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RESEARCH ARTICLE

Assessment of salt tolerance in spinach under in vitro condition

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ABSTRACT

Salinity is a major abiotic stress factor that limits the growth and yield of crops, including spinach (*Spinacia oleracea* L.) This study aimed to investigate the effects of different NaCl concentrations (0, 100, 200, and 300 mM) on the germination, growth, and physiological responses of two spinach cultivars (Yaman and Matador) under in vitro conditions. During the study, germination rate, stem fresh and dry weights (g), root fresh and dry weights (g), stem and root lengths (mm) parameters were evaluated. The results showed that all measured traits were significantly affected by salinity with differences observed among cultivars and media. The highest germination rate (75.50%) was recorded in 'Yaman', while the highest medium average (75.00%) was observed in the control treatment. Increasing salinity levels, especially at 300 mM NaCl, led to a reduction in both stem and root biomass. The highest stem fresh weight (0.180 g) and root fresh weight (0.113 g) were obtained from the control medium. Similarly, root dry weight and stem length showed significant decreases with increasing NaCl concentrations. 'Matador' showed comparatively greater tolerance under saline conditions, especially in terms of root biomass and lenght.

Key words: Abiotic stress, salinity, spinach, in vitro.

INTRODUCTION

Spinach (*Spinacia oleracea* L.) is a globally consumed leafy vegetable of considerable economic significance (Morelock and Correll, 2008). Although the exact origin of cultivated spinach remains uncertain, it is generally believed to have been domesticated approximately 2000 yr ago in Iran, formerly known as Persia (Rubatzky et al., 1997; Morelock and Correll, 2008; Ribera et al., 2021). Spinach is an annual plant species that undergoes distinct vegetative and reproductive developmental stages. It is generally sown in late winter or early spring, during which time the seedlings form a characteristic rosette of basal leaves as part of the early vegetative phase (Krarup and Moreira, 1998; Van der Vossen et al., 2004; Ribera et al., 2020). As temperatures and day length increases during summer, the plants enter the reproductive phase through bolting, producing a flowering stalk (peduncle) that can reach up to 1 m in height (Krarup and Moreira, 1998). This phase is marked by the appearance of terminal staminate flowers and/or pistillate flowers located in the axils of the bracts (Uotila, 1997). Today, a wide range of spinach cultivars are available, exhibiting diverse leaf shapes from round to hastate and surface textures, ranging from smooth to heavily crinkled (savoy type) (Morelock and Correll, 2008). Moreover, modern cultivars show varying degrees of adaptation to photoperiod and climatic conditions. Some have been bred for bolting resistance under extended warm day conditions, allowing for successful summer production (Van der Vossen et al., 2004). The increasing number of studies supports the view that consuming diets abundant in fruits and vegetables contributes to the prevention of widespread chronic illnesses, including cancer, obesity, and cardiovascular diseases. Among these, leafy green vegetables are especially valued due to their rich nutritional profiles and the bioactivity of non-essential phytochemicals they contain. Spinach is commonly evaluated as a functional food because of its rich nutritional profile such as essential vitamins, minerals and a variety of health-promoting phytochemicals. Bioactive compounds in spinach have been reported to: (i) Neutralize reactive oxygen types and protect cellular macromolecules from oxidative stress, (ii) influence the expression and function of genes associated with metabolic pathways, cell growth, inflammation, and antioxidant systems, and (iii) enhance the release of hormones that regulate satiety, thus helping to control appetite. These mechanisms contribute to spinach's well-documented anticancer, anti-obesity, blood sugar-lowering, and lipid-regulating effects. Nevertheless, its consumption remains comparatively lower than that of other leafy greens (Roberts and Moreau, 2016). Spinach is a diploid species ($2n = 2x = 12$) and highly heterozygous. It is an economically significant cool-season leafy vegetable. Global demand for spinach has been increasing, particularly due to its high nutritional value. Spinach is consumed both raw and cooked (Bhattarai and Shi, 2021).

