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Responses of pomegranate (*Punica granatum* L.) drought-tolerant rootstocks to the salinity of irrigation water

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ABSTRACT

Purpose: Pomegranate is an important crop in Iran. However, soil and water salinity in pomegranate growing areas of Iran is one of the most important abiotic stresses resulting in economically significant losses. The most effective way to deal with salinity is to develop salinity-tolerant rootstocks and cultivars and use them in areas with saline soil and water. **Research method:** The experiment was conducted as a factorial based on a completely randomized design, where three rootstocks ('Daneshgah 8', 'Daneshgah 13', and 'Daneshgah 32') and five salinity levels (irrigation water as a control treatment, and concentrations of 25, 50, 75 and 100 mM sodium chloride) were the factors. Salinity treatments were applied in June, July, and August on three-year plants. By the end of the study, some morphological, physiological, and biochemical traits were recorded. **Findings:** Under salinity stress conditions, pomegranate rootstocks showed apparent differences in measured traits. Among the evaluated rootstocks, 'Daneshgah 13' exhibited good tolerance to salinity stress due to the smaller reductions in leaf relative water content, chlorophyll content, plant height, shoot number, fresh and dry weight, as well as lower values of ion leakage, and necrotic and fallen leaves. **Research limitations:** By measuring some other physiochemical traits such as antioxidant enzymes and proline contents, the response of pomegranate drought-tolerant rootstocks to the salinity can be more clearly interpreted. **Originality/Value:** Therefore, 'Daneshgah 13' can be used as appropriate genetic resources in breeding programs for tolerance to salinity.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is one of the most important horticultural crops in Iran, which is mainly grown in arid and semi-arid regions of the country. In these areas, soil and water salinity, water deficiency, and frost damage in some years have significantly reduced the quantitative and qualitative yield of this fruit tree. The most effective way to deal with salinity is to develop salinity-tolerant rootstocks and cultivars and use them in areas with saline soil and water (Munns & Tester, 2008).

In general, pomegranate has a moderate tolerance to salinity and can tolerate water and soil salinity of 1.8 and 2.7 dS m^{-1}, respectively (Fipps, 2003). However, the decrease in rainfall and the increase in irrigation water salinity in recent years have significantly reduced the yield and quality of this tree. On the other hand, in recent years, many activities have been carried out to increase the area of pomegranate cultivation in Iran. Therefore, the introduction of rootstocks and cultivars tolerance to drought and salinity is strongly needed. In addition, modern cultural methods are evolving towards the use of clonal rootstocks instesd of seedling rootstocks that were commonly used in the past. In the previous work, we showed that the splice grafting technique could be used with higher success for stenting (simultaneous cutting and grafting) of pomegranate (ValizadehKaji et al., 2020).

Plants show various morphological, physiological, and biochemical reactions to reduce the effects of salinity stress, including decreased leaf number, leaf area, fresh and dry weight (Momenpour et al., 2015), relative leaf water content (Massai et al., 2004), chlorophyll content, and increased ion leakage (Ahmadi et al., 2019).

The effect of salinity stress on morphological, physiological, and biochemical characteristics of pomegranate trees has been reported by many researchers (Ibrahim, 2016; Soori et al., 2018; Liu et al., 2018). In the study of the effect of different salinity levels on pomegranate cultivars, it was determined that with increasing salinity, shoot height, shoot diameter, the number of leaves, percentage of green leaves, fresh and dry weight, ion leakage, necrotic and fallen leaves, photosynthesis rate, leaf relative water content, and relative chlorophyll content significantly decreased (Soori et al., 2018; Ahmadi et al., 2019). However, there is no report on the reaction of pomegranate drought-tolerant rootstocks to irrigation water salinity. In our previous work, nine rootstocks were evaluated for drought tolerance, and three rootstocks ('Daneshgah 8', 'Daneshgah 13', and 'Daneshgah 32') showed the highest drought tolerance (ValizadehKaji et al., 2020). Therefore, due to soil and water salinity and water shortage in pomegranate growing areas, this study was conducted to investigate the effect of salinity stress on morphological, physiological, and biochemical characteristics of these three drought-tolerant genotypes for use as rootstock.

