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Evaluation of Sodium, Potassium, and Chlorine Elements in Superior Interspecies Hybrids of *Pistacia atlantica* (Desf.) and *Pistacia integerrima* (Arota) Compared to the UCB1 Rootstock under Salt Stress

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ABSTRACT

The current research aimed to evaluate salinity tolerance in sixteen promising hybrids of *P. atlantica* × *P. integerrima*. The sixteen hybrids were selected for plant vigor as promising rootstocks in earlier research. Salinity tolerance was applied at 0.5, 8, and 16 dS m^{-1} on the hybrids for 4 months. Each hybrid was propagated by seed to achieve 3 replicates per hybrid. Each hybrid was evaluated for the response to salinity stress by evaluating sodium, potassium, and chlorine contents, as well as stem diameter (cm), after the salinity treatments. The UCB-1 rootstock served as the control, on which comparative assessments of the sixteen hybrids were made. The results showed that leaf and root sodium contents were lowest in 'AI6' (0.5834 mg kg^{-1}) and 'AI16' (0.9769 mg kg^{-1}), respectively. The leaf and root chlorine contents were lowest in 'AI12' (1.0658 mg kg^{-1}) and 'AI7' (1.2179 mg kg^{-1}), respectively. The highest leaf and root potassium contents were observed in 'AI13' (1.4507 mg kg^{-1}) and 'UCB1' (1.1731 mg kg^{-1}), respectively. 'AI13' showed the highest ST ratio (7.5611), indicating potentially elevated potassium content in stem tissues compared to others. The largest stem diameter was observed in 'AI4' (2.8292 cm). Thus, it can be concluded that the most salt-tolerant hybrids were 'AI4', 'AI13', 'AI2', and 'AI1'. Depending on the specific physiological trait measured, these hybrids can be potentially used for future breeding programs to increase salinity tolerance in pistachio rootstocks. These hybrids can expand orchard establishment in saline soils where the UCB-1 rootstock performs undesirably.

Introduction

The cultivation of pistachios, a crop of considerable economic and nutritional value, faces significant challenges due to the increasing impact of climate change, particularly the intensification of salinity stress in agricultural soils (Jamshidi Goharrizi et al., 2020). Rootstocks, the foundational component of pistachio cultivation, play a crucial role in determining the productivity, resilience, and overall success of these orchards. As salinity stress worsens under changing climatic conditions, the development and selection of salt-tolerant rootstocks have become vital for maintaining high yields and ensuring the sustainability of pistachio farming (Behzadi Rad et al., 2021).

Rootstock tolerance to salinity stress is essential for pistachio productivity. In regions where salinity is a growing concern, selecting rootstocks with inherent tolerance to saline environments directly influences the vigor of the grafted scion, thereby enhancing overall plant performance (Akbari et al., 2020b). High yields are dependent not only on the scion variety but also on the rootstock's ability to thrive in adverse conditions, making the choice of rootstock a critical factor in the long-term viability of pistachio orchards (Raoufi et al., 2021). Furthermore, hybrid vigor, or heterosis, is a phenomenon that can be harnessed through cross-pollination between closely related pistachio species. This practice results in rootstocks that exhibit superior growth rates, improved resistance to environmental stresses, and increased productivity compared to their parent species (Vahdati et al., 2021). The diversity of possibilities in creating various types of hybrids offers a vast array of options for developing rootstocks that can meet specific agronomic needs, particularly in the face of climate change-induced challenges.

Salinity tolerance depends on controlling the uptake of Na^+ and Cl^- from soils and the partitioning of these ions within plants. In many species, the ability of roots to exclude Na^+ and Cl^- from the transpiration stream flowing to the shoot has been linked to genetic variation associated with salinity tolerance (Zeng et al., 2019). Therefore, measurements of Na^+, K^+, or Cl^- are frequently used to screen for genetic variation in salinity tolerance, as maintaining a high uptake of K^+ is also essential. These ions can be readily extracted with dilute acid since they are not bound covalently to compounds in cells. Na^+ and K^+ can be measured using a flame photometer, by atomic absorption spectrometry, or by inductively coupled plasma (ICP)–atomic emission spectrometry in a dilute nitric acid extract. Similarly, Cl^- can be measured in the same acid extract colorimetrically using a spectrophotometer (Bagheri et al., 2021).

Among the primary breeding objectives for pistachio rootstocks, particularly when grafted to a scion with the extended juvenility of current commercial *Pistacia vera* scions, are vigor and rapid growth (Sheikhi et al., 2019). Arota, a new Iranian interspecific hybrid pistachio rootstock (*Pistacia integerrima* (male parent) × *Pistacia atlantica* (female parent)), was selected from the progeny of 222 controlled crosses made in 2013 and 2014. The most vigorous seedlings from these crosses were evaluated for 4 years and compared to UCB1, a traditionally released pistachio rootstock by the University of California Berkeley (UCB) (Ferguson and Haviland, 2016). Earlier research by the developers of Arota (Akbari et al., 2020a) indicated that 16 of the 222 seedling F_1 hybrids had statistically significant better height and diameter growth compared to UCB1. Within 5 to 7 months from seed germination, these 16 seedlings achieved a diameter sufficient for T-budding, which could facilitate orchard establishment within the first year of rootstock growth. In contrast, previous research on pistachio rootstocks showed that 'UCB-1', 'Akbari', and 'Ahmad-Aghai' were moderately tolerant to salinity stress and were recommended for rootstock perusal under moderate salinity conditions (Raoufi et al., 2020). Also, the effects of NaCl salinity on the seed germination, growth, and some physiological characteristics in 'Akbari, ''Akbari' hybrid (*P. integrrima* × 'Akbari'), 'Qazvini,' and 'Qazvini' hybrid (*P. integrrima* × 'Qazvini') were investigated in Iran, concluding that Akbari hybrids have competitive potential as rootstocks in saline conditions (Heydari et al., 2021). In Turkey, recent research showed that pistachio rootstock traits such as growth and development were evaluated for stem diameter and height, showing maximum stem length and width values in the progeny of the Ohadi cultivar, and the lowest values in the progeny of Uzun and Kırmızı hybrids (Verel et al., 2024). In Spain, *Pistacia terebinthus* L. was evaluated as a rootstock best adapted to rainfed conditions, showing that plants grafted onto *P. terebinthus* survived longer under extreme drought conditions, whereas moderate stress conditions caused their yield to be lower than that of plants grafted onto UCB-1 under the same conditions (Fernández-Suela et al., 2023). Regarding wild species, Vahdati et al. (2021) indicated that *P. integerrima* is the most vigorous rootstock now commonly used in pistachio cultivation but is also the least cold tolerant. It is rapidly being replaced by more cold- and salinity-tolerant hybrids, with particular attention being aimed at cross-pollinations between *P. integerrima* and *P. atlantica.* Other researchers noted that hybrids 'Ghazvini', 'Badami', and 'Kaleh-Ghouchi' are the most favorable *P. vera* rootstocks in Iran for tolerance to salinity and drought stress (Jamshidi Goharrizi et al., 2020). The effects of salinity were evaluated on three *P. vera* rootstocks ('Ghazvini', 'Badami', and 'Sarakhs') under different salinity treatments (0, 75, 150, and 225 Mm NaCl), thereby showing a decrease in K ions in the roots and stems of all rootstocks (Hokmabadi et al., 2005). Also, the cultivar 'Badami Zarand' and an inter-specific hybrid of *P. atlantica* × *P. vera* were more salt tolerant than 'Ghazvini' according to relevant claims in the literature (Karimi and Roosta, 2014). The results showed that concentrations of sodium (Na), chloride (Cl), magnesium (Mg), calcium (Ca), copper (Cu) and iron (Fe) in pistachio shoots increased in response to 120 mM salinity (Mohit Rabari et al., 2023).