Soil salinization is one of the most critical abiotic stresses adversely impacting plant growth and development, with profound implications for global food security. This results with the excessive accumulation of salts in the soil, predominantly driven by human-induced factors such as unsustainable irrigation practices, poor land management, and excessive fertilizer application. Increasing concentrations of sodium (Na^+), chloride (Cl^-) and other salt ions disrupt cellular homeostasis, interfere with vital physiological processes including seed germination, photosynthesis, and nutrient uptake and induce metabolic imbalances. Prolonged exposure to salinity stress can cause severe tissue damage and in extreme cases, plant death (Balasubramaniam et al., 2023). Soil salinization primarily results from the accumulation of water-soluble salts such as Na, K, Cl and sulfate (SO_2) in the root zone, which induces osmotic stress by lowering the soil water potential. This osmotic balance reduces the capacity of plant root cells to absorb water efficiently, preventing water uptake and initiating physiological drought, even in the presence of adequate soil moisture (Munns and Tester, 2008; Kamran et al., 2019; Stavi et al., 2021; Balasubramaniam et al., 2023). Soil salinity is a critical abiotic stress factor that adversely affects plant development and yield by triggering various harmful processes, particularly osmotic imbalance and ion toxicity (Negrão et al., 2017). The severity and nature of the resulting physiological and biochemical changes; however, are influenced by both the level of salinity and the specific plant species exposed to the stress (Liu et al., 2025). In addition to its nutritional importance, spinach is widely utilized as a model plant species in stress physiology research because of its distinctive characteristics, such as high photosynthetic capacity and notable tolerance to salinity (Di Martino et al., 2003; Kim et al., 2021; Liu et al., 2025).

Plants are frequently exposed to biotic and abiotic stresses that limit their growth and reduce yield, leading to significant global economic losses. Breeding for stress tolerance has been used to improve crop resilience, and plant tissue culture offers a cost-effective, efficient approach for developing and screening tolerant genotypes. In vitro selection under controlled conditions allows precise evaluation of physiological and biochemical responses to stress (Wijerathna-Yapa and Hiti-Bandaralage, 2023). This study aimed to evaluate the response of two spinach cultivars to different concentrations of salinity (NaCl) (0-control, 100, 200, and 300 mM) under in vitro conditions.

MATERIALS AND METHODS

This study was conducted at the Prof. Dr. Saadet BÜYÜKALACA Plant Tissue Culture Laboratory of Horticulture Department of Çukurova University (Adana, Türkiye). Two spinach (*Spinacia oleracea* L.) cultivars (Yaman and Matador) were used during the experiments. 'Yaman' is a semi-oriental type, with upright growth and a medium-length stem. The leaf color is very dark, shiny, and smooth. The leaf quality is high and its shelf life is long (Anonymous, 2025a). 'Matador' has large, dark green leaves with short stems, its strong plant structure is resistant to cold temperatures and yields 1.0-1.5 t ha^{-1}. It can be harvested 60-70 d after planting (Anonymous, 2025b).

Preparation of culture medium and seed sowing

Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) was used as the basal nutrient medium. Sucrose was added as a C source, and agar was included to solidify the medium. To assess the effects of salinity, sodium chloride (NaCl) was added at four concentrations: 0 (control), 100, 200, and 300 mM. The pH of the medium was adjusted to 5.8 using appropriate buffering agents before sterilization. The media were sterilized in an autoclave at 121 °C and 1.2 atm for 15 min. After sterilization, the media were poured into sterile petri dishes under aseptic conditions in a laminar flow. Spinach seeds were then sown onto the solidified media for in vitro germination and growth.

Sterilization of seed and cultivation

Spinach seeds were surface-sterilized by immersion in a 25% sodium hypochlorite solution for 20 min. After sterilization, seeds were thoroughly rinsed several times with sterile bidistilled water to eliminate any residual disinfectant. The sterilized seeds were then aseptically transferred onto the prepared culture media in a laminar flow. The cultures were incubated in a controlled growth chamber set at 25 ± 2 °C with a photoperiod of 16:8 h, under a light intensity of approximately 3000 lux.

During experiments, some parameters such as germination rate (%) (Kaya et al., 2006), fresh and dry weights of shoots and roots (g), shoot and root lengths (mm) (Keleş, 2019) were evaluated (Daşgan et al., 2002).

