating greater salinity



sensitivity (Figures 1A, C; Table 1). In contrast, the cultivars Tabana and the line D-98-10 showed similar slopes in germination (0.334 and 0.396), respectively (Figures 1B, D; Table 1).

Salinity stress significantly reduced germination rates ($p \leq 0.01$) (Supplementary 1). According to the mean comparison results, the Shabrang cultivar had the highest germination rate (0.91 seeds/day) under the control (non-saline) treatment. In contrast, the lowest germination rate (0.4 seeds/day) was observed for the D-98-8 line at 20 dS/m salinity (Table 2). The Shabrang cultivar experienced the most minor reduction in germination rate across salinity levels (30%), whereas the D-98-8 line showed the most significant decrease (48.7%). Salinity stress also had a substantial impact on seedling length ($p \leq 0.01$) (Supplementary 1), according to the mean comparison results. The Shabrang cultivar had the longest seedlings (112 cm) under the control treatment, whereas the D-98-8 line had the shortest (0.5 cm) at 20 dS/m salinity (Table 2). Seedling length was most affected by salinity compared to other traits, with reductions of 97.3%, 99.2%, 99.6%, and 97.8% in the Shabrang and Taban genotypes, as well as the D-98-8 and D-98-10 lines at 20 dS/m salinity, respectively. The Shabrang cultivar showed the smallest reduction (97.3%), while the D-98-8 line experienced the largest decrease (99.6%) in seedling length across different salinity levels.

Salinity stress substantially reduced shoot dry weight compared to the control treatment ($p \leq 0.01$) (Supplementary 1), although the extent of reduction varied among the genotypes. The mean comparison results showed that the Shabrang cultivar had the highest shoot dry weight (0.06 g) under the control conditions. The D-98-8 line exhibited the lowest shoot dry weight (0.0001 g) at 20 dS/m salinity, with a significant difference from the other lines (Table 3). Salinity stress also significantly negatively affected root dry weight ($p \leq 0.01$) (Supplementary 1). According to the mean comparison results, under control conditions, the D-98-10 line and Shabrang cultivar had the highest root dry weights, measuring 0.051 g and 0.049 g, respectively. The D-98-8 line had the lowest root dry weight (0.001 g) at 20 dS/m salinity, with a significant difference from the other lines (Table 3). The Shabrang cultivar experienced the most minor reduction in root dry weight at 89.7%, while the D-98-8 line showed the most significant decrease at 97.4% across different salinity levels. With increasing salinity stress, seedling length vigor index values decreased significantly ($p \leq 0.01$) (Supplementary 1) across all genotypes, although responses varied among cultivars. The mean comparison results indicated that the Shabrang cultivar had the highest seedling length vigor index (11,167) under control conditions. Meanwhile, the D-98-8 line recorded the lowest seedling length vigor index (0.01) at

20 dS/m salinity, with a significant difference from the Shabrang cultivar (Table 3). The Shabrang cultivar experienced the smallest reduction (99.6%), whereas the D-98-8 line exhibited the most significant reduction (100%) in seedling length vigor index across different salinity levels. Similarly, increasing salinity stress caused a significant decrease in the seedling weight vigor index, with responses varying depending on salinity level and genotype (Supplementary 1). The mean comparison results showed that the Shabrang cultivar had the highest seedling weight vigor index (11) under control conditions. In contrast, the D-98-8 line showed the lowest seedling weight vigor index (0.01) at 20 dS/m salinity, with a significant difference (Table 3). The Shabrang cultivar had the smallest reduction (99.3%), while the D-98-8 line had the most significant reduction (100%) across different salinity levels.

Although all morphological traits of seedlings decreased with increasing salinity across all cultivars, the rate of decline was greater in the D-98-8 line than in the other cultivars. The overall reduction percentage for different traits was lowest in the Shabrang cultivar at 519.8% and highest in the D-98-8 line at 595.2%. The traits with the most significant reduction percentages were shoot dry weight, seedling length vigor index, and seedling weight vigor index, each decreasing by 100%. In contrast, the Germination percentage showed the least reduction, at 6.7%, in the D-98-8 line and the Shabrang cultivar, respectively. The seedling length vigor index and shoot dry weight traits in the Shabrang cultivar, the seedling length vigor index in the Taban cultivar and D-98-10 line, as well as seedling weight vigor index, seedling length vigor index, and seedling length traits in the D-98-8 line, were the most sensitive to salt stress.

3.2 Experiment 2: seed electrical conductivity for salt tolerance screening

The analysis of variance showed that the liquid obtained from soaking the studied durum wheat varieties in distilled water had significant differences ($p \leq 0.01$) in electrical conductivity (Supplementary 2). The mean comparison revealed that the Shabrang cultivar had the lowest electrical conductivity at 0.228 dS/m-1 per gram of seed. In contrast, the D-98-8 line exhibited the highest electrical conductivity at 0.295 dS/m per gram of seed, indicating a significant difference in cellular leakage (Table 4). These results suggested that the Shabrang cultivar, known for its high vigor, had the highest physiological quality and the lowest electrical conductivity. Meanwhile, the D-98-8 line, identified as low vigor, demonstrated the lowest physiological quality with the highest electrical conductivity.

3.3 Experiment 3: effects of arbuscular mycorrhizal fungi on root architecture and phosphate concentration

The interaction of genotype, salinity, and the colonization with *Funneliformis mosseae* fungus significantly affected ($p \leq 0.01$) the average root width (Supplementary 3). Salinity stress notably reduced average root width in both the Shabrang cultivar and the D-98-8 line, irrespective of the presence or absence of the fungus.

TABLE 1 Germination percentage in different salinity levels of equations for different durum wheat genotypes.

Genotype	Equation	Slope	R ²
Shabrang	$y = -0.233x^2 + 1.4765x + 98.262$	0.233	0.902
Taban	$y = -0.334x^2 + 0.3167x + 94.095$	0.334	0.97.7
D-98-8	$y = -0.66x^2 + 0.6016x + 99.449$	0.66	0.94
D-98-10	$y = -0.396x^2 + 1.6254x + 94.852$	0.396	0.94

TABLE 2 Comparison of the means of interaction of genotype and salinity levels on germination rate and seedling length.

