JOURNAL OF HORTICULTURE AND POSTHARVEST RESEARCH
2024, VOL. 7(2), 171-182

Journal of Horticulture and Postharvest Research

Journal homepage: www.jhpr.birjand.ac.ir

University of Birjand

Effect of biofertilizer inoculation on the growth and physiological traits of Red Angel and Wonderful pomegranate plantlets under salinity stress

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ARTICLE INFO

Original Article

Article history:
Received 13 January 2024
Revised 17 February 2024
Accepted 6 April 2024

Keywords:
Cell membrane injury
Glomus mosseae
Pseudomonas fluorescens
Relative water content
Sodium chloride

DOI: 10.22077/jhpr.2024.7168.1356
P-ISSN: 2588-4883
E-ISSN: 2588-6169

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ABSTRACT

Purpose: The study aimed to explore the effects of biofertilizer inoculation on the growth and morphophysiological traits of Red Angel and Wonderful pomegranate cultivars under salinity stress. **Research method**: The experiment utilized a factorial design based on a completely randomized design with four replications to assess the effects of salinity stress at three levels (control, 4, and 8 dS/m) and biofertilizer at four levels (control, *Pseudomonas fluorescens*, *Glomus mosseae*, and *P. fluorescens* + *G. mosseae*) on pomegranate plantlets. **Findings**: The results showed that the highest percentage of symbiosis was observed in *P. fluorescens* + *G. mosseae*, with 89.16% and 90.55% in the Red Angel and Wonderful cultivars, respectively. Salinity did not have any influence on the percentage of symbiosis in both cultivars. Furthermore, the application of biofertilizers increased the stem diameter, number of lateral branches, total number of leaves, leaf fresh and dry weight, root diameter, number of lateral roots, and relative water content of leaves in both cultivars. Additionally, all biofertilizers reduced cell membrane injury at all salinity levels by approximately 40%. Salinity decreased the leaf fresh and dry weight, root fresh and dry weight, and number of lateral roots, while increasing cell membrane injury in both cultivars. **Research limitations**: No limitations were identified. **Originality/Value**: The results highlight the potential of biofertilizers in mitigating the adverse effects of salinity stress on pomegranates, particularly when *P. fluorescens* and *G. mosseae* are combined.

INTRODUCTION

Pomegranate (*Punica granatum* L.), a member of the Lythraceae family and native to Iran and North Africa, thrives in dry, semi-tropical, and Mediterranean climates (Feyzi et al., 2018; Sarkhosh et al., 2020). The global cultivation of pomegranate spans an area of over 500 thousand hectares, yielding more than six and a half million tons of various cultivars annually (FAO, 2021).

Salinity affects more than 80% of the world's land, with saline areas on the rise due to improper irrigation water management, climate change, reduced annual rainfall, increased evaporation, and the use of inappropriate irrigation systems (Zheng et al., 2009). This poses a significant challenge in agriculture, impacting plant growth and development. Salinity stress causes structural changes in plant organs, reduces chlorophyll and photosynthesis, and diminishes plant growth and efficiency (Keshavarzi et al., 2022; Poury et al., 2023). It also imposes nutritional restrictions, reduces water absorption, and disrupts ion balance, leading to decreased plant production (Anahita et al., 2015).

In pomegranate trees, salinity can result in reduced fruit formation and growth, an increase in incomplete flowers, reduced branch growth, and fruit drop (Fattahi et al., 2021). High salinity can reduce pomegranate fruit size, weight, and quality, leading to issues like cracking, browning, and sunburn (Tavousi et al., 2016; Momenpour et al., 2022). Soori et al. (2019) studied the effect of different sodium chloride concentrations in irrigation water on the growth indices and physiological characteristics of selected pomegranate cultivars. The research revealed that as salinity increased, plant height, leaf size, fresh and dry leaf weight, and roots decreased, while the percentage of leaf necrosis increased. The findings indicated that the studied cultivars exhibited acceptable tolerance to salinity up to an electrical conductivity of 6.21 dS/m. Ibrahim (2016) evaluated and compared the salinity resistance of two pomegranate cultivars, Wonderful and Manfalouty, under hydroponic cultivation conditions. The research demonstrated that under salt stress conditions, the Wonderful cultivar displayed higher chlorophyll content, branch length, and growth ratio compared to the Manfalouty cultivar.