Statistical analysis

The study was conducted as a factorial experiment arranged in a randomized plot design, with four replicates per treatment. Each replicate consisted of five petri dishes. Statistical analyses, including correlation analysis, were performed using JMP statistical software (JMP Statistical Discovery, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Table 1 presents the average germination rates of different spinach cultivars grown on media supplemented with different concentrations of NaCl. Analysis revealed that the effects of cultivar and medium, as well as the Cultivar × Medium interaction, were all significant. Among the media, the highest average germination rate was observed on 0 mM medium (75.00%), while the lowest was recorded on 300 mM medium (46.00%). In terms of cultivar averages, the highest germination rate was found in 'Yaman', with an average of 75.50%.

The shoot fresh weight values of different spinach cultivars grown on nutrient media containing different concentrations of NaCl are presented in Table 2. According to the analyses, the medium factor had a significant effect on shoot fresh weight. The highest average shoot fresh weight was recorded on medium 0 mM with 0.180 g. This was followed by media 100 mM (0.129 g), 2000 mM (0.106 g), and 3000 mM (0.075 g), respectively.

Table 1. Effect of different NaCl concentrations in nutrient media on germination percentage of two spinach cultivars. $LSD_{cultivar}$: 1.685***; LSD_{medium}: 1.946***; $LSD_{cultivar×medium}$: 3.370***; $^{***}P \leq 0.001$. Averages followed by different letters are significantly different.

	Yaman	Matador	Medium average
mM	%	%	%
0	92.0^{a}	62.0^{d}	75.0^{A}
100	72.0^{b}	58.0^{e}	66.0^{B}
200	70.0^{bc}	36.0^{f}	52.0^{C}
300	68.0^{c}	20.0^{g}	46.0^{D}
Cultivar average	75.5^{A}	44.0^{B}	

Table 2. Effect of different NaCl concentrations in nutrient media on fresh stem weights of two spinach cultivars. $LSD_{cultivar}$: ^{ns}; LSD_{medium}: 0.187***; $LSD_{cultivar×medium}$: ns; ^{ns}: nonsignificant; $^{***}P \leq 0.001$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	g	g	g
0	0.195	0.164	0.180^{A}
100	0.107	0.152	0.129^{B}
200	0.103	0.109	0.106^{B}
300	0.077	0.073	0.075^{C}
Cultivar average	0.121	0.124	

Table 3 shows the shoot dry weight values of different spinach cultivars grown on nutrient media containing different concentrations of NaCl. Based on the analysis, the effect of medium was significant. Medium averages revealed that the highest value was recorded in medium 0 mM (0.0121 g), while the lowest was observed in medium 300 mM (0.0059 g).

The findings related to the root fresh weight of different spinach cultivars grown on nutrient media supplemented with different concentrations of NaCl are presented in Table 4. Statistical analysis indicated that both cultivar and medium had significant effects on root fresh weight. Among the cultivars, the highest average root fresh weight was recorded in Matador (0.096 g). In terms of media, the highest average value was observed in the control 0 mM with 0.113 g.

Table 5 presents the root dry weight values of different spinach cultivars grown on nutrient media containing various concentrations of NaCl. Analysis showed that cultivar average, medium average, and the Cultivar × Medium interaction had significant effects on root dry weight. Among the media, the highest average root dry weight was recorded in the control medium with 0.008 g, while the lowest was observed in medium 300 mM with 0.003 g. For cultivar averages, the highest root dry weight was obtained from 'Matador' with 0.006 g. Regarding the interaction between cultivar and medium, the highest value was also found in 'Matador' grown on medium 0 mM with 0.012 g.

Table 3. Effect of different NaCl concentrations in nutrient media on dry stem weights of two spinach cultivars. $LSD_{cultivar}$: ^{ns}; LSD_{medium}: 0.040^{**}; $LSD_{cultivar×medium}$: ns; ^{ns}: nonsignificant; $^{**}P \leq 0.01$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	g	g	g
0	0.0103	0.0138	0.0121^{A}
100	0.0096	0.0113	0.0105^{AB}
200	0.0067	0.0073	0.0070^{BC}
300	0.0058	0.0060	0.0059^{C}
Cultivar average	0.0081	0.0096	

Table 4. Effect of different NaCl concentrations in nutrient media on fresh root weights of two spinach cultivars. $LSD_{cultivar}$: 0.235^{**}; LSD_{medium}: 0.271^{**}; $LSD_{cultivar×medium}$: ^{ns}; ^{ns}: nonsignificant, $^{**}P \leq 0.01$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	g	g	g
0	0.062	0.164	0.113^{A}
100	0.047	0.084	0.066^{B}
200	0.039	0.078	0.059^{B}
300	0.029	0.055	0.042^{B}
Cultivar average	0.044B	0.096A	