Earlier research on 'Arota' inspired suggestions to improve the measurement of salinity tolerance in the 16 promising hybrids by measuring several physiological parameters to scale the tolerance of each hybrid and compare their tolerance to that of UCB1. Thus, the current research aimed to evaluate the salinity tolerance of the 16 promising hybrids by measuring the values of sodium, potassium, and chorine in the roots and leaves of the hybrids. It was hypothesized that the hybrids can perform better than the UCB1 when measured for physiological traits. While the importance of pistachio rootstocks cannot be overstated, especially in the context of escalating salinity stress driven by climate change, the strategic selection and development of rootstocks that combine salinity tolerance with hybrid vigor are essential for sustaining high yields and ensuring the future success of pistachio cultivation.

Material and methods

Sample collection and preparation

To obtain controlled crosses, using 37 female *P. atlantica* and six male *P. integerrima* trees, 37 female trees were pollinated with the pollen of six male trees. On each female tree, three separate branches were used for each cross, resulting in a total of 18 branches on each female tree and 666 pollinations for 222 crosses. Although the selected parents producing Arota are both heterozygous, a marked uniformity was displayed among the resulting hybrid seedlings. The names of the companies that made the hybridizations and evaluated the F_1 hybrids, Arezobahar (ARO) and Tat (TA), were combined to form the name Arota. The average germination percentage for UCB1 was 75.5% vs. 91.6% for Arota seeds. From the 222 crosses, sixteen promising hybrids were selected based on morphological characteristics (Akbari et al., 2020a). These hybrids were named as AI6, AI7, AI2, AI12, AI10, AI8, AI11, AI13, AI3, AI4, AI14, AI5, AI16, AI1, AI9, and AI15. Each hybrid was propagated by seed to achieve 3 replicates per hybrid. The salinity treatment had three levels, 0.5, 8 and 16 dS m^{-1}, which was applied to the plants for 4 months. The leaf and root samples of each hybrid were sent to the laboratory in three replicates to analyze the amount of chlorine, sodium and potassium elements in the roots and leaves. After collecting leaf and root samples from the sixteen hybrids, the collected samples were washed with deionized water to remove any surface contaminants, and then dried at 70 °C in a forced-air oven for 48 h to achieve a constant weight.

Determination of sodium (Na^+) content

Dried leaf and root samples were ground to a fine powder using a mortar and pestle. Approximately 0.5 g of each sample was weighed and placed into a digestion tube. A digestion method using concentrated nitric acid (HNO_3) was employed. Samples were first digested at 120 °C for 1 h, followed by an additional digestion with hydrogen peroxide (H_2O_2) at 160 °C until the solution became clear. The digested material was then filtered and diluted to a final volume of 50 mL with deionized water. Sodium content was quantified using a flame photometer (Sherwood 420, Sherwood Scientific Ltd, Cambridge, UK). Calibration standards ranging from 0 to 100 mg L^{-1} Na^+ were prepared from a sodium chloride (NaCl) stock solution, and sample concentrations were determined against this calibration curve. Sodium content was expressed as mg kg^{-1} of dry weight.

Determination of potassium (K^+) content

Potassium content was determined from the same digested extracts used for sodium analysis. The potassium concentration was measured using the same Flame Photometer (Sherwood 420). Calibration standards for potassium ranged from 0 to 200 mg L^{-1} K^+, prepared from a potassium chloride (KCl) stock solution. Potassium content was expressed as mg kg^{-1} of dry weight.

Determination of chlorine (Cl^-) content

Chlorine content in the pistachio leaves and roots was determined using the argentometric titration method. Approximately 0.5 g of dried and ground sample was extracted with 50 mL of deionized water by shaking at room temperature for 2 h. The extract was filtered, and the chlorine content was determined by titrating with a standard silver nitrate ($AgNO_3$) solution (0.01 N), using potassium chromate (K_2CrO_4) as an indicator. The endpoint was identified by the appearance of a persistent reddish-brown color.

The chlorine content was calculated using the formula, and the results were expressed as mg kg^{-1} of dry weight.

$$Cl^{-}\ content\ (mg\ kg^{-1}) = \frac{(Volume\ of\ AgNO_3 \times Normality\ of\ AgNO_3 \times 35.45 \times 1000)}{Sample\ weight\ (g)}$$

In this research, two-year-old seedlings, as the progeny of 16 superior interspecies hybrids (*P. integerrima* as paternal parent and *P. atlantica* as the maternal parent), which were superior in terms of growth among 96 hybrids, were subjected to salt stress. To investigate the reaction of pistachio rootstocks to the salinity stress caused by NaCl, salinity treatments were performed at 3 levels with greenhouse water salinity as the control (with EC 0.5 dS m^{-1}), EC 8 dS m^{-1}, and EC 16 dS m^{-1} in 3 repetitions as a factorial experiment in a completely random basic design. For each treatment, 3 seedlings were evaluated as 3 replications. Irrigation treatments were applied every other day with a constant amount for all seedlings. The second factor of treatments were hybrid rootstocks.

At the beginning and before applying the salinity treatment, the diameter and height indices of the seedlings were measured. The diameter of the seedlings was evaluated from a height of 5 cm above ground. Growth indices of seedling diameter and height were measured monthly. The salinity treatment with three control salinity levels, 8 dS m^{-1}, and 16 dS m^{-1} continued for 4 months. The leaf and root samples of each hybrid were analyzed in three replicates to determine the amount of chlorine, sodium and potassium elements in the roots and leaves.

Data analysis

All measurements were performed in triplicate for each sample type (leaves and roots) to ensure accuracy and reproducibility. The data were analyzed using statistical software (e.g., SPSS, Version 25.0), and the mean values along with standard deviations were calculated. Statistical significance was determined using one-way analysis of variance (ANOVA) ($P<0.05$).

Results

Leaf sodium content

The leaf sodium content varied among the hybrids, with the highest content observed in 'AI15' (1.7988 mg kg^{-1}) and the lowest in 'AI6' (0.5834 mg kg^{-1}). The majority of the hybrids exhibited moderate leaf sodium levels, with values mostly ranging between 0.7 and 1.2 mg kg^{-1}. 'AI6' (0.5834 mg kg^{-1}) had the lowest sodium content, significantly different from all other hybrids ($P\leq0.05$). 'AI7' (0.7720 mg kg^{-1}), 'AI2' (0.7761 mg kg^{-1}), 'AI12' (0.7822 mg kg^{-1}), and UCB1 (0.8128 mg kg^{-1}) were grouped together, showing no significant differences among them but were significantly different from 'AI6' and hybrids with higher sodium content. 'AI10' (0.8738 mg kg^{-1}), 'AI8' (0.8983 mg kg^{-1}), 'AI11' (0.9640 mg kg^{-1}), 'AI13' (0.9738 mg kg^{-1}), 'AI3' (0.9826 mg kg^{-1}), 'AI4' (0.9894 mg kg^{-1}), 'AI16' (1.0065 mg kg^{-1}), 'AI5' (1.0940 mg kg^{-1}), 'AI14' (1.1474 mg kg^{-1}), and 'AI1' (1.1599 mg kg^{-1}) all showed significant differences from each other. 'AI9' (1.2584 mg kg^{-1}) and 'AI15' (1.7988 mg kg^{-1}) had the highest sodium content, with significant differences from almost all other hybrids ($P\leq0.05$) (Table 1).