The growth-promoting rhizosphere bacteria, including *Azetobacter*, *Azospirillum*, and *Pseudomonas*, are vital soil microorganisms that stimulate plant growth through direct and indirect mechanisms (Azarmi-Atajan & Sayyari-Zohan, 2022). *P. fluorescens* bacteria, in particular, optimize cell processes, produce a wide range of plant growth regulators, organic acids, and exopolysaccharides, and facilitate the absorption of essential nutrients, demonstrating adaptation and resistance for survival and growth in saline conditions (Saleem et al., 2012).

Symbiotic fungi offer an effective method for enhancing resistance to adverse environmental conditions, particularly salt stress, by increasing seedling survival, growth, and resistance to abiotic stresses such as salinity (Seifi et al., 2014). Arbuscular mycorrhizal (AM) fungi play a significant role in improving plant growth under saline conditions by reducing the adverse effects of salinity stress on plants through various physical, nutritional, physiological, and cellular effects. Pomegranate has been shown to be a suitable host for mycorrhizal fungi, with their use resulting in increased plant establishment, improved shoot and root growth, enhanced chlorophyll production in pomegranate tissue culture, and increased resistance to adverse moisture conditions and osmotic stress (Bompadre et al., 2015). Inoculation with AM fungi has been found to enhance the growth of pomegranate trees and improve their vegetative and nutritional characteristics in saline conditions (Parvin et al., 2017).

The use of biological stimulants is an effective strategy for mitigating the adverse effects of salinity stress on plants. AM fungi improving the nutritional, physiological, and morphological conditions of the host plant and enhance the plant's resistance to salt stress through symbiosis and various other mechanisms (Evelin et al., 2019). Similarly, growth-stimulating bacteria in symbiosis with the root environment can enhance the physical conditions of the rhizosphere, modify the selectivity of sodium and potassium, produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and employ specialized mechanisms to directly or indirectly improve plant growth and development (Dominguez-Nunez et al., 2013). However, there is a lack of comprehensive research on the effect of the symbiotic relationship of AM fungi and plant growth-promoting bacteria (PGPB) on pomegranate cultivars under salinity stress conditions. Therefore, the aim of this experiment was to assess the influence of a PGPB (*Pseudomonas fluorescens*) and an AM fungi (*Glomus mosseae*), as well as their combination, on some morphological and physicochemical traits in pomegranate cultivars Red Angel and Wonderful under salinity conditions. The Red Angel is a popular cultivar known for its distinctive flavor and beautiful appearance. The Wonderful cultivar is popular for its large, sweet, and tangy arils, as well as its high antioxidant content. Both cultivars are adaptable to various growing conditions (Hooks et al., 2021; Yan-hui et al., 2022).

MATERIALS AND METHODS

Plant materials and treatments

The research was conducted in 2021 at the Department of Horticulture Science, Gorgan University of Agricultural Sciences and Natural Resources in Gorgan, Iran. The experiment utilized a factorial design based on a completely randomized design with four replications and two pots for each replication (each pot had one rooted cutting). The first factor comprised three levels of irrigation water salinity: non-saline control, 2.5, and 6.4 g/l of NaCl, corresponding to EC values of 1.4, 4, and 8 dS/m, respectively. The second factor consisted of four levels of biofertilizer: non-inoculated control, *P. fluorescens*, *G. mosseae*, and *P. fluorescens* + *G. mosseae*. In January, one-year-old hardwood cuttings from Red Angel and Wonderful cultivars were prepared and rooted in 7 L pots filled with a 1:1 mixture of agricultural soil and sand. The potting mixture had the following composition: N (0.11 mg/kg), K (625 mg/kg), P (98 mg/kg), pH of 7.1, and EC of 1.1 dS/m. To ensure sterility, the prepared potting mixture was autoclaved at 121°C and 1.5 bar for one hour. The plantlets were cultivated in a controlled greenhouse environment with a relative humidity of 80 ± 5% and a daytime temperature of 28 ± 5°C.