MATERIALS AND METHODS

Plant materials and condition of salinity stress

The experiment was performed in a research greenhouse at the Department of Horticultural Sciences, Arak University (34°4′26″ N; 49°40′36″ E). Mean temperatures of day and night were 26 and 19 °C, respectively, and the relative humidity ranged from 60 to 70%. The experiment was conducted as a factorial based on a completely randomized design (CRD), where three rootstocks ('Daneshgah 8', 'Daneshgah 13', and 'Daneshgah 32') and five salinity levels (irrigation water as a control treatment, and concentrations of 25, 50, 75 and 100 mM sodium chloride; equivalent to 0.66, 3.35, 6.21, 9.72, and 14.35 dS m^{-1}, respectively) were the factors. Different concentrations of sodium chloride were added to the irrigation water. Each treatment consisted of at least three replicates.

Table 1. Soil mineral contents, physical and chemical properties of the experimental vineyard

Soil texture	Clay	Silt	Sand	Organic matter	EC (ds m^{-1})	pH	P	K	Zn	Mn	Fe	Cu
	(%)						(ppm)					
Sandy clay loam	10	28	62	2.9	2.1	7.2	15	280	0.62	2.8	2.7	0.51

Salinity treatments were applied in June, July, and August on three-year plants. Different sodium chloride solutions were used twice a week in the amount of 1.5 liters per 10-liter pot for three months. This quantity of solution was previously determined to be optimal to salinity stress. Solutions of sodium chloride were added gradually to avoid sudden shock and plasmolysis. For this purpose, except for the control treatment, first, the plants were irrigated with 25 mM sodium chloride treatment. In the following stages in the plants that had to be treated with higher salinity, the salt concentration was gradually increased until finally to the desired salinity level. Some physical and chemical traits of soil associated with this research are presented in Table 1.

Measurements

By the end of the study, some morphological, physiological, and biochemical traits were recorded.

To measure the percentage of necrotic leaves, at the end of the experiment, the number of necrotic leaves was counted and divided by the total number of leaves. Also, to measure the percentage of fallen leaves, during the experiment, the number of fallen leaves until the end of the experiment was recorded and divided by the total number of leaves.

Leaf relative water content

Leaf relative water content (LRWC) was determined using the following formula (1) (Farooqi et al., 2000):

$$\text{LRWC} = [(\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight})] \times 100 \qquad (1)$$

Electrolyte leakage measurement

Two leaves per rootstock were placed into 50 mL tubes containing 40 mL of distilled water for 24 h at the ambient temperature (24 °C±1) under constant shaking at 125 rpm. Then the first solution electrical conductivity (EC1) was measured by EC meter. Samples were subsequently autoclaved at 120 °C for 20 min, allowed to cool to ambient temperature, and electrical conductivity was measured again (EC2). The electrical conductivity of bud samples remaining at 4 °C for 24 h was considered as control (EC0). Relative electrolyte leakage (REL) was calculated using the formula 2 (ValizadehKaji et al., 2020):

$$\text{REL} = [(\text{EC1} - \text{EC0}) / (\text{EC2} - \text{EC0})] \times 100 \qquad (2)$$

Chlorophyll contents

To determine chlorophyll content approx. 0.5 g of leaf samples were ground with 20 mL of 80% acetone and was centrifuged at 5,000 rpm for 5 min, and the supernatant was transferred to a 100-mL volumetric flask. The absorbance of the supernatant was assayed using a UV–visible spectrophotometer (Cary Win UV 100; Varian, Sydney, Australia) (Lichtenthaler, 1987). Wavelengths of 646.8 nm for chlorophyll b (Chl-b) and 663.2 nm for chlorophyll a (Chl-a) were used for determining chlorophyll concentrations, and contents were expressed as mg g^{-1} FW leaf (Fresh Weight).

Statistical analysis

The data were analyzed using the GLM procedure of SAS software (Version 9.1), and significant differences were tested at $P \leq 0.05$ using Duncan's multiple ranges. Before statistical analysis, the expressed data as percentages were subjected to arcsine transformation, and the original values of all transformed data were presented.

RESULTS AND DISCUSSION

There were significant interactions between rootstocks and salinity levels on all measured parameters; therefore, only mean comparisons for interactions are shown.