	Salinity (ds/m)	Shabrang	Genotype Taban	D-98-8	D-98-10
Germination rate (Seed/day)	Control	0.91 ^a ± 0.04	0.86 ^{ab} ± 0.07	0.78 ^{abc} ± 0.02	0.88 ^{ab} ± 0.006
	2	0.86 ^{bc} ± 0.02	0.83 ^{bcd} ± 0.05	0.75 ^{cde} ± 0.02	0.84 ^{abc} ± 0.04
	4	0.85 ^{bcd} ± 0.03	0.71 ^{defg} ± 0.02	0.71 ^{efg} ± 0.03	0.79 ^{def} ± 0.02
	6	0.81 ^{cde} ± 0.03	0.7 ^{efghi} ± 0.01	0.68 ^{ghi} ± 0.02	0.73 ^{efg} ± 0.04
	8	0.76 ^{def} ± 0.04	0.68 ^{ghij} ± 0.03	0.61 ^{ijk} ± 0.05	0.64 ^{hij} ± 0.03
	12	0.72 ^{efg} ± 0.06	0.65 ^{hijk} ± 0.06	0.48 ^{klm} ± 0.006	0.62 ^{hijk} ± 0.04
	16	0.68 ^{ghij} ± 0.04	0.57 ^{klm} ± 0.04	0.46 ^{mno} ± 0.03	0.52 ^{lmn} ± 0.02
	18	0.67 ^{ijk} ± 0.007	0.5 ^{lmn} ± 0.03	0.44 ^{nop} ± 0.03	0.49 ^{mno} ± 0.02
	20	0.63 ^{klm} ± 0.03	0.49 ^{mno} ± 0.04	0.4 ^{opq} ± 0.04	0.46 ^{nop} ± 0.04
	Control	112 ^a ± 0.3	106.5 ^b ± 0.8	106.6 ^b ± 0.8	105.3 ^b ± 1.3
Seedling length (cm)	2	31 ^{cde} ± 0	31.5 ^{cd} ± 0.3	32.3 ^c ± 0.7	29.6 ^{def} ± 0.6
	4	29.3 ^{efg} ± 0.3	27.5 ^{gh} ± 0.9	31.6 ^{cd} ± 0.6	26.4 ^{hi} ± 0.7
	6	28.6 ^{fg} ± 0.8	23.6 ^j ± 1.3	9.25 ⁿ ± 0.6	23.5 ^j ± 0.3
	8	25.4 ^{ij} ± 0.4	20.8 ^k ± 1.5	8.6 ⁿ ± 0.8	19.9 ^k ± 0.5
	12	15.6 ^l ± 0.6	16.9 ^l ± 0.3	7.4 ⁿ ± 0.4	11.4 ^m ± 0.4
	16	7.6 ⁿ ± 0.6	4.3 ^o ± 0.8	4.5 ^o ± 0.7	3.9 ^{op} ± 0.5
	18	4 ^{op} ± 0.5	4.4 ^o ± 0.5	0.6 ^q ± 0.06	2.5 ^{opq} ± 0.3
	20	3 ^{op} ± 0.2	0.7 ^q ± 0.1	0.5 ^q ± 0.05	2.2 ^{pq} ± 0.4

Means with similar letters in each column are not significantly different at the 5% probability level.

The average root width in the Shabrang variety and the D-98-8 line decreased from 0.052 to 0.039 (a reduction of 25%) and from 0.047 to 0.027 (a decrease of 42.5%), respectively, with increasing salinity in the absence of fungus. However, in the presence of the fungus, average root width in the Shabrang variety decreased slightly from 0.058 to 0.046, and in the D-98-8 line, it decreased from 0.052 to 0.044. The Shabrang variety, characterized by stronger vigor and reduced cell leakage, successfully formed a symbiosis with fungi under saline conditions (Figures 2, 3).

The interaction effects of genotype and the presence of the fungus on the number of connected components, network length, and root colonization were statistically significant ($p \leq 0.01$) (Supplementary 3). Based on the mean comparison results, the Shabrang cultivar had the highest number of connected components (34.1), a network length of 752 cm, and 60% root colonization in treatments with the fungus. In contrast, the D-98-8 line showed 26.1 connected components, 714 cm in network length, and 52.4% root colonization. Therefore, according to the results, mycorrhizal fungi colonized the salinity-tolerant Shabrang cultivar 14% more than the salinity-sensitive D-98-8 line, thereby increasing the number of root-connected components and network length in Shabrang compared to D-98-8. These observations suggest that the arbuscular mycorrhizal fungus colonized the salt-tolerant Shabrang cultivar more effectively than the salt-sensitive D-98-8 line, indicating that Shabrang was more successful in establishing a symbiotic relationship with the fungus (Figure 4). Without the fungus, the number of connected components and the network length in the Shabrang cultivar decreased to 26.3 and 678 cm, respectively, representing reductions of 29.5% and 10.9% compared

to when the fungus was present. Similarly, in the D-98-8 line, the number of connected components and network length without the fungus were 22.25 and 620 cm, respectively, showing decreases of 17.1% and 15.1% compared to when the fungus was present (Table 5).

The interaction between genotype and salinity significantly influenced network length ($p \leq 0.01$) (Supplementary 3). The mean comparison results indicate that the Shabrang cultivar had the longest network length (892) under control conditions without salinity. In contrast, the D-98-8 line displayed the shortest network length (387) at a salinity of 20 dS/m. The salinity-tolerant cultivar Shabrang showed a higher network length than the salinity-sensitive cultivar D-98-8 across all salinity levels (Figure 5).

The interaction effects of fungal presence and salinity on network length distribution, overall network length, root colonization, and root phosphate concentration were statistically significant ($p \leq 0.01$) (Supplementary 3). Based on the mean comparison results, the highest network length distribution (2.49) and total network length (927 cm) were observed in the non-saline treatment (control) with fungus. However, as salinity increased in the presence of fungus, network length distribution and total network length decreased to 1.02 and 474 cm, respectively, representing reductions of 59% and 48.8%. In conditions without fungus, as salinity increased, the network length distribution and total network length decreased from 2.06 and 815 cm to 0.54 and 400 cm, respectively, indicating reductions of 73.7% and 50.9%. This suggests that fungus has a positive effect in mitigating the impact of salinity (Figures 6, 7).