The bacterium *P. fluorescens* was obtained from the Department of Soil Biology at the Soil and Water Research Institute in Tehran, Iran. A liquid inoculation suspension (75 ml) containing the bacteria were added to each pot (Aalipour et al., 2018). The *G. mosseae* AM fungi were sourced from Toran Biotechnology Company in Shahroud, Iran. The inoculum included rhizosphere soil, AM fungi spores (approximately 50-60 spores per gram of dry clay soil), as well as hyphae, arbuscules, and root segments from the host plant *Trifolium repens* L. Each pot was inoculated with 100 g of fungi powder mixed with 6 kg of the potting mixture.

Once the plantlets had established and achieved sufficient growth, salinity stress was gradually introduced over a 10-week period. To prevent sudden shock to the plantlets, the salt concentration in the irrigation water was gradually increased until it reached the final desired concentration (Hariadi et al., 2011). The pots were irrigated once a week, and the amount of water applied was adjusted based on changes in pot weight. Regular monitoring of substrate EC was conducted every week following irrigation to ensure effective management of soil

salinity, preventing excessive salt accumulation in the soil over time. At the end of the experiment, the EC of the potting mixture was measured.

Morphophysiological assessments

The quantification of symbiosis percentage involved staining fresh root segments three months after inoculation and examining them under a microscope to identify and quantify the presence of symbiotic structures, following the procedures outlined by Phillips and Hayman (1970). Stem and root diameters were measured in millimeters using a digital caliper (El-Khawaga & Yossef, 2013). The number of leaves, lateral branches, and lateral roots were recorded at the end of the experiment. After determining the fresh weight of leaves and roots, the leaf dry weight and roots were obtained by following the method of Ganjeali and Kafi (2007) after placing the plant samples at 70°C for 48 hours. The relative water content (RWC) of leaves was determined using the method described by Dhanda and Sethil (1998). Cell membrane injury was assessed based on the method outlined by Sairam et al. (1997). In this method, the conductivity of leaf samples was measured before and after autoclaving to evaluate cellular injury using the related equation.

Data analysis

The data was statistically analyzed using SAS (version 9.3), and mean comparisons were conducted using the Duncan's multiple range test. Prior to the analysis, the data underwent suitable transformations to meet the assumptions of normality and homogeneity of variance.

RESULTS AND DISCUSSION

The results of the study showed that the interaction effect of biofertilizers (*P. fluorescens*, *G. mosseae*, and *P. fluorescens* + *G. mosseae*) and salinity treatments on the percentage of symbiosis and the morphological traits, including stem diameter, the number of lateral branches, total number of leaves, fresh and leaf dry weight, root diameter, number of lateral roots, fresh and dry weight of roots in both the Red Angel and Wonderful cultivars were statistically non-significant. Consequently, the main effects of the biofertilizers and salinity treatments were assessed. However, the interaction effect of biofertilizer and salinity on RWC of leaves and cell membrane injury was found to be significant in both cultivars, and therefore, the interaction effects of the treatments were compared.

Table 1. The effect of biofertilizer and salinity on symbiosis, stem diameter and number of lateral branches in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Symbiosis (%)		Stem diameter (mm)		Lateral branches (n)	
	Red Angel	Wonderful	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Control	29.66±0.89^{d}	30.11±1.28^{d}	3.05±0.15^{c}	3.83±0.21^{d}	0.91±0.08^{d}	1.41±0.15^{d}
P. fluorescens	87.41±0.51^{b}	88.25±0.73^{b}	5.33±0.23^{b}	5.81±0.23^{b}	2.83±0.17^{b}	3.08±0.15^{b}
G. mosseae	80.00±0.98^{c}	80.35±1.20^{c}	5.33±0.19^{b}	5.66±0.22^{c}	2.25±0.13^{c}	2.83±0.21^{c}
P. fluorescens + *G. mosseae*	89.16±0.78^{a}	90.55±1.11^{a}	6.33±0.14^{a}	6.25±0.13^{a}	3.16±0.21^{a}	3.58±0.19^{a}
Salinity (dS/m)	P=0.156	P=0.431	P=0.144	P=0.594	P=0.131	P=0.371
Control	70.81±6.64	71.03±6.66	4.56±0.30	5.36±0.32	2.13±0.30	2.81±0.27
4	71.56±6.23	71.38±6.41	4.33±0.29	5.37±0.27	2.13±0.26	2.78±0.25
8	72.31±6.11	73.50±5.98	4.33±0.29	5.31±0.30	2.05±0.18	2.63±0.24