Morphological traits

Salinity had a negative effect on plant height, and with increasing sodium chloride concentration, plant height decreased (Table 2). However, 'Daneshgah 13' showed the highest tolerance to salinity, so that only at a concentration of 100 mM sodium chloride, the height of this rootstock was significantly reduced compared to the control and at different levels of salinity showed the least amount of height reduction. In contrast, the highest percentage of height reduction (13.26%) was related to 'Daneshgah 8' (Table 2). Plant height is highly dependent on the growing environment. Because the plant needs enough water to grow, if the required water supply is not provided, there will be a reduction in height due to a decrease in the turgor pressure of the growing cells (Munns &Tester, 2008).

Table 2. Changes in morphological charastristics of three pomegranate rootstocks at different levels of salinity

Sodium chloride (mM)	Rootstocks	Plant height (cm)	Shoot number	Fresh weight (g)	Dry weight (g)	Necrotic leaves (%)	Fallen leaves (%)
0	Daneshgah 8	120.13 abc	7.86 cd	980.66 a	410.66 a	0.00 g	0.00 g
	Daneshgah 13	120.50 ab	7.00 def	962.33 bc	402.33 b	0.00 g	0.00 g
	Daneshgah 32	121.16 a	10.06 a	860.66 g	359.00 e	0.00 g	0.00 g
25	Daneshgah 8	119.73 abc	7.80 cd	973.00 ab	412.66 a	0.00 g	0.00 g
	Daneshgah 13	120.33 ab	6.96 defg	959.00 cd	399.00 b	0.00 g	0.00 g
	Daneshgah 32	119.86 abc	9.83 a	856.00 gh	361.00 e	0.00 g	0.00 g
50	Daneshgah 8	112.83 ef	7.30 de	951.66 cd	410.33 a	6.13 d	2.40 e
	Daneshgah 13	118.86 abc	6.90 defg	949.66 d	385.66 c	2.30 f	1.50 f
	Daneshgah 32	116.60 cd	9.70 a	846.33 h	351.00 f	3.58 e	2.46 e
75	Daneshgah 8	109.06 g	6.16 fg	902.00 f	390.33 c	15.90 b	3.56 d
	Daneshgah 13	117.06 bcd	6.70 efg	929.33 e	370.33 d	3.76 e	2.10 ef
	Daneshgah 32	112.00 efg	9.26 ab	830.33 i	330.66 f	6.73 d	5.26 c
100	Daneshgah 8	104.20 h	4.36 h	847.66 h	341.00 g	32.86 a	11.46 a
	Daneshgah 13	114.90 de	5.96 g	907.00 f	349.66 f	3.90 e	3.43 d
	Daneshgah 32	110.26 fg	8.43 bc	779.66 j	300.66 i	11.53 c	8.43 b
P-value							
Sodium chloride		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Rootstock		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Sodium chloride × Rootstock		0.0016	0.0113	<0.0001	<0.0001	<0.0001	<0.0001

Mean values followed by the similar letters within a column are not significantly different from each other at P ≤ 0.05 (Duncan's multiple range test).

Table 3. Changes in chlorophyll contents of three pomegranate rootstocks at different levels of salinity

Sodium chloride (mM)	Rootstocks	Chlorophyll a (mg g FW $^{-1}$)	Chlorophyll b (mg g FW $^{-1}$)	Total chlorophyll (mg g FW $^{-1}$)
0	Daneshgah 8	0.84 bcd	0.21 ab	1.05 b
	Daneshgah 13	0.88 a	0.22 a	1.11 a
	Daneshgah 32	0.86 ab	0.20 bc	1.06 b
25	Daneshgah 8	0.78 e	0.20 bc	0.98 c
	Daneshgah 13	0.88 a	0.21 ab	1.10 a
	Daneshgah 32	0.85 abc	0.20 bc	1.05 b
50	Daneshgah 8	0.70 g	0.17 d	0.87 e
	Daneshgah 13	0.85 bcd	0.20 bc	1.05 b
	Daneshgah 32	0.83 cd	0.18 d	1.01 c
75	Daneshgah 8	0.60 h	0.13 f	0.73 f
	Daneshgah 13	0.82 d	0.19 cd	1.01 c
	Daneshgah 32	0.77 e	0.15 e	0.93 d
100	Daneshgah 8	0.50 i	0.08 h	0.59 h
	Daneshgah 13	0.76 e	0.14 e	0.91 d
	Daneshgah 32	0.73 f	0.11 g	0.84 f
P-value				
Sodium chloride		<0.0001	<0.0001	<0.0001
Rootstock		<0.0001	<0.0001	<0.0001
Sodium chloride × Rootstock		<0.0001	0.0006	<0.0001

Mean values followed by the similar letters within a column are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test).