Leaf potassium content

Leaf potassium content was highest in 'AI13' (1.4507 mg kg^{-1}) and lowest in 'AI6' (0.9240 mg kg^{-1}). Other hybrids such as 'AI8' (1.3169 mg kg^{-1}) and 'AI2' (1.2642 mg kg^{-1}) also had relatively high potassium levels, while many others clustered around 1.0 to 1.2 mg kg^{-1}. 'AI6' (0.9240 mg kg^{-1}), 'AI12' (0.8885 mg kg^{-1}), 'UCB1' (0.9653 mg kg^{-1}), and 'AI5' (0.9576 mg kg^{-1}) were grouped together, showing no significant differences among them ($P\leq0.05$). 'AI7' (1.1429 mg kg^{-1}), 'AI3' (1.1636 mg kg^{-1}), 'AI11' (1.1863 mg kg^{-1}), 'AI16' (1.1438 mg kg^{-1}), and 'AI14' (1.1827 mg kg^{-1}) formed another group with similar potassium levels, significantly different from those in the previous group. 'AI2' (1.2642 mg kg^{-1}), 'AI1' (1.2711 mg kg^{-1}), and 'AI9' (1.2056 mg kg^{-1}) showed no significant differences among themselves but were different from others with lower or higher levels. 'AI13' (1.4507 mg kg^{-1}) had the highest potassium content, significantly different from all other hybrids ($P\leq0.05$) (Table 1).

Leaf chlorine content

The leaf chlorine content showed a wide range, from 'AI12' (1.0658 mg kg^{-1}) to 'AI16' (2.0169 mg kg^{-1}). Hybrids such as 'UCB1' (1.0591 mg kg^{-1}) and 'AI12' (1.0658 mg kg^{-1}) had the lowest chlorine levels, while 'AI4' (1.8032 mg kg^{-1}) and 'AI11' (1.8075 mg kg^{-1}) had significantly higher levels. 'AI12' (1.0658 mg kg^{-1}) and UCB1 (1.0591 mg kg^{-1}) had the lowest chlorine content, with no significant differences between them ($P\leq0.05$). 'AI6' (1.3272 mg kg^{-1}) and 'AI10' (1.2687 mg kg^{-1}) were similar but significantly different from the group with the lowest content. 'AI7' (1.4237 mg kg^{-1}), 'AI2' (1.5689 mg kg^{-1}), 'AI8' (1.5667 mg kg^{-1}), 'AI13' (1.5958 mg kg^{-1}), 'AI3' (1.5714 mg kg^{-1}), and 'AI1' (1.5556 mg kg^{-1}) formed a group with no significant differences among themselves but were significantly different from those with lower and higher chlorine levels. 'AI11' (1.8075 mg kg^{-1}), 'AI4' (1.8032 mg kg^{-1}), 'AI5' (1.7566 mg kg^{-1}), 'AI9' (1.8732 mg kg^{-1}), 'AI14' (1.9971 mg kg^{-1}), and 'AI16' (2.0169 mg kg^{-1}) represented the highest chlorine content levels, with significant differences from most other hybrids ($P\leq0.05$) (Table 1).

Table 1. Duncan's multiple range test indicating 17 pistachio rootstock varieties, including UCB1 and 16 hybrid varieties, their average values of leaf sodium content (mg kg^{-1}), leaf potassium content (mg kg^{-1}), leaf chlorine content (mg kg^{-1}), root sodium content (mg kg^{-1}), root potassium content (mg kg^{-1}), root chlorine content (mg kg^{-1}), root K/Na ratio, leaf K/Na ratio, ST ratio, and stem diameter (cm). Data represent mean values of three salinity levels (EC1: 0.5 dS m^{-1}, EC2: 8 dS m^{-1}, and EC3: 16 dS m^{-1}). Hybrid varieties are named as AI6, AI7, AI2, AI12, UCB1, AI10, AI8, AI11, AI13, AI3, AI4, AI14, AI5, AI16, AI1, AI9, and AI15.

Variety	Leaf sodium content	Leaf potassium content	Leaf chlorine content	Root sodium content	Root potassium content	Root chlorine content	Root K/Na ratio	Leaf K/Na ratio	ST ratio	Stem diameter (cm)
AI6	0.5834^{a}	0.9240^{a}	1.3272^{b}	1.0862^{cd}	0.8464^{b}	1.6890^{h}	0.9192^{g}	1.4812^{h}	4.4070^{f}	2.6850^{cd}
AI7	0.7720^{b}	1.1429^{b}	1.4237^{bc}	1.2373^{f}	0.8950^{cd}	1.2179^{a}	0.7687^{de}	1.6113^{i}	5.4014^{g}	1.7750^{ab}
AI2	0.7761^{b}	1.2642^{bc}	1.5689^{cd}	1.0247^{b}	0.8442^{b}	1.3065^{b}	0.8948^{g}	1.6815^{i}	4.3831^{f}	2.5658^{bcd}
AI12	0.7822^{b}	0.8885^{a}	1.0658^{a}	1.3303^{h}	0.9439^{def}	1.7002^{h}	0.7205^{cd}	1.1470^{de}	2.6853^{de}	1.6408^{a}
UCB1	0.8128^{b}	0.9653^{a}	1.0591^{a}	1.6324^{n}	1.1731^{j}	1.9110^{j}	0.7254^{cd}	1.1586^{e}	2.6326^{cde}	1.7917^{ab}
AI10	0.8738^{bc}	0.9012^{a}	1.2687^{b}	1.5075^{l}	0.9316^{def}	1.9152^{j}	0.6499^{ab}	1.0442^{c}	2.7921^{de}	2.3158^{abcd}
AI8	0.8983^{bcd}	1.3169^{c}	1.5667^{cd}	1.1032^{d}	0.9720^{fghi}	1.3195^{b}	0.9236^{g}	1.5245^{h}	3.3790^{e}	1.9883^{abcd}
AI11	0.9640^{cd}	1.1863^{b}	1.8075^{e}	1.0446^{b}	0.9470^{efg}	1.5461^{f}	1.1856^{i}	1.2519^{f}	2.7360^{de}	2.6808^{cd}
AI13	0.9738^{cde}	1.4507^{d}	1.5958^{d}	1.2807^{g}	0.9617^{efghi}	1.3773^{c}	0.7510^{d}	1.6763^{i}	7.5611^{h}	2.0617^{abcd}
AI3	0.9826^{cde}	1.1636^{b}	1.5714^{cd}	1.4093^{j}	1.0009^{hi}	1.4205^{d}	0.7200^{cd}	1.3930^{g}	5.0684^{fg}	2.0267^{abcd}
AI4	0.9894^{cde}	1.1964^{bc}	1.8032^{e}	1.5523^{m}	0.9178^{de}	1.5933^{g}	0.6130^{ab}	1.2404^{f}	4.6427^{fg}	2.8292^{d}
AI16	1.0065^{de}	1.1438^{b}	2.0169^{f}	0.9769^{a}	0.9337^{def}	1.5108^{ef}	1.0470^{h}	1.3575^{g}	2.1095^{bcd}	1.9717^{abc}
AI5	1.0940^{ef}	0.9576^{a}	1.7566^{e}	1.1587^{e}	0.9960^{ghi}	1.4860^{e}	0.8796^{fg}	0.8938^{b}	1.0358^{a}	2.7158^{cd}
AI14	1.1474^{fg}	1.1827^{b}	1.9971^{f}	1.1957^{e}	1.0105^{i}	1.4956^{e}	0.9036^{g}	1.1128^{cde}	1.6451^{ab}	2.5450^{bcd}
AI1	1.1599^{fg}	1.2711^{bc}	1.5556^{cd}	1.0630^{bc}	$.8600^{bc}$	1.2981^{b}	0.8295^{ef}	1.1138^{cde}	1.8844^{bc}	2.6158^{bcd}
AI9	1.2584^{g}	1.2056^{bc}	1.8732^{ef}	1.4529^{k}	0.9568^{efgh}	1.8139^{i}	0.6681^{bc}	1.0626^{cd}	2.7853^{de}	2.3842^{abcd}
AI15	1.7988^{h}	1.2003^{bc}	1.7106^{de}	1.3699^{i}	0.7940^{a}	1.5164^{ef}	0.6014^{a}	0.6838^{a}	1.4359^{ab}	2.5050^{bcd}

Duncan's multiple range test shows significant differences in each column ($P \leq 0.05$). Similar letters in each column indicate insignificant differences ($P \leq 0.05$).