Table 5. Effect of different NaCl concentrations in nutrient media on dry root weights of two spinach cultivars. $LSD_{cultivar}$: 0.0189^{***}; LSD_{medium}: 0.022^{***}; $LSD_{cultivar×medium}$: 0.038^{*}; $^{*}P < 0.05$; $^{***}P \leq 0.001$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	g	g	g
0	0.005^{bc}	0.012^{a}	0.008^{A}
100	0.004^{c}	0.007^{b}	0.005^{B}
200	0.002^{c}	0.004^{c}	0.003^{C}
300	0.002^{c}	0.003^{c}	0.003^{C}
Cultivar average	0.003^{B}	0.006^{A}	

Table 6 presents the average shoot length values of different spinach cultivars grown on nutrient media containing different concentrations of NaCl. Analysis revealed that all examined factors had a significant effect on shoot length. In terms of cultivar averages, the longest shoot was observed in 'Matador' with 48.31 mm, while the shortest was recorded in 'Yaman' with 36.85 mm. Among the media, the highest average shoot length was obtained on medium 0 mM with 58.00 mm.

Table 7 presents the average root length values of different spinach cultivars grown on nutrient media supplemented with different concentrations of NaCl. According to the analysis, both the cultivar and medium had significant effects on root length. Among the media, the greatest root elongation was observed in the control treatment with 79.58 mm, while the shortest was recorded in medium 300 mM with 41.01 mm. For cultivar averages, the longest root length was found in 'Matador' with 75.71 mm.

Table 6. Effect of different NaCl concentrations in nutrient media on stem lengths of two spinach cultivars. $LSD_{cultivar}$: 1.261***; LSD_{medium}: 1.456***; $LSD_{cultivar×medium}$: 2.522**; $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	mm	mm	mm
0	44.40^{bc}	71.60^{a}	58.00^{A}
100	36.95^{cd}	52.13^{b}	44.54^{B}
200	34.31^{cd}	36.69^{cd}	35.50^{C}
300	31.74^{d}	32.84^{d}	32.29^{D}
Cultivar average	36.85^{B}	48.31^{A}	

Table 7. Effect of different NaCl concentrations in nutrient media on stem lengths of two spinach cultivars. $LSD_{cultivar}$: 1.075***; LSD_{medium}: 1.241***; $LSD_{cultivar×medium}$: ^{ns}; ^{ns}: nonsignificant; $^{***}P \leq 0.001$. Averages followed by different letters are significantly different.

Salinity	Yaman	Matador	Medium average
mM	mm	mm	mm
0	54.19^{cd}	104.94^{a}	79.58^{A}
100	38.26^{de}	83.30^{b}	60.78^{B}
200	37.48^{de}	63.03^{c}	50.25^{C}
300	30.46^{e}	51.56^{cd}	41.01^{D}
Cultivar average	40.10^{B}	75.71^{A}	

Figure 1 illustrates the correlations among the measured parameters of different spinach cultivars grown on nutrient media containing different concentrations of NaCl. A strong positive correlation was observed between root fresh weight and root dry weight (r = 0.81), shoot length (r = 0.80), and root length (r = 0.84). Similarly, root dry weight showed strong positive correlations with shoot length (r = 0.83) and root length (r = 0.85).