The highest root colonization percentage (76%) was observed in the non-saline (control) treatment with fungus, while the lowest

TABLE 3 Comparison of the average genotype interaction and various salinity levels on shoot dry weight, root dry weight, seedling vigor index, and seedling weight.

	Salinity (ds/m)	Shabrang	Genotype Taban	D-98-8	D-98-10
Shoot dry weight (g)	Control	0.06 ^a ± 0.001	0.054 ^b ± 0.005	0.059 ^a ± 0.001	0.047 ^{cde} ± 0.001
	2	0.049 ^c ± 0.003	0.049 ^c ± 0.001	0.043 ^{efg} ± 0.002	0.046 ^{cde} ± 0.001
	4	0.048 ^{cd} ± 0.005	0.043 ^{efg} ± 0.001	0.039 ^{gh} ± 0.001	0.038 ^{hi} ± 0.002
	6	0.044 ^{def} ± 0.001	0.039 ^{hi} ± 0.002	0.027 ^l ± 0.003	0.034 ^j ± 0.001
	8	0.04 ^{fgh} ± 0.001	0.033 ^{jk} ± 0.002	0.022 ^{mn} ± 0.001	0.035 ^{ij} ± 0.002
	12	0.029 ^{kl} ± 0.001	0.025 ^{lm} ± 0.001	0.015 ^{op} ± 0.001	0.019 ^{no} ± 0.003
	16	0.019 ⁿ ± 0.003	0.013 ^{pq} ± 0.003	0.01 ^q ± 0.004	0.018 ^{no} ± 0.001
	18	0.005 ^{rs} ± 0.002	0.003 st ± 0.002	0.0001 ± 0.00005	0.009 ^{qr} ± 0.001
	20	0.002 st ± 0.0001	0.001 ^t ± 0.001	0.0001 ± 0.00002	0.003 st ± 0.002
Root dry weight (g)	Control	0.049 ^a ± 0.001	0.035 ^{efg} ± 0.001	0.039 ^{cd} ± 0.001	0.051 ^a ± 0.001
	2	0.038 ^{cde} ± 0.002	0.038 ^{cde} ± 0.001	0.037 ^{def} ± 0.001	0.044 ^b ± 0.001
	4	0.035 ^{efg} ± 0.002	0.029 ^j ± 0.003	0.041 ^{bc} ± 0.001	0.036 ^{defg} ± 0.002
	6	0.037 ^{def} ± 0.003	0.029 ^{ij} ± 0.002	0.033 ^{gh} ± 0.001	0.03 ^{hij} ± 0.002
	8	0.034 ^{fg} ± 0.002	0.023 ^l ± 0.001	0.032 ^{ghi} ± 0.001	0.028 ^{jk} ± 0.001
	12	0.024 ^{kl} ± 0.001	0.022 ^{lm} ± 0.002	0.019 ^m ± 0.001	0.022 ^{lm} ± 0.002
	16	0.009 ^o ± 0.004	0.007 ^{op} ± 0.001	0.008 ^{op} ± 0.002	0.014 ⁿ ± 0.002
	18	0.007 ^{op} ± 0.001	0.007 ^{op} ± 0.002	0.003 ^{qr} ± 0.0005	0.007 ^{op} ± 0.001
	20	0.005 ^{pq} ± 0.001	0.002 ^{qr} ± 0.0006	0.001 ^r ± 0.0003	0.003 ^{qr} ± 0.0005
Seedling length vigor index	Control	11167 ^a ± 33.3	10658 ^b ± 82	10429 ^b ± 237	10533 ^b ± 133
	2	3100 ^{cde} ± 3.1	3158 ^{cde} ± 36	3233 ^c ± 72	2967 ^{def} ± 60
	4	2933 ^{ef} ± 33.3	2750 ^{fgh} ± 90	3166 ^{cd} ± 66	2642 ^{hi} ± 74
	6	2867 ^{fg} ± 88.1	2367 ⁱ ± 134	906 ^{lm} ± 80	2358 ⁱ ± 30
	8	25427 ^{hi} ± 41.6	2087 ^j ± 156	867 ^{lm} ± 88	1945 ^j ± 22
	12	1567 ^k ± 66.6	1653 ^k ± 36	628 ⁿ ± 50	1048 ^l ± 94
	16	704 ^{mn} ± 101.4	388 ^o ± 71	96 ^{pqr} ± 30	225 ^{opqr} ± 166
	18	267 ^{opq} ± 36	284 ^{op} ± 50	2.2 ^r ± 0.5	24 ^r ± 9
	20	36 ^{qr} ± 18.1	1.1 ^r ± 0.09	0.001 ^r ± 0.0001	17 ^r ± 9
Seedling weight vigor index	Control	11 ^a ± 0.14	9 ^{cde} ± 0.05	9.6 ^{bc} ± 0.13	10 ^b ± 0.2
	2	9 ^{def} ± 0.55	8.8 ^{def} ± 0.34	8.1 ^{fgh} ± 0.05	9.1 ^{bcd} ± 0.23
	4	8.4 ^{def} ± 0.12	7.3 ⁱ ± 0.54	8.1 ^{fgh} ± 0.11	7.4 ^{hi} ± 0.29
	6	8.2 ^{efg} ± 0.23	6.8 ^{ij} ± 0.46	5.7 ^{kl} ± 0.14	6.4 ^{jk} ± 0.1
	8	7.5 ^{ghi} ± 0.37	5.7 ^{kl} ± 0.41	5.5 ^l ± 0.14	6.2 ^{kl} ± 0.15
	12	5.4 ^l ± 0.23	4.7 ^m ± 0.12	2.9 ^o ± 0.18	3.7 ⁿ ± 0.2
	16	2.7 ^o ± 0.42	1.8 ^p ± 0.11	0.37 ^{rs} ± 0.08	1.2 ^{pq} ± 0.71
	18	0.9 ^{qr} ± 0.24	0.7 ^{qrs} ± 0.19	0.004 ^s ± 0.001	0.14 ^{rs} ± 0.04
	20	0.08 ^s ± 0.01	0.01 ^s ± 0.001	0.001 ^s ± 0.0001	0.04 ^s ± 0.02

Means with the same letters in each column are not significantly different at the 5% probability level.

(31%) was observed at 20 dS/m salinity, with a significant difference between the two. Therefore, root colonization decreased by 45% as salinity increased (Figure 8).