Root sodium content

Root sodium content varied significantly, with the highest in 'UCB1' (1.6324 mg kg^{-1}) and the lowest in 'AI16' (0.9769 mg kg^{-1}). Hybrids such as 'AI9' (1.4529 mg kg^{-1}) and 'AI4' (1.5523 mg kg^{-1}) also exhibited high sodium content, whereas others such as 'AI2' (1.0247 mg kg^{-1}) had lower levels. 'AI16' (0.9769 mg kg^{-1}) had the lowest sodium content, significantly different from all other hybrids ($P \leq 0.05$). 'AI2' (1.0247 mg kg^{-1}), 'AI11' (1.0446 mg kg^{-1}), 'AI1' (1.0630 mg kg^{-1}), and 'AI8' (1.1032 mg kg^{-1}) formed a group with no significant differences among them. 'AI7' (1.2373 mg kg^{-1}), 'AI14' (1.1957 mg kg^{-1}), and 'AI5' (1.1587 mg kg^{-1}) showed significant differences compared to other groups. 'AI12' (1.3303 mg kg^{-1}), 'AI15' (1.3699 mg kg^{-1}), 'AI3' (1.4093 mg kg^{-1}), 'AI9' (1.4529 mg kg^{-1}), 'AI10' (1.5075 mg kg^{-1}), 'AI4' (1.5523 mg kg^{-1}), and 'UCB1' (1.6324 mg kg^{-1}) had the highest sodium content, with significant differences from most other hybrids ($P \leq 0.05$) (Table 1).

Root potassium content

Root potassium content was highest in 'UCB1' (1.1731 mg kg^{-1}) and lowest in 'AI15' (0.7940 mg kg^{-1}). The majority of the hybrids had potassium levels ranging between 0.8 and 1.0 mg kg^{-1}, with 'AI5' (0.9960 mg kg^{-1}) and 'AI14' (1.0105 mg kg^{-1}) being notable exceptions with slightly higher levels. 'AI15' (0.7940 mg kg^{-1}) had the lowest potassium content, significantly different from all other hybrids ($P \leq 0.05$). 'AI6' (0.8464 mg kg^{-1}), 'AI2' (0.8442 mg kg^{-1}), and 'AI1' (0.8600 mg kg^{-1}) formed a group with no significant differences among themselves. 'AI7' (0.8950 mg kg^{-1}), 'AI4' (0.9178 mg kg^{-1}), 'AI16' (0.9337 mg kg^{-1}), 'AI10' (0.9316 mg kg^{-1}), 'AI12' (0.9439 mg kg^{-1}), and 'AI11' (0.9470 mg kg^{-1}) had moderate potassium levels with no significant differences among several values. 'AI5' (0.9960 mg kg^{-1}), 'AI3' (1.0009 mg kg^{-1}), 'AI14' (1.0105 mg kg^{-1}), and UCB1 (1.1731 mg kg^{-1}) had the highest potassium content, with significant differences from most other hybrids ($P \leq 0.05$) (Table 1).

Root chlorine content

The root chlorine content ranged from 1.2179 mg kg^{-1} in 'AI7' to 1.9152 mg kg^{-1} in 'AI10'. The lowest chlorine content was observed in 'AI7' (1.2179 mg kg^{-1}), while the highest was in 'AI10' (1.9152 mg kg^{-1}). Several other hybrids, such as 'AI12' (1.7002 mg kg^{-1}) and 'UCB1' (1.9110 mg kg^{-1}), also had high chlorine levels. 'AI7' (1.2179 mg kg^{-1}) and 'AI8' (1.3195 mg kg^{-1}) had the lowest root chlorine content, significantly different from most other hybrids. 'AI2' (1.3065 mg kg^{-1}) and 'AI1' (1.2981 mg kg^{-1}) showed no significant differences from each other but differ from those with lower or higher content ($P \leq 0.05$). 'AI13' (1.3773 mg kg^{-1}), 'AI3' (1.4205 mg kg^{-1}), 'AI16' (1.5108 mg kg^{-1}), 'AI15' (1.5164 mg kg^{-1}), 'AI14' (1.4956 mg kg^{-1}), 'AI5' (1.4860 mg kg^{-1}), and 'AI11' (1.5461 mg kg^{-1}) were grouped with moderate chlorine levels but showed significant differences from those with the lowest and highest levels. 'AI12' (1.7002 mg kg^{-1}), 'AI10' (1.9152 mg kg^{-1}), and 'UCB1' (1.9110 mg kg^{-1}) exhibited the highest root chlorine content, with significant differences from most other hybrids ($P \leq 0.05$) (Table 1).

Root K/Na ratio

The root K/Na ratio exhibited significant variability across the studied pistachio rootstock varieties. The highest root K/Na ratio was observed in the variety AI16 (1.0470), followed closely by AI11 (1.1856). On the other hand, the lowest ratio was noted in the AI15 rootstock (0.6014). UCB1, a widely used commercial variety, displayed a moderate value (0.7254). Notably, AI1 also achieved a relatively higher ratio of 0.8295. These differences highlight variability in the ability of the rootstocks to maintain potassium over sodium in root tissues, which may have implications for salinity tolerance and nutrient uptake efficiency (Table 1).

Leaf K/Na ratio

Among the leaf K/Na ratios, AI15 exhibited the lowest leaf K/Na ratio at 0.6838, suggesting a relatively higher sodium accumulation or lower potassium retention in stems for this variety. In contrast, AI13 and AI2 recorded the highest ratios of 1.6763 and 1.6815, respectively. UCB1 maintained a moderately low value of 1.1586, indicating its average performance under salinity stress. The significant variation across varieties, from 0.6838 in AI15 to 1.6815 in AI2, reflects the differential capacity of the rootstocks to modulate ion balance in the stems (Table 1).

Stem diameter

Stem diameter varied across the hybrids, with 'AI4' having the largest diameter (2.8292 cm) and 'AI12' the smallest (1.6408 cm). Other hybrids with large stem diameters included 'AI5' (2.7158 cm) and 'AI11' (2.6808 cm). Hybrids such as 'AI12' and 'AI7' (1.7750 cm) had relatively small diameters. 'AI12' (1.6408 cm) had the smallest stem diameter, significantly different from all other hybrids ($P \leq 0.05$). 'AI7' (1.7750 cm), UCB1 (1.7917 cm), and 'AI16' (1.9717 cm) showed no significant differences among themselves but were significantly different from those with smaller or larger diameters. 'AI8' (1.9883 cm), 'AI14' (2.5450 cm), 'AI13' (2.0617 cm), 'AI3' (2.0267 cm), 'AI9' (2.3842 cm), 'AI15' (2.5050 cm), 'AI10' (2.3158 cm), 'AI11' (2.6808 cm), and 'AI5' (2.7158 cm) were grouped with larger diameters but showed significant differences from each other. 'AI4' (2.8292 cm) had the largest stem diameter, significantly different from almost all other hybrids ($P \leq 0.05$) (Table 1).

Physiological responses to EC values

The effects of three different salinity treatments, i.e., 0.5 ds m^{-1} (EC1), 8 ds m^{-1} (EC8), and 16 ds m^{-1} (EC16), were evaluated on various physiological traits of 17 pistachio varieties (Table 2). The analysis highlighted the changes in these traits due to the different salinity levels and the significant differences among them ($P \leq 0.05$).

Leaf sodium content

The lowest leaf sodium content was observed at the lowest salinity level (EC1: 0.7301), indicating minimal sodium uptake under these conditions. As salinity increased to EC8 (1.0867 mg kg^{-1}), there was a significant rise in leaf sodium content, reflecting that moderate salinity stress considerably enhanced sodium accumulation. The highest leaf sodium content (1.1608 mg kg^{-1}) was recorded at the highest salinity level (EC16), showing a strong response to elevated salinity stress, significantly differing from both EC1 and EC8 ($P \leq 0.05$) (Table 2).