Turhan et al. (2011) investigated the effect of varying salinity levels (0, 4.7, 9.4, and 14.1 dS m^{-1}) on the germination of cabbage and cauliflower. Findings indicated that higher salt concentrations celarly suppressed seed germination rates in both species. Singh and Jakhar (2018) evaluated different concentrations of 24-epibrassinolide (24-EBL) treatments (10^{-5}, 10^{-7}, 10^{-9}, and 10^{-11} M) (pre-soaked) on germination-related parameters in seeds of *Vigna mungo*. They reported that salinity stress—especially at elevated levels such as 16 and 20 dS m^{-1}—had a substantial negative impact on seed germination parameters. Abdel-Farid et al. (2020) investigated the effects of different NaCl concentrations (25, 50, 100, and 200 mM) on seed germination and seedling growth of cucumber and tomato under both in vitro and in vivo conditions. Their findings indicated that 200 mM NaCl slowed the germination process in cucumber seeds. In tomato, all tested NaCl concentrations significantly affected germination percentage, germination time and seedling development. Kara et al. (2024) tested different NaCl concentrations (0, 50, 100, 150, and 200 mM) on tomato development under in vitro conditions; the highest germination percentages were observed in the control medium (0 mM NaCl) with 80.00%, followed by 76.00% at 50 mM, and 75.60% at 100 mM. Naik et al. (2025) investigated the effects of salt stress on plant growth in *Spinacia oleracea* using hydroponic method. A significant difference was observed in seed germination percentage between the control and salt-treated seeds. The germination rate was recorded as 59.6 ± 1.45% in the control. However, with increasing salinity concentrations, a decline in germination percentage was observed with 14 ± 2% at 10 dS m^{-1} after 10 d.

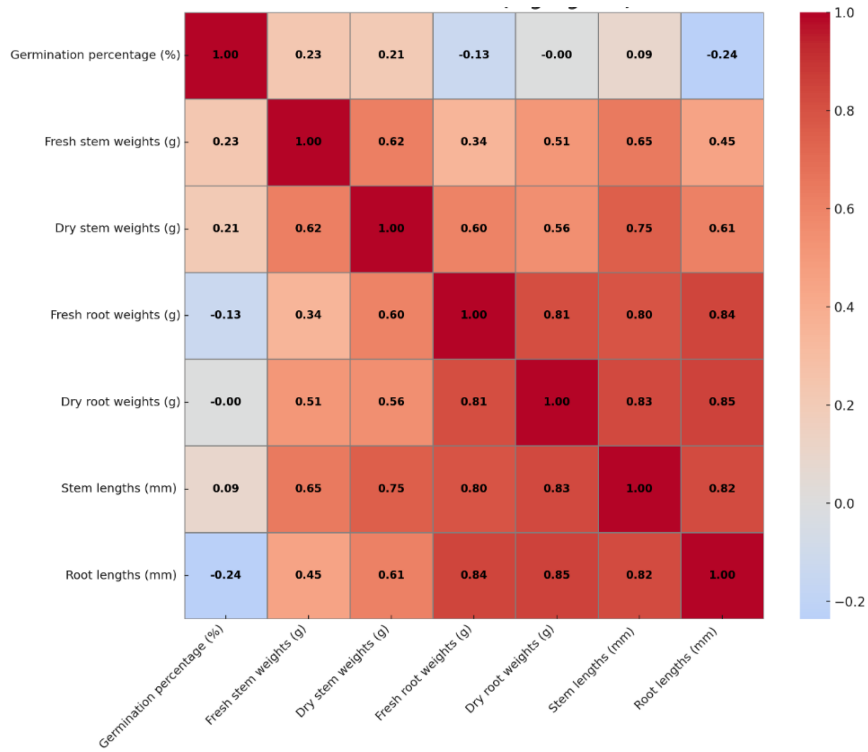

Figure 1. Correlation matrix of morphological parameters in spinach cultivars under salinity stress.