The highest root phosphate concentration (3.28 mg/g dry weight) was observed in non-saline treatments (control) with fungus. However, as salinity increased in the presence of the fungus, the concentration decreased to 1.45 mg/g dry weight, a 55.7% reduction. Without fungus, root phosphate concentration

dropped from 2.57 mg/g dry weight to 0.76 mg/g dry weight with increasing salinity, a 70.4% decrease. This illustrates the fungus's positive role in reducing the impact of salinity on root phosphate levels (Figure 9). The main effect of genotype on network length distribution and root phosphate concentration was significant at the 1% probability level (Supplementary 3). Based on the mean comparison results, the Shabrang cultivar showed the highest network length distribution at 1.8, while the D-98-8 line had the

TABLE 4 Mean comparison of electrical conductivity of four durum wheat genotypes.

Genotype	Mean (dS m ⁻¹ g ⁻¹ of seed)
Shabrang	0.228 ^d ± 0.005
Taban	0.25 ^c ± 0.002
D-98-8	0.295 ^a ± 0.004
D-98-10	0.262 ^b ± 0.001

Means with similar letters in each column are not significantly different at a 5% probability level.

lowest at 1.56, with a significant difference between them (Figure 10). Additionally, the salt-tolerant Shabrang exhibited the highest root phosphate concentration at 1.86 mg/g dry weight, whereas the salt-sensitive D-98-8 had the lowest at 1.7 mg/g dry weight (Figure 11). The main effect of salinity on the number of connected components was significant at the 1% probability level (Supplementary 3). According to the mean comparison, the control treatment had the highest number of connected components at 45.3, while at 20 dS/m salinity, the lowest was 0.97, with a significant difference (Figure 12).

4 Discussion

Salinity stress is a major environmental constraint that significantly alters root system architecture and decreases plant

growth and productivity by limiting water and nutrient uptake (Farooq et al., 2024). In this study, salinity stress notably impacted several root architecture traits, including mean root width, network length, network length distribution, and the number of connected components. Apparent differences were observed between the salinity-tolerant cultivar Shebrang and the salinity-sensitive line D-98-8. Additionally, inoculation with Funneliformis mosseae played an important role in shaping root system architecture under stress conditions by modulating these responses.

4.1 Identification of salt-tolerant and salt-sensitive genotypes at early growth stages

Experiments 1 and 2 aimed to identify salinity-tolerant and salinity-sensitive genotypes of durum wheat during early growth stages, providing a physiological and morphological basis for later mycorrhizal studies. In this research, salinity stress significantly reduced germination percentage, germination rate, seedling length, root dry weight, shoot dry weight, and vigor indices, with the degree of reduction varying strongly by genotype. Similarly, researchers observed that germination percentage and rate declined in various durum wheat and sorghum genotypes under increasing salinity stress (Borlu et al., 2018; Dehnavi et al., 2020). These findings align with our results and further support the idea that salinity, depending on its level, can inhibit or delay germination without entirely stopping it (Gulzar and Khan, 2001; Ouji et al., 2015). Salinity damages the structure of plant cells, tissues, and organs. Additionally, it is reported to limit access to essential nutrients by creating osmotic effects, ultimately restricting root emergence and

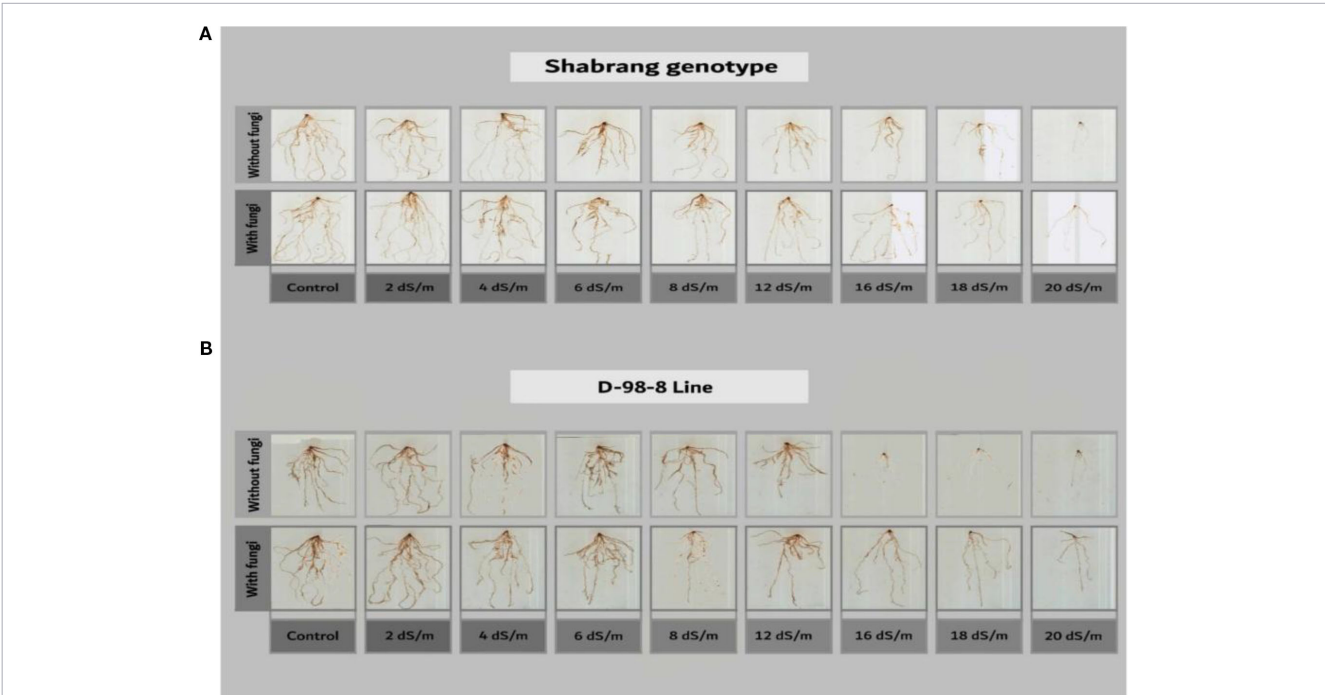


FIGURE 2 Changes in the root architecture of the Shabrang (A) cultivar and line D-98-8 (B) were observed with increasing salinity and inoculation with mycorrhizal fungi compared to the control.