Under salinity conditions, the number of shoots decreased (Table 2). Compare to unstressed plants, the reduction in shoot number in 'Daneshgah 8' was significant at concentrations of 75 (a 21.62% decrease) and 100 mM (a 44.52% decrease) sodium chloride, but the reduction in shoot number in 'Daneshgah 13' and 'Daneshgah 32' was significant only at 100 mM sodium chloride. The lowest and highest reductions in shoot number were related to 'Daneshgah 13' and 'Daneshgah 8', respectively, so that at a concentration of 100 mM sodium chloride, the reduction in shoot number in 'Daneshgah 13' and 'Daneshgah 8'compared to control plants was 44.36% and 14.85%, respectively (Table 1).

With increasing sodium chloride concentration, the fresh and dry weight of the plants decreased; however, 'Daneshgah 13' showed the lowest reduction of wet and dry weight compared to the other two rootstocks. In contrast, the highest reduction of wet and dry weight belonged to 'Daneshgah 8' (Table 2). These results indicate that 'Daneshgah 13' maintained its growth characteristics better under salinity stress and its tolerance to salinity stress was higher than the other two rootstocks.

The concentration of 25 mM sodium chloride did not affect the percentage of necrotic and fallen leaves, but at concentrations of 50, 75, and 100 mM sodium chloride, the percentage of necrotic and fallen leaves increased significantly in all rootstocks (Table 2). The highest and lowest percentages of necrotic and fallen leaves were recorded in 'Daneshgah 8' and 'Daneshgah 13', respectively (Table 2). The major damage of salinity is related to the accumulation of sodium ions in leaf tissue, which leads to necrosis and aging of leaves, followed by reduced crop growth in a short time (Munns, 2002).

In accordance with the results achieved in this research, a decrease in morphological traits under salinity stress conditions has been reported in pomegranate and some fruit trees (Naeini et al., 2005; Ibrahim, 2016; Tavousi et al., 2016; Momenpour & Imani, 2018). Assessing the effect of salinity stress on different pomegranate cultivars, Soori et al. (2018) reported that pomegranate cultivars show different reactions to different salinity levels and the cultivar of Malas Yousofkhani, due to its superiority in most traits related to salinity tolerance, had a higher tolerance than other cultivars to salinity. The decrease in morphological indices is due to ion toxicity and an increase in the osmotic potential of the soil solution. Salinity stress, such as drought, due to the interference of the osmotic mechanism, restricts access to water and rapidly reduces the growth process of the plant, and causes metabolic changes that are similar to the effects of drought stress (Momenpour et al., 2015).

Chlorophyll contents

Under salinity stress and increasing sodium chloride concentration, the contents of chlorophyll a, chlorophyll b, and total chlorophyll decreased in all rootstocks (Table 3). Compared with unstressed plants, the decrease in chlorophyll content in 'Daneshgah 13'was less than the other two rootstocks. 'Daneshgah 8' also showed the largest reduction in chlorophyll content (Table 3). Under salinity stress conditions, a decrease in chlorophyll contents have been detected in different plants, including pomegranate (Ibrahim, 2016), strawberry (Kaya et al., 2002), and grapevine (Sivritepe & Eris, 1999) in which is in parallel to the results obtained in the recent study (Fig. 1). In pomegranate, it was found that the content of chlorophyll in susceptible cultivars under salinity stress showed a greater decrease than tolerant cultivars, which is consistent with the results of this study (Soori et al., 2018). Decreased chlorophyll content of leaves due to salinity stress may be associated with increased activity of the chlorophyll-degrading enzyme (Chlorophyllase) (Rao & Rao, 1981). In addition, the decrease in chlorophyll content at high salinity may be due to inhibition of the uptake, transport, or use of magnesium and nitrate ions in plants (Ibrahim, 2016).