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

Table 2. The effect of biofertilizer and salinity on number of leaves, leaf fresh weight, and leaf dry weight in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Leaves (n)		Leaf fresh weight (g)		Leaf dry weight (g)	
	Red Angel	Wonderful	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
Control	110.41±1.31^{d}	115.91±2.01^{d}	0.48±0.02^{d}	0.59±0.02^{d}	0.23±0.01^{c}	0.27±0.01^{d}
P. fluorescens	177.58±1.73^{a}	183.98±2.17^{a}	0.58±0.02^{c}	0.68±0.02^{c}	0.33±0.01^{b}	0.38±0.01^{b}
G. mosseae	162.75±2.24^{c}	170.75±2.15^{c}	0.62±0.01^{b}	0.70±0.01^{b}	0.34±0.01^{a}	0.37±0.01^{c}
P. fluorescens + *G. mosseae*	173.66±1.55^{b}	180.50±2.31^{b}	0.67±0.01^{a}	0.77±0.01^{a}	0.34±0.01^{a}	0.39±0.01^{a}
Salinity (dS/m)	P<0.001	P=0.069	P=0.008	P=0.019	P<0.001	P<0.001
Control	159.56±6.92^{a}	162.75±6.92	0.63±0.02^{a}	0.65±0.02^{a}	0.32±0.01^{a}	0.36±0.01^{a}
4	157.00±7.42^{b}	160.06±7.53	0.56±0.02^{b}	0.64±0.02^{b}	0.31±0.01^{b}	0.34±0.01^{b}
8	151.75±6.84^{c}	159.25±7.39	0.55±0.02^{c}	0.62±0.02^{c}	0.29±0.01^{c}	0.34±0.01^{b}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

Table 3. The effect of biofertilizer and salinity on root diameter and number of lateral roots in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Root diameter (mm)		Lateral roots (n)	
	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001
Control	1.66±0.14^{c}	2.66±0.14^{d}	22.25±0.59^{d}	24.33±0.62^{d}
P. fluorescens	2.33±0.14^{b}	3.16±0.17^{c}	32.08±0.63^{b}	34.16±0.64^{b}
G. mosseae	2.33±0.28^{b}	3.33±0.28^{b}	28.50±0.76^{c}	33.66±0.81^{c}
P. fluorescens + *G. mosseae*	2.83±0.21^{a}	3.66±0.23^{a}	42.91±0.75^{a}	44.91±0.87^{a}
Salinity (dS/m)	P<0.001	P<0.001	P<0.001	P=0.003
Control	2.87±0.18^{a}	3.16±0.19^{a}	33.12±2.13^{a}	35.22±2.20^{a}
4	1.93±0.14^{c}	3.00±0.14^{b}	30.62±1.91^{b}	32.75±1.90^{b}
8	2.06±0.19^{b}	2.81±0.18^{b}	30.51±1.97^{b}	32.56±1.90^{b}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

The study revealed that the highest percentage of symbiosis was observed in *P. fluorescens* + *G. mosseae*, with 89.16% and 90.55% in the Red Angel and Wonderful cultivars, respectively (Table 1). These findings are consistent with previous research by Al-Khaliel (2010), who reported symbiosis with *Glomus* spp. fungi in most plants under various environmental stress conditions. Esna-Ashari and Bahrami (2018) also noted that the combination of fungi and bacteria led to increased nodulation and nutrient absorption under osmotic and salinity stress conditions. In both cultivars, salinity did not have any influence on the percentage of symbiosis. Notably, even in the absence of biofertilizer treatments, non-inoculated plants exhibited a certain degree of symbiosis (Table 1). This could be attributed to potential contamination from indigenous bacteria and AM species present in the irrigation water and greenhouse environment, a finding consistent with previous research (Aseri et al., 2008; Eftekhari et al., 2012). These findings underscore the adaptability of pomegranate cultivars to form symbiotic relationships with beneficial microorganisms, even under salinity stress. The consistent symbiosis percentages across salinity treatments highlight the complex interactions between pomegranate plants and microbial communities