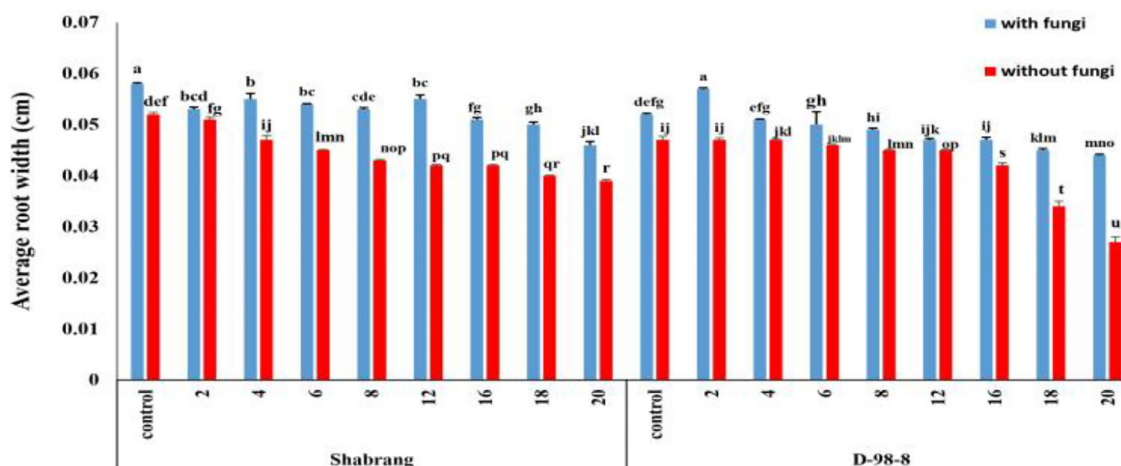


FIGURE 3

Shows the interactions between two durum wheat genotypes, fungi, and different salinity levels on average root width. Means marked with the same letters in each column are not significantly different at the 5% probability level.

seedling growth (Krishnamurthy et al., 2007; Abari et al., 2011; Bilgili et al., 2011). Early growth responses to salinity stress are considered reliable indicators of how plants will perform later under stress, as they influence seedling establishment, root system development, and nutrient uptake. The superior performance of the Shabrang cultivar during germination and early seedling growth under stress conditions suggests it has a greater ability to maintain cell integrity and water uptake in saline environments, likely contributing to the increased root development observed in Experiment 3.

4.2 Seed vigor and membrane stability as early indicators of salinity tolerance

The electrical conductivity test evaluates the integrity of cell membranes, which is connected to seed vigor (Ujjainkar et al., 2021). In other words, the electrical conductivity of the solution

obtained by soaking seeds in water can indicate seed vigor. Since seed vigor is related to salinity tolerance (Khajeh-Hosseini et al., 2003), cell membrane permeability is also linked to salinity tolerance. In this study, electrical conductivity (EC) testing provided a clear physiological basis for differentiating salt-tolerant and salt-sensitive durum wheat genotypes at the early growth stage. The lower EC values observed in the salt-tolerant genotype (Shabrang) suggest greater membrane stability, reflecting higher seed vigor compared to the salt-sensitive genotype (D-98-8). This membrane integrity likely reduces solute leakage under saline conditions, enabling more efficient water uptake and quicker membrane repair during germination. The differences in EC between genotypes correspond with their varying germination and seedling vigor at different salinity levels. Genotypes with lower EC show higher germination rates, seedling lengths, and vigor indices. This supports the idea that membrane integrity is a key factor in salt tolerance during early development. Conversely, higher EC values in the salt-sensitive genotype indicate increased membrane permeability and impaired cell repair mechanisms, which may lead to reduced germination and weaker seedling development under saline conditions. These findings confirm that the EC test not only indicates overall seed quality but also serves as a reliable physiological screening method for predicting salt tolerance in durum wheat. The correlation between EC results and seedling responses under salt stress further validates this approach for selecting genotypes before conducting detailed physiological and morphological evaluations.

4.3 Salinity-induced modifications of the root

The observed decrease in average root width, network length, and number of connected components with increasing salinity in this study matches previous reports indicating that salinity, through osmotic stress and ion toxicity, limits root growth, affects cell expansion, and disrupts meristem activity (Munns and Tester, 2008). Narrower roots and reduced network connectivity at high salinity are likely caused by impaired

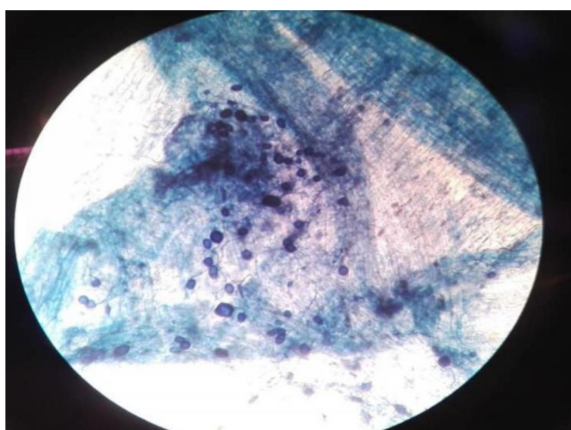


FIGURE 4

Trypan blue staining of the durum wheat root system showing arbuscular mycorrhizal colonization. Nikon light microscopy, magnification 20x.

TABLE 5 compares the means of interaction between the two genotypes and the fungus regarding the number of connected components, network length, and root colonization.

Genotype	Fungi	Number of connected components	Network length (cm)	Root colonization (%)
Shabrang	With fungi	34.1 ^a ± 2.7	752 ^a ± 28	60 ^a ± 3.3
	Without fungi	26.3 ^b ± 2.2	678 ^c ± 27	0
D-98-8	With fungi	26.1 ^b ± 2.3	714 ^b ± 30	52.4 ^b ± 3.1
	Without fungi	22.3 ^c ± 2.1	620 ^d ± 30	0

Means with the same letters in each column are not significantly different at a 5% probability level.