Leaf potassium content

Leaf potassium content was at its lowest under the EC1 treatment (1.0673 mg kg^{-1}), suggesting that lower salinity may have led to reduced potassium uptake or retention in the leaves. A slight, statistically insignificant increase in leaf potassium content was observed under EC8 (1.0845 mg kg^{-1}), indicating that moderate salinity did not significantly affect potassium levels. However, at the highest salinity level (EC16), there was a notable increase in leaf potassium content that reached the maximum value (1.2649 mg kg^{-1}), which may suggest a compensatory mechanism to counteract sodium toxicity ($P \leq 0.05$) (Table 2).

Leaf chlorine content

The lowest leaf chlorine content was observed under the EC1 treatment (1.2161 mg kg^{-1}), reflecting minimal chlorine accumulation under these conditions. There was a significant increase in leaf chlorine content under EC8, reaching 1.7685 mg kg^{-1}, indicating that moderate salinity led to higher chlorine accumulation. The chlorine content remained high and similar under EC16 (1.7745 mg kg^{-1}), suggesting that chlorine levels plateaued under high salinity stress ($P \leq 0.05$) (Table 2).

Root sodium content

Root sodium content was lowest under the EC1 treatment (0.9675 mg kg^{-1}), indicating minimal sodium uptake by the roots in low salinity conditions. A significant increase in root sodium content was observed under EC8 (1.3003 mg kg^{-1}), showing that moderate salinity stress led to greater sodium accumulation. The highest root sodium content was recorded under EC16 (1.5132 mg kg^{-1}), highlighting the strong impact of high salinity on root sodium accumulation ($P \leq 0.05$) (Table 2).

Root potassium content

Root potassium content reached its highest level under the EC1 treatment (0.9368 mg kg^{-1}), suggesting that lower salinity favored potassium retention in the roots. There was no significant change in root potassium content between EC1 and EC8 (0.9355 mg kg^{-1}), indicating that moderate salinity did not strongly affect root potassium levels. A slight increase in root potassium content was observed under EC16 (0.9486 mg kg^{-1}), though this change was not statistically significant, suggesting stable potassium levels across the different salinity treatments ($P \leq 0.05$) (Table 2).

Root chlorine content

The lowest root chlorine content was recorded under the EC1 treatment (1.1868 mg kg^{-1}), indicating minimal chlorine accumulation in low salinity conditions. A significant increase in root chlorine content was noted under EC8 (1.5755 mg kg^{-1}), reflecting higher chlorine accumulation as salinity increased. The highest root chlorine content was observed under EC16 (1.8467 mg kg^{-1}), highlighting a strong response to high salinity stress, with levels significantly differing from EC1 and EC8 ($P \leq 0.05$) (Table 2).

Table 2. Water salinity treatments of 0.5 ds m^{-1} (EC1), 8 ds m^{-1} (EC8), and 16 ds m^{-1} (EC16). Data represent mean values of all 17 varieties in response to the salinity treatments and their effects on leaf sodium content (mg kg^{-1}), leaf potassium content (mg kg^{-1}), leaf chlorine content (mg kg^{-1}), root sodium content (mg kg^{-1}), root potassium content (mg kg^{-1}), root chlorine content (mg kg^{-1}), root K/Na ratio, leaf K/Na ratio, ST ratio, and stem diameter (cm).

EC value	Leaf sodium content	Leaf potassium content	Leaf chlorine content	Root sodium content	Root potassium content	Root chlorine content	Root K/Na ratio	Leaf K/Na ratio	ST ratio	Stem diameter (cm)
EC1	0.7301^{a}	1.0673^{a}	1.2161^{a}	0.9675^{a}	0.9368^{a}	1.1868^{a}	1.0587^{c}	1.5774^{c}	3.9194^{c}	2.5272^{a}
EC8	1.0867^{b}	1.0845^{a}	1.7685^{b}	1.3003^{b}	0.9355^{a}	1.5755^{b}	0.7412^{b}	1.2303^{b}	3.3300^{b}	2.3156^{ab}
EC16	1.1608^{c}	1.2649^{b}	1.7745^{b}	1.5132^{c}	0.9486^{a}	1.8467^{c}	0.6356^{a}	0.9748^{a}	2.7361^{a}	2.0577^{b}

Duncan’s multiple range test shows significant differences in each column ($P \leq 0.05$). Similar letters in each column indicate insignificant differences ($P \leq 0.05$).

ST ratio

The ST ratio is defined as the ratio of potassium to sodium in the shoots to the ratio of potassium to sodium in the roots, which may represent a combination metric related to sodium and potassium accumulation across tissues. This parameter varied widely among the hybrids. AI13 showed the highest ST ratio (7.5611), indicating potentially elevated potassium content in stem tissues compared to others. UCB1 displayed a moderate ST ratio of 2.6326. This variability in ST ratio among rootstocks provides further insights into their differential physiological responses to salinity stress.

Stem diameter

The largest stem diameter was observed under the EC1 treatment (2.5272 cm), suggesting optimal growth conditions with low salinity. Under EC8 (2.3156 cm), stem diameter slightly decreased, though the change was not statistically significant, indicating that moderate salinity had a minor impact on stem growth. The smallest stem diameter was recorded under EC16 (2.0577 cm), showing that high salinity stress negatively affected stem growth, with a significant difference from EC1 ($P \leq 0.05$) (Table 2). Figure 1 is a depiction of seedlings from d 94 of the salinity experiment, which shows the leafless hybrid UCB1 (left) and the AI12 hybrid (right) in response to salinity stress. On the 120th d, the UCB1 hybrid had no leaves and the number of leaves in the AI12 hybrid was very few (Fig. 1).

EC × hybrid interaction effects

The interaction effects between EC and hybrid variety were statistically significant for all dependent variables except diameter ($p = 0.409$), suggesting that the impact of EC is influential on most of the traits depending on the hybrid variety. Regarding sodium, potassium, chloride concentrations, and potassium: sodium ratios in leaves and roots, as well as the ST variable, the interaction effects were highly significant ($p = 0.000$). This indicates that the response of each variety to EC varies significantly across these physiological factors. Leaf Na, K, and Cl had F-values of 19.838, 17.455, and 31.601, respectively, suggesting a substantial influence of EC-variety interactions on leaf ion content. Root Na, K, and Cl exhibited similarly strong interactions, with F-values of 56.460, 13.799, and 37.034, respectively, indicating that EC influenced root ion content variedly across the varieties. Root K/Na ratio showed the highest F-value (40.426), suggesting that EC-variety interactions significantly affected ion balance in roots (Table 3).

Fig. 1. AI12 hybrid (right) and UCB1 (left) without leaves.

Table 3. ANOVA results for the interaction between salinity treatment, expressed as electrical conductivity (EC), and plant variety across each dependent variable. Each interaction effect was analyzed for significance at the P<0.05 level.

Dependent Variable	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Diameter	EC * Variety	25.303	32	0.791	1.048	0.409
Leaf Na	EC * Variety	9.285	32	0.290	19.838	0.000
Leaf K	EC * Variety	7.632	32	0.238	17.455	0.000
Leaf Cl	EC * Variety	25.800	32	0.806	31.601	0.000
Leaf K/Na	EC * Variety	5.251	32	0.164	23.787	0.000
Root Na	EC * Variety	2.857	32	0.089	56.460	0.000
Root K	EC * Variety	.984	32	0.031	13.799	0.000
Root Cl	EC * Variety	1.993	32	0.062	37.034	0.000
Root K/Na	EC * Variety	39.573	32	1.237	40.426	0.000
ST	EC * Variety	611.823	32	19.119	31.727	0.000

Figure 2 illustrate the interaction effects between EC (electrical conductivity) treatments and four pistachio hybrids (‘AI1’, ‘AI2’, ‘AI4’, and ‘AI13’) concerning the potassium-to-sodium (K/Na) ratio in the leaf tissues. Statistical analysis using Duncan’s test (at P ≤ 0.05) reveals significant differences in K/Na ratios among the hybrids under varying EC treatments. The highest interaction effects were observed in hybrids AI13 (7.983) and AI2 (5.230) with EC1. In Figure 3, the interaction effects are displayed for the K/Na ratio in root tissues. Using Duncan’s test (P≤0.05), significant differences in the root K/Na ratios among hybrids under various EC treatments showed the highest interaction in AI2 (1.227) and AI1 (0.970) with the EC1 treatment and AI2 (0.987) with the EC2 treatment. Figure 4 displays the interaction effects regarding ST values (salt tolerance). The highest ST value (15.840) was observed in AI13 with EC1. Also, with EC2, the highest interaction for ST value was observed in AI4 (4.647) and AI13 (4.613). With EC3, the highest interactions for ST values was observed in AI4 (6.080) and AI2 (5.220).