The study's results also indicated an increase in stem diameter with the use of biofertilizer (Table 1). The highest stem diameter of the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* (6.33 and 6.25 mm, respectively). These findings align with the research of Williams et al. (2010), who reported that inoculation with AM fungi under increased levels of salinity stress led to an increase in stem diameter due to enhanced production of hormones, cell division and elongation, water and nutrient absorption, cell

development, and photosynthesis. Additionally, the independent effect of salinity on stem diameter was statistically non-significant in both cultivars. This could be attributed to the specific tolerance of these cultivars to salinity stress. Pomegranate plants may have developed mechanisms to cope with moderate salinity levels without significant changes in stem diameter.

According to the research findings, the independent effect of biofertilizer on the number of lateral branches in both cultivars was found to be significant (Table 1). The application of biofertilizers led to an increase in the number of lateral branches in both cultivar (Table 3). The highest number of lateral branches in the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* (3.16 and 3.58, respectively). These results are in line with the findings of Tadayon and Maafpourian (2018), who reported that biofertilizer and mycorrhiza inoculation under salinity stress conditions resulted in a 20.8% increase in the number of lateral branches of the studied genotypes. They also demonstrated that inoculation with *P. fluorescens* and *Azospirillum* spp increased the number of lateral branches of studied genotypes through nitrogen fixation and the production of growth regulators such as auxin. In contrast, the independent effect of salinity on the number of lateral branches of both cultivars was not found to be significant (Table 3). The lack of significant effect of salinity on the number of lateral branches in pomegranate cultivars could be attributed to the genetic resilience of these cultivars to salinity stress. Additionally, the experimental conditions, such as the duration of exposure to salinity or the specific growth stage of the plants during the study, could have influenced the results.

The effect of salinity on the total number of leaves was significant only in the Red Angel cultivar, with the lowest number of total leaves observed at 8 dS/m salinity (151.75) (Table 2). Salinity mainly influences the number of leaves through stimulating leaf abscission. Elevated salinity levels commonly lead to water stress in plants, prompting leaf abscission. Nevertheless, biofertilizers can improve the plant's ability to withstand stress and manage salinity-induced challenges. In contrast, the application of biofertilizers led to an increase in the total number of leaves in the Red Angel and Wonderful cultivars, with the highest total number of leaves observed in *P. fluorescens* (177.58 and 183.98, respectively). These results are consistent with the findings of Fattahi and Mohammadkhani (2019), who reported that the use of biofertilizer is an effective strategy in reducing salinity losses, and with the treatment of *G. mosseae* and *G. versiform*, the total number of leaves in citrus seedlings increased. Additionally, the results of this research align with the findings of Dominguez-Nunez et al. (2013), who reported that the combined use of *P. fluorescens*, *G. mosseae*, and *G. intraradices* led to an increase in the number of leaves in silver cedar genotypes through increased absorption of water and nutrients, synthesis of auxin, gibberellic acid, and cytokinin, and production of antioxidant enzymes.

With the application of biofertilizer, an increase in leaf fresh and dry weight was observed (Table 2), with the highest values in *P. fluorescens* + *G. mosseae*. Conversely, salinity led to a decrease in both leaf fresh and dry weight, with the lowest values observed at 8 dS/m salinity. In a study by Bahrani et al. (2020), it was noted that at the highest concentration of salinity, the leaf fresh weight decreased by 32% in grape studied genotypes due to a decrease in cell water content. Additionally, Wen-Bo et al. (2008) reported that inoculation with *G. mosseae* resulted in an increase in the leaf fresh and dry weight of iris due to enhanced absorption of water and nutrients by the roots and an increase in the root surface area with the hyphae of the fungi under salt stress conditions. The results are also consistent with the findings of Naseri et al. (2012), who demonstrated that the combination of *P. putida* bacteria and *Funneliformis mosseae* fungi increased the leaf fresh and dry weight of *Trigonella foenum-graceum* medicinal plant by 52% compared to the control treatment under osmotic stress conditions.