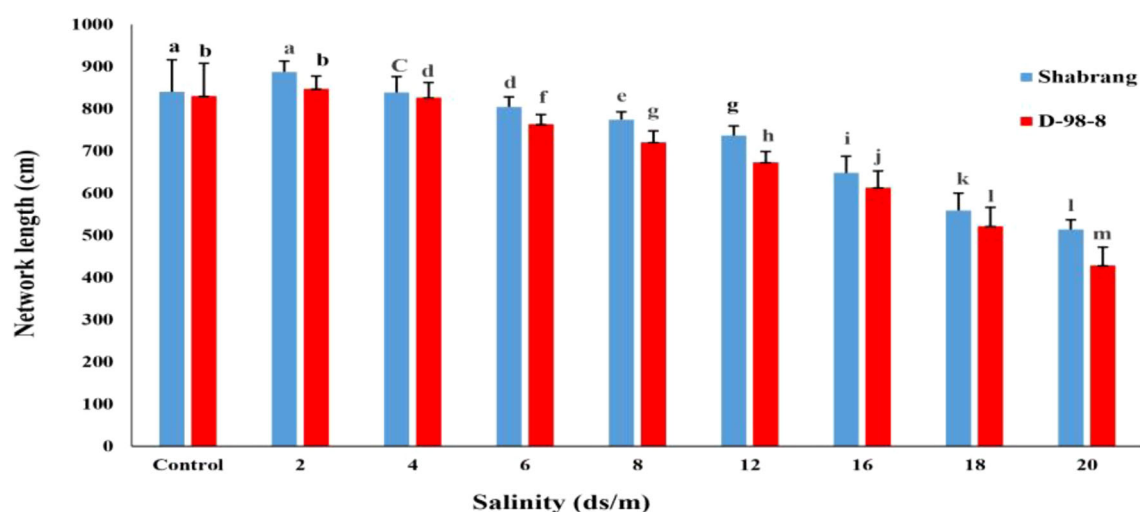


FIGURE 5

The relationship between genotype and different salinity levels influences network length. Means marked with the same letters in each column are not significantly different at the 5% probability level.

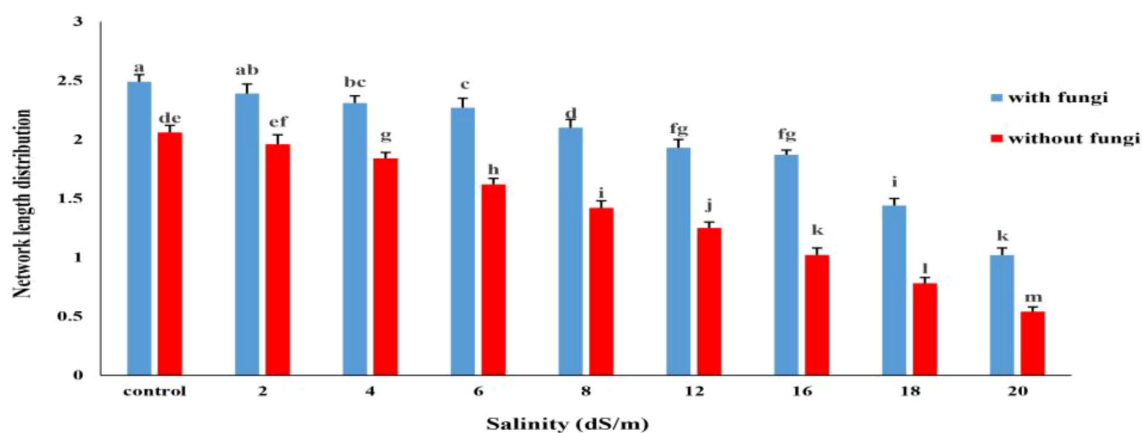


FIGURE 6

The relationship between fungi and different salinity levels affects the network length distribution. Means with similar letters in each column are not significantly different in terms of the probability level.

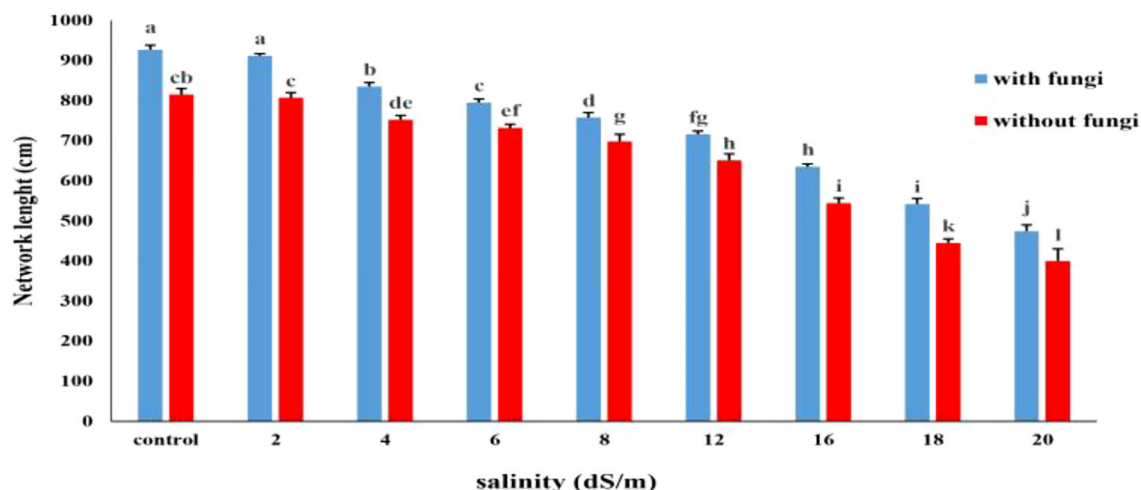


FIGURE 7

Shows how fungi and different salinity levels affect network length. Means with the same letters in each column are not significantly different at a 5% probability level.

cortical cell expansion and decreased carbon allocation to the root, which together hinder the development of a comprehensive root system. The decline in root architectural parameters was more pronounced in the salinity-sensitive line D-98-8 than in the cultivar Shabrang, demonstrating that root architecture adaptability depends on genotype in response to salinity stress. Salt-tolerant genotypes create a more efficient root system under stress by maintaining root growth, supporting vascular development, and reducing membrane damage [Koevoets et al. \(2016\)](#). The higher network length and number of connected components observed in the Shabrang cultivar across different salinity levels suggest a greater capacity to maintain root system integrity, which may explain its increased tolerance to salt stress.