Jamil et al. (2007) reported a decrease in radish fresh weight under saline conditions. Similarly, Kapoor and Pande (2015) investigated the effect of NaCl-induced salinity (0, 50, 100, 150 and 200 mM) on the growth and physiological parameters of a leafy vegetable fenugreek ‘RMt-1’. Their assessment included a range of parameters such as germination percentage, shoot and root lengths, shoot/root ratio, leaf and branch numbers, vigor index, fresh and dry biomass, moisture content, relative water content, and photosynthetic pigment concentrations. The findings showed a clear decline for all measured parameters with increasing salinity levels. Based on the results, it was concluded that ‘RMt-1’ exhibits sensitivity to increasing salt concentrations. Fardus et al. (2018) evaluated the mitigating effects of exogenous salicylic acid (1 mM SA) on salt-induced stress in two wheat cultivars (BARI Gom 21 and BARI Gom 25) through a pot experiment involving various salinity levels (50-200 mM NaCl), both with and without SA supplementation. It was found that increasing salt concentrations affected plant height, shoot number and biomass accumulation, while SA application partially alleviated these effects. Similarly, Seth (2018) reported that 100 mM NaCl significantly inhibited shoot and root elongation, as well as fresh and DM production in tomato varieties. Altuner et al. (2022) determined that increasing salt concentrations caused a decline in root dry weight in barley varieties. Manjavachi et al. (2022) investigated the effects of osmopriming on salt stress tolerance in *Petroselinum crispum* seeds by evaluating physiological seed quality and antioxidant enzyme activity. Seeds were treated with polyethylene glycol (PEG) solutions of different osmotic potentials and then exposed to different NaCl concentrations. Results showed that increasing salinity led to a linear decrease in germination rate and germination time. Decreasing in seedling length, fresh and dry weight provided more evident at osmotic potentials of -0.4 MPa and lower. Baktemur (2023) aimed to investigate the effects of nutrient media containing different concentrations of NaCl (0, 50, 100, 150, 200, and 250 mM) on the development of squash under in vitro conditions. The highest root fresh weight was observed in the 150 mM treatment (3.78 g), while the lowest was recorded at 250 mM (2.03 g).

In a study conducted by Çamlıca and Yaldız (2017), it was aimed to determine the response of basil plants to different salt concentrations from 0 to 240 mM with 20 mM increments. Root length varied between 0.08 and 5.07 cm, while shoot length ranged from 0.1 to 5.82 cm. The highest shoot and root lengths were observed at 20 mM except control, whereas the lowest values were recorded at 240 mM. In lentil, seedling growth decreased with increasing salinity stress (Foti et al., 2018). Öner and Kırlı (2018) reported that increasing NaCl concentrations resulted in approximately a 46% decrease in radicle length. In another study, Afsar et al. (2020) observed that salt stress caused notable decreases in shoot length, plant height, leaf number and both fresh and dry biomass in arugula plants compared to control conditions. Avcı et al. (2020) also reported that rising salinity levels led to a reduction in root fresh weight in cotton varieties grown under in vitro conditions.

Similar to the literature summary presented here, all parameters measured in our study were significantly affected by increasing salinity. Specifically, 300 mM NaCl resulted in a decrease in both shoot and root biomass. Variety 2 showed relatively greater tolerance to saline conditions in terms of root biomass and length. It is quite expected that plant species would be affected by rising salt levels. However, as with all studies on this subject, our aim was to determine the level of this tolerance, which vary among plant species. The greatest advantage of tissue culture experiments is their speed compared to pot or field experiments and ease of testing for a wide range of genotypes and salt concentrations. Sidek et al (2024), 'MARDI Siraj 297' rice was subjected to in vitro callus selection under 0-150 mM NaCl for 5 mo, and tolerant lines were identified based on morphological and biochemical traits.

CONCLUSIONS

This study showed that salinity has a deep effect on the early developmental stages of spinach, significantly influencing germination rate, stem fresh and dry weights, root fresh and dry weights, as well as stem and root lengths under in vitro conditions. Increasing NaCl concentrations, especially at 300 mM, resulted in a clear decline in stem and root fresh and dry weights, as well as stem and root lengths. The highest values for all parameters evaluated including the highest stem fresh weight (0.180 g) and root fresh weight (0.113 g) were obtained in the control medium (0 mM NaCl). While 'Yaman' exhibited the highest germination rate (75.50%), 'Matador' showed comparatively better performance under saline conditions, particularly in root lengths. This suggests a genotype-dependent variation in salt tolerance mechanisms, probably related to differential ionic regulation or osmotic adjustment capabilities. The findings obtained from this study emphasize genotype screening under controlled conditions as a preliminary step for identifying salt-tolerant genotypes before testing pot or field experiments.

Author contributions

Conceptualization: M.U., E.K., A.Ş., H.T., G.B. Methodology: M.U., A.Ş., E.K., H.T., G.B. Software: E.K., G.B. Validation: E.K., H.T., G.B. Formal analysis: E.K., G.B. Investigation: M.U., E.K., H.T., G.B. Data curation: E.K., G.B. Writing-original draft: G.B. Writing-review & editing: H.T., G.B. Visualization: G.B. Supervision: G.B. Project administration: G.B. Funding acquisition: G.B. All co-authors reviewed the final version and approved the manuscript before submission.

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