Furthermore, Mardhiah et al. (2016) reported that salinity led to a significant decrease in photosynthesis, biomass, and shoot growth, resulting in a decrease in leaf dry weight and shoots.

The results also revealed that the lowest root diameter in the Red Angel and Wonderful cultivars was observed at 8 dS/m salinity (2.06 and 2.81 mm, respectively) (Table 3). With the application of biofertilizer, the root diameter of both cultivars increased, with the largest root diameter observed in *P. fluorescens* + *G. mosseae* (2.83 and 3.66 mm, respectively). These findings align with the results of Khosrovjerdi et al. (2013), who reported that application of *Sinorhizobium meliloti* × *Azotobacter chrococcum* × *Azospirillum lipoferum* × *P. fluorescens* and the AM fungi of *G. mosseae* and *F. mosseae*, individually and in a binary combination, led to an increase in the root diameter of chickpeas under salinity condition. The synergistic relationship between AM fungi and bacteria stimulates the growth of nitrogen fixers and the production of plant hormones such as auxin, cytokinin, and gibberellin, resulting in an increase in root diameter and the number of lateral roots.

The increase in salinity resulted in a decrease in the number of lateral roots (Table 3). The lowest number of lateral roots in the Red Angel and Wonderful cultivars was observed at 8 dS/m salinity (30.51 and 32.56, respectively). However, the application of biofertilizer increased the number of lateral roots, with the highest numbers observed in *P. fluorescens* + *G. mosseae* (42.91 and 44.91, respectively). These findings are consistent with the results of Naseri et al. (2012), who demonstrated that under osmotic stress conditions, treatment with *F. mosseae* increased biomass and the number of lateral roots by enhancing the absorption of nutrients from the soil solution. They also concluded that the number of lateral roots is highly dependent on the growth environment, as salt stress reduces the water content of cells and makes their elongation difficult. Furthermore, they reported that the combined treatment of *F. mosseae* and *P. putida* increased root length and the number of lateral roots through the expansion of the root system and soil exploration by external hyphae in the hairy roots and the root surface. Lateral roots play a crucial role in water and nutrient uptake, anchoring plants in the soil, and providing stability to the plant. Boosting root diameter and lateral roots with biofertilizers can enhance nutrient absorption, water uptake, and plant resilience. This leads to higher yields, better stress tolerance, and improved overall plant performance, benefiting agricultural sustainability.

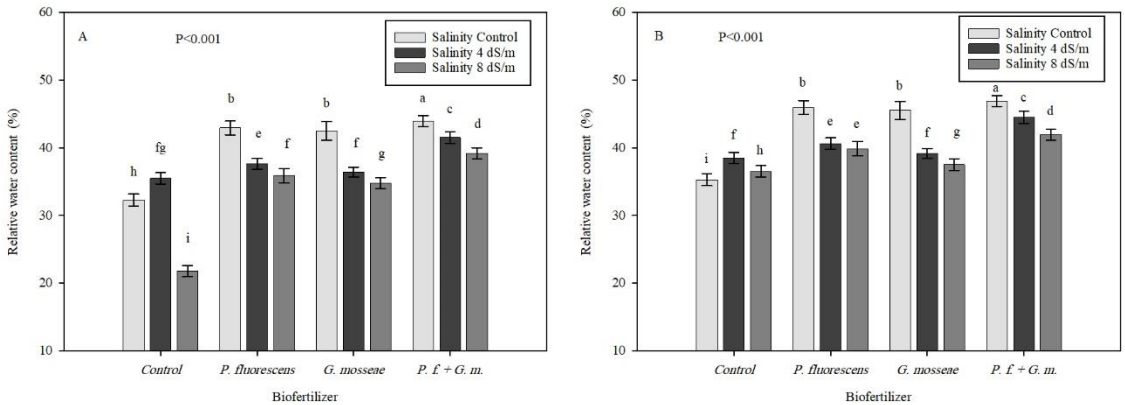

Fig. 1. The interaction effect of biofertilizer and salinity on relative water content of leaves in pomegranate plantlets of Red Angel (A) and Wonderful (B) cultivars. Different letters represent significant differences at P=0.01. Error bars indicate ± standard error.