4.4 Role of AMF in improving root system architecture under salinity

Inoculation with mycorrhizal fungi significantly reduced the adverse effects of salinity on root architectural traits, as shown by increased network length, network length distribution, and the number of connected components in inoculated plants compared to uninoculated controls. These findings support the beneficial role of mycorrhizal fungi in promoting root growth and structural complexity under abiotic stress conditions ([Begum et al., 2019](#); [Evelin et al., 2019](#)). Mycorrhizal fungi can improve root architecture by enhancing nutrient uptake, especially phosphorus, regulating plant hormone levels (e.g., auxins and cytokinins), and improving

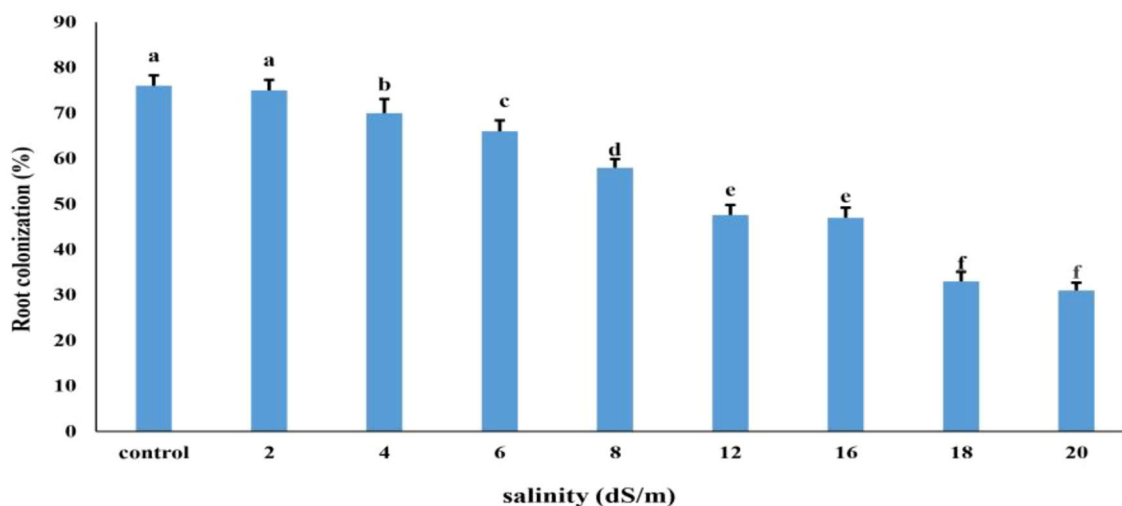


FIGURE 8

The effect of different salinity levels on root colonization is observed. Means with similar letters in each column are not significantly different at a 5% probability level.

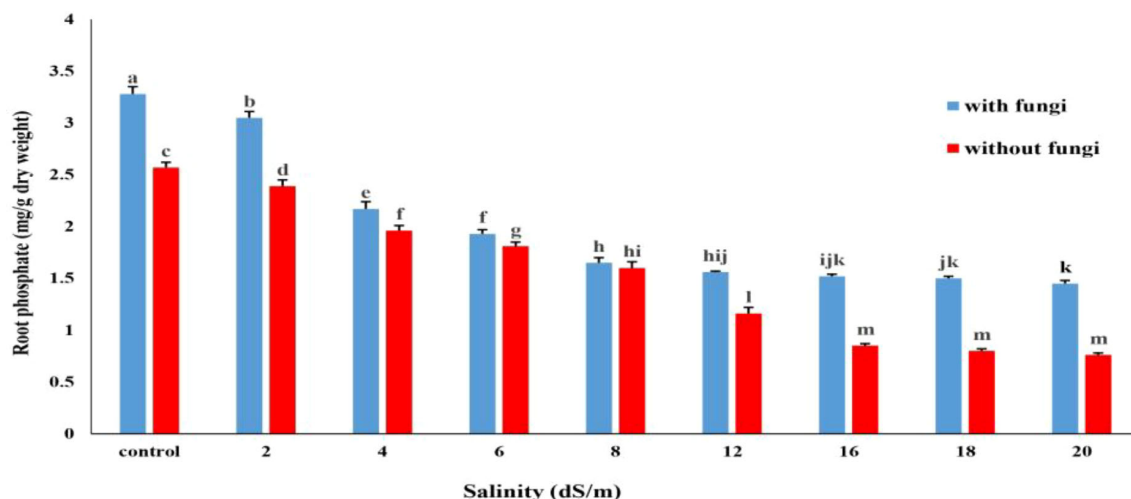


FIGURE 9

The relationship between fungi and different salinity levels affects root phosphate. Means with similar letters in each column are not significantly different at a 5% probability level.

osmotic regulation (Smith and Smith, 2012; Pedranzani et al., 2016). In this study, the positive association between increased network length and higher root phosphate concentration in mycorrhizal treatments suggests that enhanced phosphorus uptake helped sustain a more extensive, interconnected root system under salt stress.

4.5 Genotype- dependent mycorrhizal responsiveness

A key and significant finding of this study is the improvement in root colonization, network length, and the number of connected components in the salt-tolerant cultivar Shabrang compared to the salt-sensitive line D-98-8 in the presence of AMF. This finding indicates that mycorrhizal response is strongly genotype-dependent, as previously reported for wheat and other cereals (Sawers et al., 2017; Gao and Zhang, 2025). The higher colonization efficiency in Shabrang may be due to stronger root vigor, reduced cell membrane leakage, and a root biochemical

environment that is more conducive to fungal establishment under saline conditions. Salt-tolerant genotypes often allocate more carbon to roots and symbionts, thereby enhancing AMF colonization and performance even under stress conditions (Chaudhary et al., 2025). Consequently, the improved symbiotic performance in the Shabrang cultivar likely helped sustain root network complexity and nutrient absorption capacity under saline conditions.