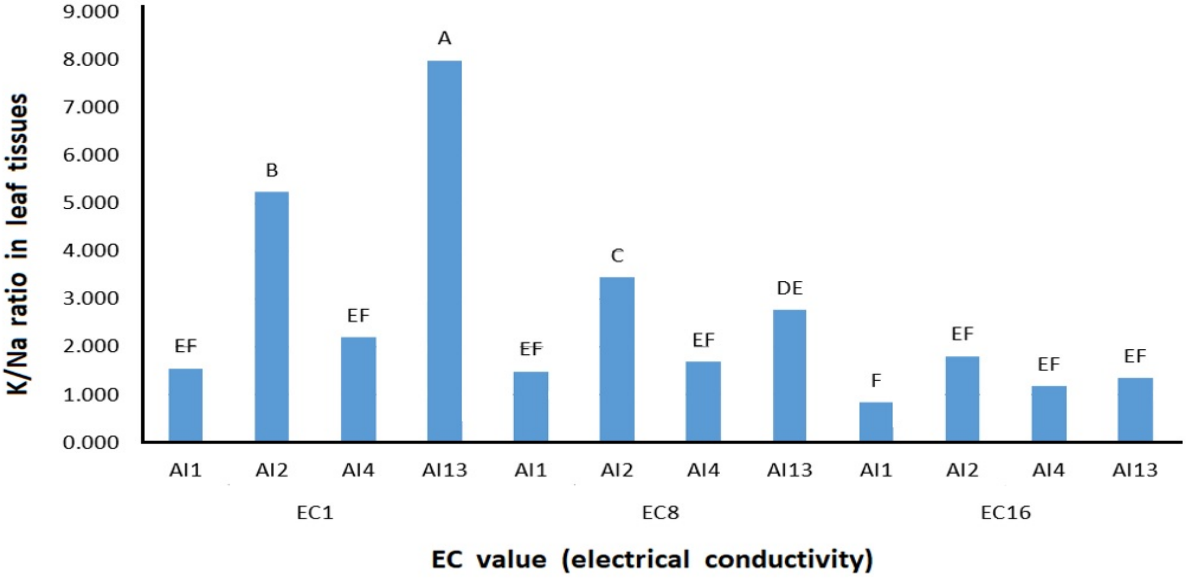

Fig. 2. Interaction effects of salinity treatments based on electric conductivity (EC1: 0.5 dS m^{-1}, EC2: 8 dS m^{-1}, and EC3: 16 dS m^{-1}) and four superior pistachio hybrids (‘AI1’, ‘AI2’, ‘AI4’, and ‘AI13’) in terms of K/Na in leaf tissues of the four superior hybrids. Significant differences are based on Duncan’s test (P≤0.05). Different letters on each column indicate significant differences.

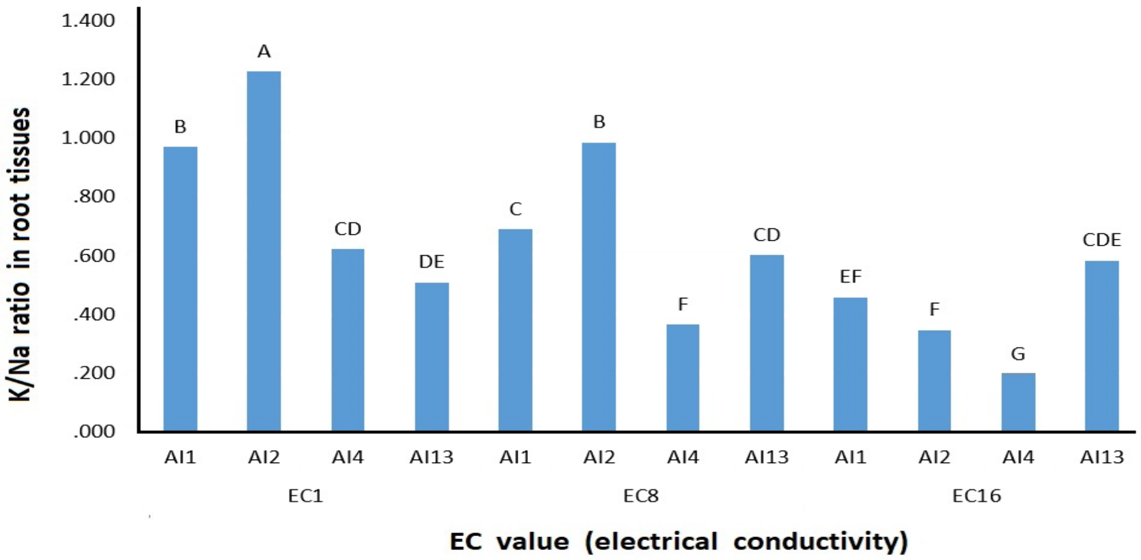

Fig. 3. Interaction effects of salinity treatments based on electric conductivity (EC1: 0.5 dS m^{-1}, EC2: 8 dS m^{-1}, and EC3: 16 dS m^{-1}) and four superior pistachio hybrids ('AI1', 'AI2', 'AI4', and 'AI13') in terms of K/Na in root tissues of the four superior hybrids. Significant differences are based on Duncan's test (P≤0.05). Different letters on each column indicate significant differences.

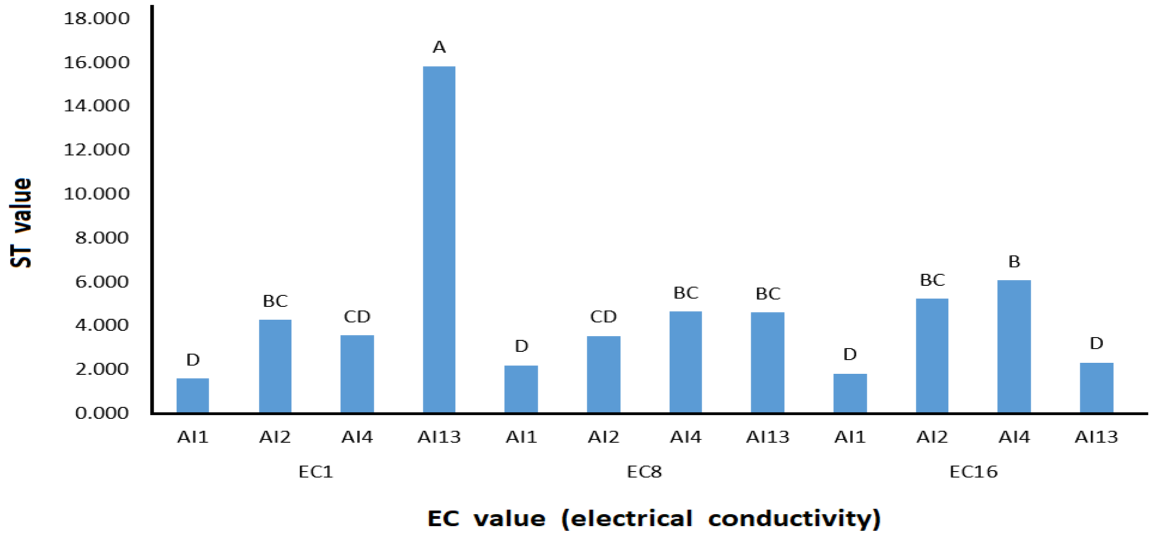

Fig. 4. Interaction effects of salinity treatments based on electric conductivity (EC1: 0.5 dS m^{-1}, EC2: 8 dS m^{-1}, and EC3: 16 dS m^{-1}) and four superior pistachio hybrids ('AI1', 'AI2', 'AI4', and 'AI13') in terms of ST values of the four superior hybrids. The ST value is defined as the ratio of potassium to sodium in the shoots to the ratio of potassium to sodium in the roots. Significant differences are based on Duncan's test (P≤0.05). Different letters on each column indicate significant differences.