Table 4. The effect of biofertilizer and salinity on root fresh and dry weight in pomegranate plantlets of Red Angel and Wonderful cultivars.

Treatments	Root fresh weight (g)		Root dry weight (g)	
	Red Angel	Wonderful	Red Angel	Wonderful
Biofertilizer	P<0.001	P<0.001	P<0.001	P<0.001
Control	39.20±0.90^{d}	41.20±0.90^{d}	18.61±0.61^{c}	19.27±0.64^{d}
P. fluorescens	44.33±0.63^{c}	46.24±0.67^{c}	23.55±0.42^{b}	24.33±0.43^{c}
G. mosseae	45.30±0.63^{b}	47.30±0.63^{b}	23.49±0.51^{b}	25.83±0.51^{a}
P. fluorescens + *G. mosseae*	46.03±0.50^{a}	48.03±0.50^{a}	24.89±0.60^{a}	25.41±0.64^{b}
Salinity (dS/m)	P<0.001	P<0.001	P<0.001	P<0.001
Control	45.59±0.54^{a}	47.59±0.54^{a}	24.26±0.62^{a}	24.86±0.64^{a}
4	43.71±0.98^{b}	45.71±0.98^{b}	22.26±0.73^{b}	23.30±0.73^{b}
8	41.84±0.85^{c}	43.78±0.86^{c}	21.01±0.68^{c}	21.70±0.72^{c}

Different letters in each column represent significant differences at P=0.01. Mean values ± standard error.

The increase in salinity resulted in a significant decrease in the root fresh and dry weight in both cultivars (Table 4), with the lowest weights observed at 8 dS/m salinity. However, with the application of biofertilizer, particularly *P. fluorescens* + *G. mosseae*, the root fresh and dry weight of both cultivars increased. These findings are consistent with the results of Rydlová et al. (2016), who demonstrated that inoculation with AM fungi increased root fresh and dry weight under salinity conditions. Additionally, Soori et al. (2019) reported a 57% decrease in the fresh root weight of the Shahwar pomegranate cultivar due to the decrease in cellular water content and root expansion area with an increase in salinity. The results are also in line with the findings of Cruz and Husain (2008), who concluded that AM fungi increased the root fresh and dry weight of many plants due to the effect of mycorrhiza on the absorption of nutrients such as nitrogen, phosphorus, and potassium. Furthermore, Esna-Ashari and Bahrami (2018) reported that inoculation with AM fungi, including *G. hoi*, *G. intraradices*, and *G. mosseae*, increased the root fresh and dry weight of *Poncirus trifoliata* seedlings under salinity conditions. Bahrani et al. (2020) also demonstrated a decrease in the root dry weight of Chafteh grape cultivar with an increase in osmotic stress caused by salinity.

The application of biofertilizers increased the RWC of leaves at all salinity levels (Fig. 1). The highest RWC of leaves in the Red Angel and Wonderful cultivars was observed in *P. fluorescens* + *G. mosseae* at the non-saline control (43.9% and 46.9%, respectively). However, the lowest RWC in the Red Angel cultivars was observed at 8 dS/m salinity and non-inoculated control (21.79%), while in the Wonderful cultivar, the lowest RWC of leaves was observed at zero salinity and non-inoculated control (35.29%). Increasing the RWC through biofertilizer application is practically significant as it indicates improved water status in plants. This enhancement in water retention can contribute to enhanced salinity tolerance by helping plants maintain proper hydration levels even under saline conditions. These results are consistent with the findings of Kumar et al. (2015), who demonstrated a 26% increase in the RWC in pomegranate genotypes under salinity conditions with treatment using AM fungi. Similarly, Ghasemi and Zahedi (2018) showed an increase in the RWC in all sorghum genotypes under salinity stress as a result of inoculation with mycorrhiza. However, they noted that mycorrhiza did not have a significant effect on the RWC in some genotypes under non-saline conditions. Additionally, Alipour et al. (2019) reported an increase in the RWC in *Cupressus arizonica* as a result of the combination of *P. fluorescens*, *G. mosseae*, and *G. intraradices*, attributed to the increase in root length and changes in root morphology for water search. The observed increase in RWC suggests improved water uptake and retention, which may contribute to enhanced salinity tolerance and overall plant resilience.