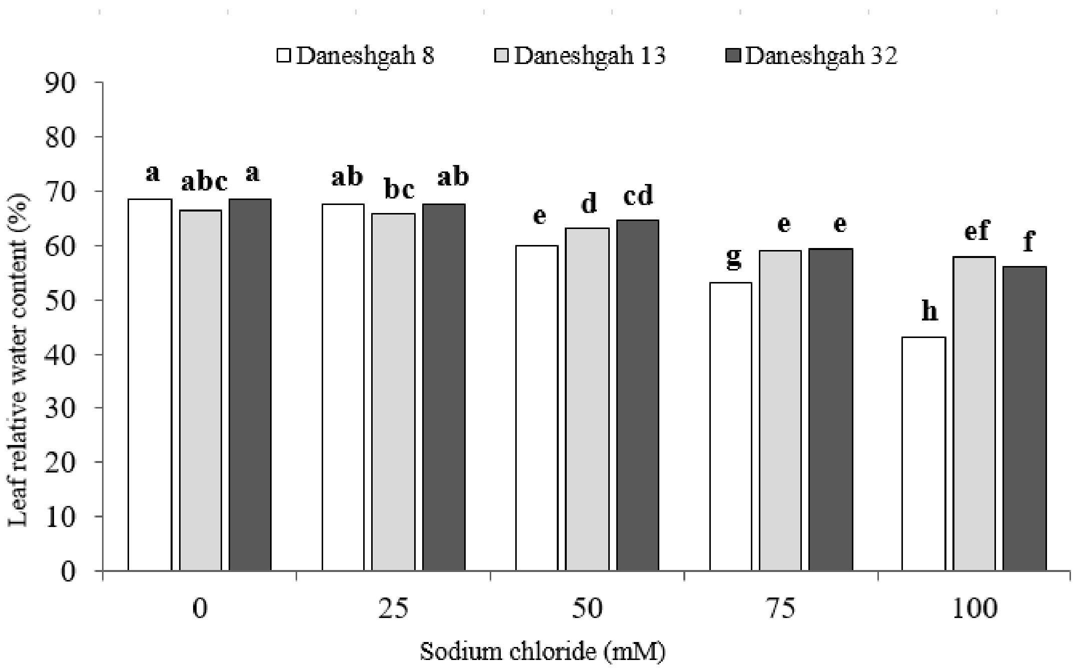

Fig. 1. Leaf relative water content in the leaves of three pomegranate rootstocks under different salinity conditions. Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments.

Leaf relative water content

As shown in Figure 1, the leaf relative water content decreased with increasing salinity. At the concentration of 25 mM sodium chloride, the reduction of leaf relative water content in all three rootstocks was not significant compared to control plants. At concentrations of 50, 75, and 100 mM sodium chloride, the lowest and highest percentages of decrease in leaf relative water content were related to ‘Daneshgah 13’ and ‘Daneshgah 8’, respectively (Fig. 1). In accordance with the results achieved in this research, a decrease in leaf relative water content under salinity stress conditions has been reported in some fruit trees, such as prunus (Massai et al., 2004) and almond (Shibili et al., 2003). Under salinity stress conditions, tolerant pomegranate cultivars have higher leaf relative water content than susceptible cultivars (Soori et al., 2018). The decrease in leaf's relative water content is the decrease in water uptake from the soil due to soil salinity, which upsets the balance between the two processes of water uptake and transpiration, and as a result, plant water is reduced (Soori et al., 2018). In addition, salinity reduces the relative water content and osmotic potential of leaf cell sap through the gradual accumulation of sodium ions. Reduction in the leaf relative water content reduces the water required for morphological and physiological processes such as cell elongation, the opening of stomata, and photosynthetic processes (Ahmadi et al., 2019).