4.6 Interaction effects of salinity and AMF

The significant interaction between salinity and fungal presence on root architectural traits and root phosphate concentration highlights the benefits of mycorrhizal fungi symbiosis. Although salinity stress reduced root colonization and root architectural parameters at higher levels, mycorrhizal plants outperformed non-mycorrhizal plants across all salinity treatments. This indicates that mycorrhizal fungi mitigated, but did not eliminate, the adverse effects of salinity stress on root architecture. Similar

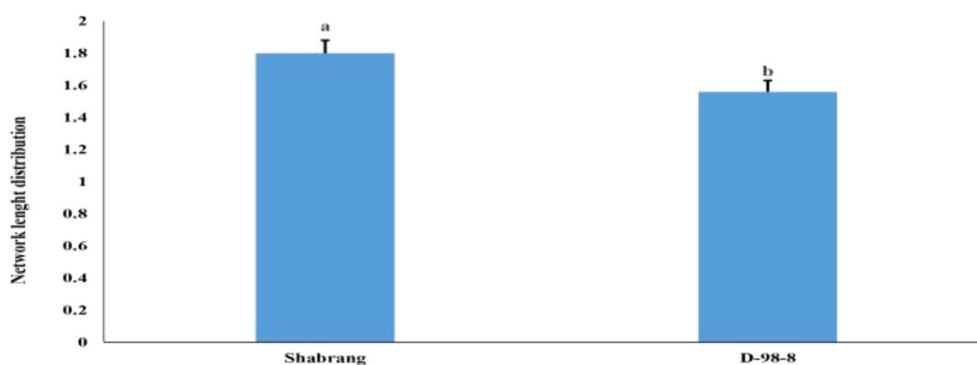


FIGURE 10

The main effect of genotype on network length distribution was significant. Means with similar letters in each column are not significantly different at a 5% probability level.

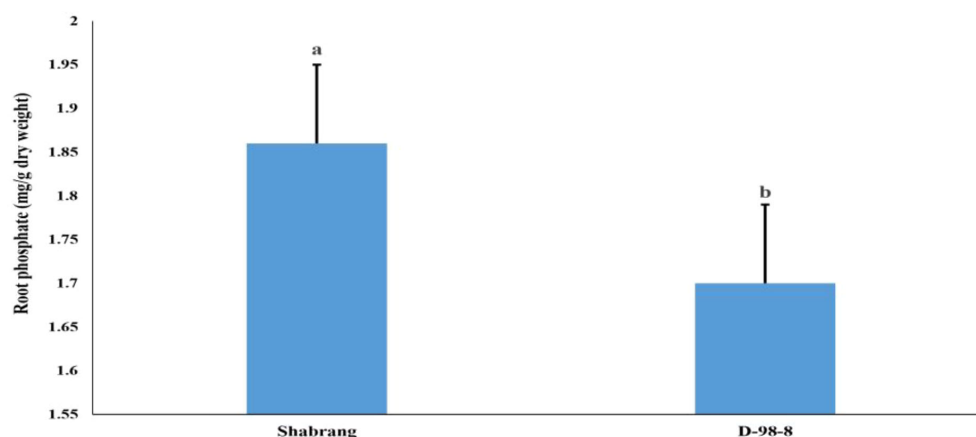


FIGURE 11

The primary effect of genotype on phosphate concentration in the root. Means with similar letters in each column are not significantly different at the 5% probability level.

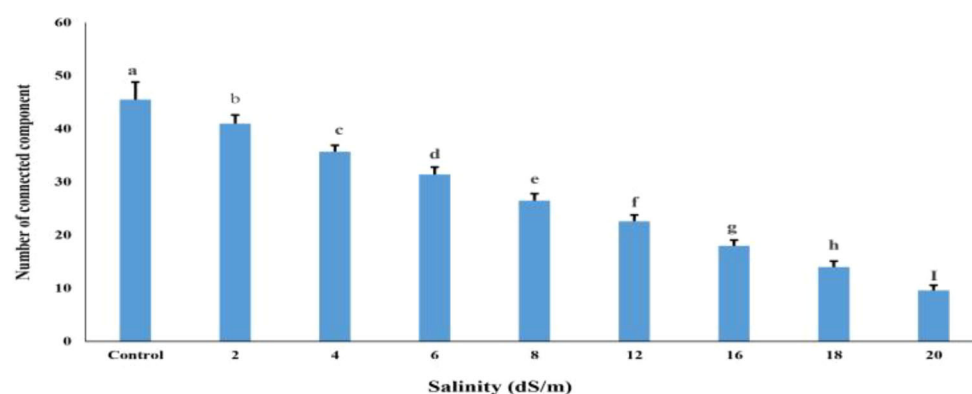


FIGURE 12

The main effect of different salinity levels on the number of connected components. Means with similar letters in each column are not significantly different at the 5% probability level.

findings have been reported in previous studies, suggesting that mycorrhizal fungi can partly counteract the reduction in root growth caused by salinity stress by enhancing nutrient uptake, antioxidant defenses, and ionic balance (Ruiz-Lozano et al., 2012; Chandrasekaran et al., 2019). The observed decrease in the rate of network length decline and phosphate concentration under stress conditions in mycorrhizal plants confirms the protective role of mycorrhizal fungi in more saline environments.

5 Conclusions

Our results indicated that the cell leakage rate in durum wheat cultivars is linked to their tolerance to germination salinity. In other words, durum wheat cultivars with lower cell leakage demonstrate greater resilience to salinity stress during germination. Therefore,

the cell leakage rate serves as an effective screening tool for salinity tolerance during germination in durum wheat. Furthermore, durum wheat cultivars with higher salinity tolerance and lower cell leakage are better at forming symbiotic relationships with arbuscular mycorrhizal fungi under salinity stress. This symbiosis helps the plant develop stronger roots under stress, leading to improved soil establishment and increased stress tolerance. Notably, the salinity-tolerant cultivar Shabrang formed a more robust symbiosis with arbuscular mycorrhizal fungi and was more effective at enhancing root traits under stress conditions. These findings underscore the potential of arbuscular mycorrhizal fungi as a practical strategy to improve salt-stress tolerance in durum wheat. This approach can reduce stress-related losses for farmers and support the development of sustainable agriculture in saline environments. Additionally, the seed screening method based on cell leakage rate is quick and reliable, saving time, particularly when analyzing large seed batches.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

NZ: Conceptualization, Methodology, Writing – original draft, Visualization. MT: Conceptualization, Methodology, Writing – original draft, Visualization. MZ: Formal analysis, Investigation, Writing – original draft. BD: Validation, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Correction note

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Supplementary material

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