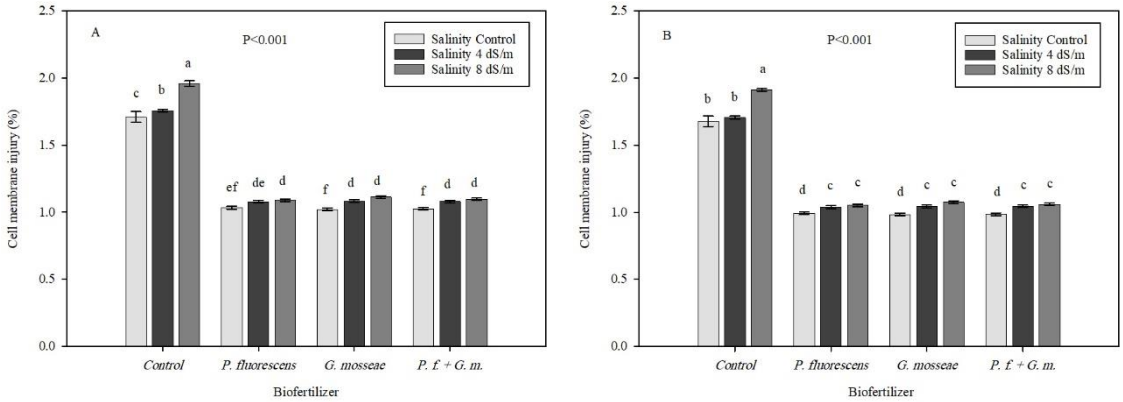

Fig. 2. The interaction effect of biofertilizer and salinity on cell membrane injury in pomegranate plantlets of Red Angel (A) and Wonderful (B) cultivars. Different letters represent significant differences at P=0.01. Error bars indicate ± standard error.

At all salinity levels, the application of biofertilizer resulted in a decrease in cell membrane injury (Fig. 2). The highest injury in the Red Angel and Wonderful cultivars was observed in the non-inoculated control at 8 dS/m salinity (1.96% and 1.91%, respectively), followed by the non-inoculated control at 4 dS/m salinity. These results are consistent with the findings of Ziaei et al. (2020), who reported that the use of AM fungi increased the membrane stability index and decreased cell membrane injury by 16% in all studied iris genotypes under salt stress. Additionally, the results align with the findings of Shirinzadeh et al. (2021), who reported that inoculation with *F. mosseae*, *F. caledonius*, *Rhizophagus intraradices*, and *R. iranicus* prevented the production of free radicals and cell membrane peroxidation, thereby preserving cell membrane proteins and enzymes and decreasing cell membrane injury in pear genotypes under salinity conditions. These results emphasize the function of biofertilizers in maintaining membrane integrity during periods of stress. Biofertilizers can enhance the plant's stress tolerance by promoting the synthesis of protective compounds such as antioxidants and osmoprotectants. These compounds help to stabilize cell membranes and protect them from damage caused by salinity stress.

CONCLUSION

The study revealed that the symbiosis percentage was highest in the Red Angel and Wonderful cultivars when using *P. fluorescens* + *G. mosseae* biofertilizer, and salinity did not affect this percentage in either cultivar. All studied biofertilizers contributed to increased stem and root diameter, number of lateral branches, roots, and leaves, as well as leaf and root fresh and dry weight, and relative water content in both cultivars. Additionally, biofertilizers reduced cell membrane injury at all salinity levels. These findings highlight the complex interplay between biofertilizer application and pomegranate physiological responses under salinity stress. Overall, the research results demonstrated that the applied biofertilizers, particularly *P. fluorescens* + *G. mosseae*, had positive effects in mitigating the harmful influences of salinity in both cultivars. Conducting a comparative analysis of different biofertilizers and their effects on pomegranate tree growth and development under varying abiotic stresses would provide valuable insights and contribute to the existing body of knowledge.

Conflict of interest

The authors declare that they have no conflict of interest to report.

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