Discussion

Salinity stress is a significant environmental factor affecting plant physiology, particularly in terms of ion homeostasis, water relations, and nutrient balance. The results presented in this study indicated how the various hybrids responded to salinity treatments, particularly focusing on the accumulation of sodium (Na^+), potassium (K^+), and chlorine (Cl^-) in the leaves and roots, as well as variations in stem diameter. The impact of salinity treatments on various physiological traits in plants is crucial for understanding how different salinity levels influence plant growth, development, and survival (Mohammed and Salih, 2024). The results from the current experiment on pistachio hybrids under three salinity treatments, i.e., EC1 (0.5 dS m^{-1}), EC8 (8 dS m^{-1}), and EC16 (16 dS m^{-1}), demonstrated significant variations in the sodium, potassium, and chlorine contents of both leaves and roots, as well as changes in stem diameter, which are vital indicators of plant health under stress. It is essential to acknowledge the physiological mechanisms that plants employ to manage salinity stress, as these mechanisms significantly influence genotype selection and evaluation.

Tolerant genotypes may adopt diverse strategies to cope with salinity, including sodium and chlorine excretion at the root surface or sequestration within specific tissues. For instance, the ability to store excess sodium and chlorine in root or vascular parenchyma tissues prevents their translocation to aerial parts, thus mitigating potential toxic effects on photosynthetic machinery. Another mechanism involves compartmentalization within leaf vacuoles or the apoplast, effectively reducing cytoplasmic ion concentrations and maintaining cellular homeostasis. In such cases, although leaf sodium levels may appear elevated, visible toxicity symptoms like necrosis or chlorosis may be absent due to efficient ion compartmentalization (Helalia et al., 2021).

The implications of these physiological strategies must be carefully considered when evaluating genotypes for hybridization. A high leaf sodium concentration alone is not indicative of susceptibility unless corroborated by signs of toxicity or impaired physiological functions. The lack of such nuanced analysis in the current dataset undermines the validity of the proposed hybrids' superiority. Comprehensive studies assessing ion dynamics, tissue-specific ion localization, and tolerance mechanisms are essential to provide a robust foundation for introducing superior hybrids (Vahdati, 2021).

Salinity stress often leads to the excessive accumulation of sodium ions (Na^+) in plant tissues, which can be detrimental to cellular processes. Sodium competes with potassium, an essential nutrient, disrupting enzymatic activities and protein synthesis (Kamiab, 2020). The significant variation in leaf sodium content among hybrids suggests differential tolerance to salinity. For instance, 'AI15' exhibited the highest leaf sodium content (1.7988), which could indicate a potential inefficiency in sodium exclusion mechanisms or a strategy to compartmentalize sodium in vacuoles to mitigate its toxic effects (Mohit Rabari et al., 2023). In contrast, 'AI6' showed the lowest sodium accumulation (0.5834), reflecting a more efficient exclusion or sequestration mechanism that prevents excessive sodium from reaching the leaves. Similarly, root sodium content varied across hybrids, with 'UCB1' having the highest sodium content (1.6324), indicating a potential role for root tissues in sodium sequestration. Root tissues often serve as the first line of defense against sodium entry, where selective ion transporters and channels modulate sodium uptake and translocation (Fattahi et al., 2021). The variation in sodium content among hybrids points to genetic differences in these regulatory mechanisms, which could be exploited for breeding salt-tolerant crops. Salinity stress is known to disrupt the ionic balance within plant tissues, primarily through the excessive accumulation of sodium ions (Na^+), which can be toxic to plants. The current experiment's findings aligned with the available literature, showing that as salinity levels increased, there was a significant rise in sodium content in both leaves and roots. Specifically, the highest sodium content was observed at the highest salinity level (EC16) for both leaves and roots, indicating that elevated salinity leads to greater sodium uptake and accumulation. This observation is consistent with the findings of Helalia et al. (2021) who noted that sodium accumulation in plant tissues is a common response to saline conditions, which can lead to ionic toxicity, osmotic stress, and ultimately, reduced plant growth and productivity. Osmotic stress and salinity stress are interrelated but distinct phenomena in plant physiology, each with unique mechanisms and effects. Osmotic stress occurs when the water potential outside plant cells decreases, making it difficult for the plant to absorb water. This stress can result from various conditions, such as high salinity, drought, or any factor that alters water balance. The primary cause of osmotic stress is an increased solute concentration in the root environment or dehydration due to reduced water availability. This leads to water moving out of plant cells or failing to move in, causing reduced turgor pressure, decreased leaf expansion, and stunted growth. To cope, plants may activate osmotic adjustment mechanisms, such as accumulating compatible solutes like proline and glycine betaine. Osmotic stress is common in environments where water availability is limited and is not exclusive to saline conditions (Bagheri et al., 2021).

In contrast, salinity stress refers to the adverse effects of high salt concentrations, particularly sodium chloride, in soil or water on plant growth and physiology. Salinity stress has two primary components: osmotic effects and ion toxicity. The osmotic effects arise from salts increasing the soil solution's osmotic potential, indirectly causing osmotic stress by reducing water availability. Ion toxicity results from the excessive absorption of ions like sodium and chloride, disrupting cellular ion balance and enzyme activities. These effects often lead to chlorosis, necrosis, reduced photosynthesis, and nutrient imbalances, which can impair growth and yield. Salinity stress is common in coastal areas, irrigated farmlands, and arid regions with naturally saline soils (Nezami et al., 2021).

Potassium is crucial for maintaining cell turgor, enzyme activation, and photosynthesis. Under salinity stress, the maintenance of high potassium levels in plant tissues is critical for plant survival and growth (Karimi et al., 2020). The results show that 'AI13' had the highest leaf potassium content (1.4507), while 'AI6' had the lowest (0.9240). The ability to maintain higher potassium levels under saline conditions is a key indicator of salinity tolerance, as it suggests the effective functioning of potassium transporters that prevent the loss of this vital nutrient (Khalilpour et al., 2021). In roots, 'UCB1' again showed the highest potassium content (1.1731), whereas 'AI15' had the lowest (0.7940). The ability to retain potassium in root tissues under salinity stress is essential, as it supports osmotic adjustment and helps in maintaining the overall ionic balance within the plant. Hybrids with higher root potassium content might possess more efficient potassium transport systems, such as high-affinity potassium transporters (HKT1), which play a crucial role in minimizing sodium uptake and enhancing potassium retention (Behzadi Rad et al., 2021). Potassium (K^+) plays a critical role in plant physiological processes, including osmoregulation, enzyme activation, and stomatal function. The observed changes in potassium content under different salinity treatments are indicative of the plant's attempt to maintain ionic balance and mitigate the toxic effects of sodium accumulation. The slight, though statistically insignificant, increase in leaf potassium content under moderate salinity (EC8) may suggest an initial compensatory response to counteract sodium toxicity, as noted in the literature (Shabala and Pottosin, 2014). However, the significant increase in leaf potassium content at the highest salinity level (EC16) could imply an adaptive mechanism where the plant enhances potassium uptake to sustain cellular functions despite the high sodium levels. In roots, potassium content remained relatively stable across treatments, which could indicate a robust mechanism for potassium retention in the root system, crucial for maintaining overall plant health under salinity stress (Ouni et al., 2022).