Relative electrolyte leakage

Based on obtained results, the percentage of ion leakage in the leaves of all studied rootstocks increased with increasing sodium chloride concentration (Fig. 2). However, the increase in ion leakage of all three rootstocks at a concentration of 25 mM sodium chloride was not significant compared to the unstressed plants. Compared to control plants, at salinity levels of 50, 75, and 100 mM sodium chloride, the highest ion leakage was related to ‘Daneshgah 8’, in which the percentage of ion leakage increased by 68.21, 138.74, and 275.17%, respectively. In contrast, the lowest ion leakage rate was related to ‘Daneshgah 13’, which at salinity levels of 50, 75, and 100 mM sodium compared to control plants, the rate of ion leakage increased to 28.11, 60.03, and 96.27, respectively (Fig. 1). In accordance with the results achieved in this research, an increase in the percentage of ion leakage under salinity stress conditions has been reported in some fruit trees, such as pomegranate (Ahmadi et al., 2019) and okra (Besma & Denden, 2012). Environmental stresses such as salinity and drought in the first stage cause damage to cell membranes; therefore, due to salinity stress, membrane permeability is also affected. Ion leakage of the membrane has been suggested by some researchers as an effective indicator in determining the degree of salinity resistance (Ashraf & Ali, 2008). Ion leakage is higher in salinity-sensitive plants than in resistant plants (Soori et al., 2018). In the present study, ‘Daneshgah 13’ had less ion leakage than the other two cultivars, indicating more tolerance to salinity.

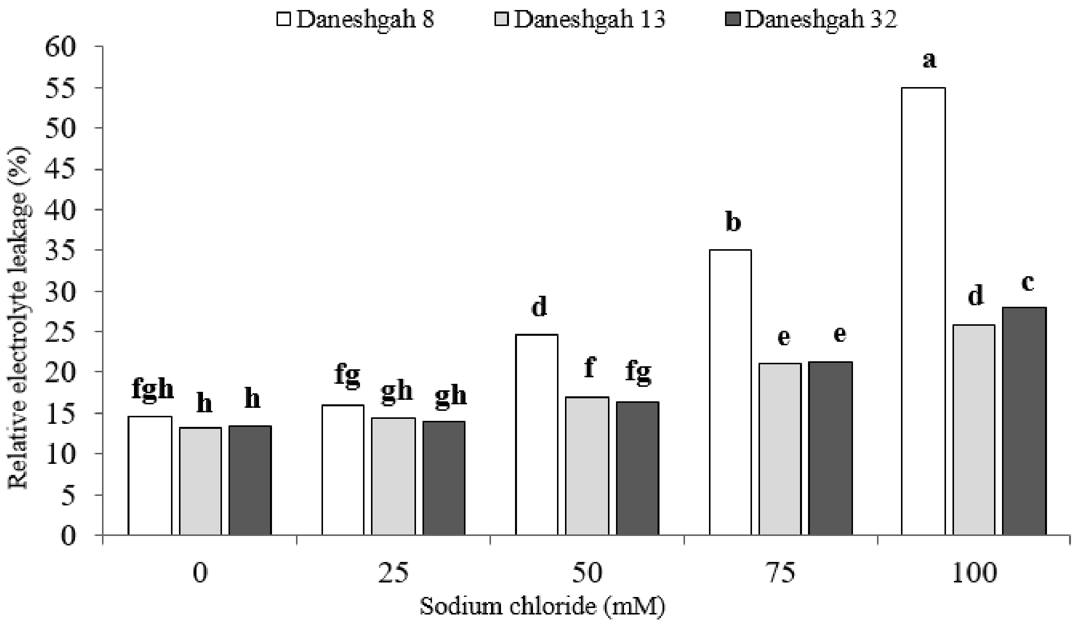

Fig. 2. The percentage of ion leakage in the leaves of three pomegranate rootstocks under different salinity conditions. Different letters at the top of columns indicate significant differences ($P \leq 0.05$) among treatments.

CONCLUSION

In response to salinity stress, different morphological and physio-chemical reactions were observed among three pomegranate rootstocks. 'Daneshgah 13' exhibited appropriate tolerance to salinity stress. The greater tolerance of 'Daneshgah 13' was associated with the preservation of morphological characteristics, the smaller reductions in leaf relative water content and chlorophyll values, as well as lower values of electrolyte leakage. The use of salinity-tolerant rootstocks could result in better production of pomegranate, particularly in areas with saline water and soil.

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Conflict of interest

The authors have no conflict of interest to report.

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