Chlorine (Cl^-) is another ion that accumulates under saline conditions. High chlorine content can be as harmful as sodium, leading to ion toxicity, osmotic stress, and disruption of photosynthesis (Kallsen and Parfitt, 2022). The results indicated a wide range of chlorine content in the leaves, with 'AI16' having the highest level (2.0169) and 'AI12' the lowest (1.0658). The variation in chlorine content among the hybrids suggests differing capacities for chlorine uptake and compartmentalization. Hybrids that accumulated less chlorine, such as 'AI12', may possess mechanisms for selective chloride exclusion or enhanced vacuolar sequestration, which are critical for reducing the toxic effects of salinity. The experiment showed a marked increase in chlorine content in both leaves and roots as salinity levels rose, with the highest chlorine content recorded at EC16. This pattern of chlorine accumulation is consistent with previous work on pistachio seedlings (Bagheri et al., 2021), who highlighted that excessive chlorine uptake under saline conditions can disrupt photosynthesis, impair nutrient uptake, and cause leaf chlorosis. The plateauing of leaf chlorine content between EC8 and EC16 suggests that chlorine accumulation may reach a threshold beyond which further increases in salinity do not significantly enhance uptake, potentially due to the plant's regulatory mechanisms that limit excessive ion accumulation to avoid severe toxicity. In roots, chlorine content was highest in 'AI10' (1.9152) and lowest in 'AI7' (1.2179). The accumulation of chlorine in roots can be indicative of a protective strategy where roots act as a barrier, limiting the translocation of chlorine to aerial parts. Hybrids with lower root chlorine content may exhibit improved selectivity in chloride transport, preventing excessive buildup in the roots and shoots (Fazely-salmani et al., 2020).

The ability of plants to maintain a higher K/Na ratio in roots is a crucial trait for salinity tolerance, as excessive sodium can inhibit potassium uptake, impairing metabolic functions such as enzyme activation and osmoregulation (Eskandari Torbaghan, 2023). In this study, the observed variability in root K/Na ratios among the pistachio varieties suggests differential salinity tolerance. The highest ratio in AI16 and AI11 varieties indicates their superior ability to maintain potassium levels despite sodium stress. This aligns with findings in other woody perennials, where increased potassium retention under salinity contributes to improved growth and stress mitigation (Tavakkoli et al., 2011). Conversely, varieties like AI15 exhibited lower root K/Na ratios, implying that these genotypes are more vulnerable to sodium interference with potassium uptake. The moderate performance of UCB1 (0.7254) highlights its adaptability, though it may not be as salt-tolerant as some hybrids with higher ratios. These results suggest that selecting rootstocks with higher root K/Na ratios, such as AI16, could enhance salinity tolerance by promoting ionic homeostasis at the root level (Vahdati, 2021).

The K/Na ratio in stems reflects the plant's ability to regulate ion transport from roots to shoots, which is essential for maintaining metabolic functions under salinity (Shabala and Cuin, 2008). The high leaf K/Na ratios in AI2 and AI13 suggest efficient exclusion of sodium from translocation tissues or effective potassium uptake, thus ensuring that essential physiological processes in the shoot tissues are not compromised. Elevated potassium in stems helps preserve turgor, facilitates photosynthesis, and supports stomatal regulation, which is critical for plants under salt stress (Shahzad et al., 2012). On the other hand, lower ratios, such as those observed in AI5 (0.8938) and AI15 (0.6838), indicate a tendency for these varieties to accumulate sodium in the stems, which can disrupt cellular processes and reduce plant vitality (Shabala and Cuin, 2008). The moderate ratio for UCB1 (1.1586) aligns with previous studies that report average salt tolerance in this rootstock, suggesting it can manage sodium to a reasonable extent but may not be optimal for highly saline conditions (Tavakkoli et al., 2011).

The ST ratio can be considered a measure of ion homeostasis across tissues, indicating how well plants compartmentalize sodium and maintain potassium distribution. A high ST ratio, such as that in AI13 (7.5611), reflects significant potassium accumulation in stems, whereas if the amount of sodium accumulation was higher would negatively impact plant performance and If excess sodium is not sequestered in vacuoles or effectively excluded from metabolically active areas (Eskandari Torbaghan, 2023). This finding suggests that AI13 may prioritize stem storage of potassium over sodium uptake, potentially increasing its growth potential under salinity. The moderate ST ratio of UCB1 (2.6326) further supports its classification as a generally adaptable rootstock with balanced, though not exceptional, performance under saline conditions. Varieties with lower ST ratios, such as AI1, may not be suited for long-term cultivation in saline soils as they cannot exhibit in ionic regulation (Zeng et al., 2019).

The significant interaction effects observed between EC and hybrid variety on the ion concentrations and ratios underscore the complex influence of salinity and pistachio genetics on nutrient uptake and allocation. The non-significant effect on diameter may indicate that morphological responses to EC are less variable across pistachio varieties, in contrast to ion uptake and balance, which are highly sensitive to both EC and genetic factors. These findings support the importance of considering both EC levels and pistachio rootstock in optimizing nutrient management strategies. The significant interaction effects between EC and variety on most measured plant characteristics show the complex role of both factors in influencing ion concentrations and nutrient balance. These findings provide insights into how salinity management could be tailored to specific varieties, potentially enhancing nutrient profiles and stress tolerance. Hybrids with higher ST values likely possess enhanced physiological or biochemical mechanisms that confer resilience under saline conditions, such as osmotic adjustment or ion compartmentalization. Identifying hybrids with higher ST values is crucial for the selection of salt-tolerant varieties, which could improve crop yield and sustainability in saline environments. The observed interactions may also inform targeted strategies for managing salinity stress in pistachio cultivation.

Salinity stress also impacts plant morphology, with stem diameter serving as a representation of overall plant vigor. The results show a wide range of stem diameters among the hybrids, with 'AI4' having the largest (2.8292 cm) and 'AI12' the smallest (1.6408 cm). The reduction in stem diameter under salinity stress is often attributed to osmotic stress, reduced water uptake, and impaired cell expansion (Sherafati and Torbaghan, 2023). Larger stem diameters in certain hybrids suggest better adaptation to saline conditions, possibly through enhanced osmotic adjustment and water retention mechanisms. The reduction in stem diameter observed under increasing salinity levels reflects the overall negative impact of salinity on plant growth. High salinity levels (EC16) significantly reduced stem diameter, indicating that the plants were under considerable stress, which likely impeded cell expansion and division. This finding is in line with previous research that salinity stress adversely affects vegetative growth by disrupting water uptake, reducing cell turgor, and impairing nutrient transport, ultimately leading to stunted growth (Nezami et al., 2021).

Conclusion

The various responses of the pistachio hybrids to salinity, as reflected in the varied sodium, potassium, and chlorine contents, underscores the complexity of pistachio diversity in responses to abiotic stress. Hybrids that maintained lower sodium and chlorine levels while retaining higher potassium content were likely to exhibit greater salinity tolerance. The findings from this study can inform breeding programs aimed at enhancing salinity tolerance in pistachios, particularly by selecting for traits associated with efficient ion regulation and osmotic adjustment. Understanding the genetic and physiological basis of these traits is crucial for developing hybrids that can thrive in saline environments, contributing to sustainable orchard establishment in salt-affected areas. In summary, the promising salinity responses of the pistachio hybrids, particularly 'AI4', 'AI13', 'AI2', and 'AI1', highlighted their superiority against the UCB-1 rootstock. These hybrids can become a valuable source for rootstock application and thus expand orchard establishment in areas where UCB-1 performed weakly. Future research can consider crossing these promising hybrids with each other to further optimize salinity tolerance in their progeny.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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