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Salinity responses in 24 guar genotypes are linked to multigenic regulation explaining the complexity of tolerance mechanisms *in planta*

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Abstract

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is an economically important, drought-tolerant legume crop affected by moderate to high salinity. Guar has various industrial applications, including gum extracted from seeds that is widely used in the oil and natural gas industries. In this investigation, we evaluated the salinity tolerance of different guar genotypes and their relation to genetic mechanisms regulating guar responses to salinity stress. We screened 24 guar genotypes in a greenhouse lysimeter system under control (electrical conductivity (EC) = 1.46 dS m^{-1}) and high salinity (EC = 13.65 dS m^{-1}) treatments. Both length and biomass of shoots were significantly affected by salinity compared to roots, indicating higher shoot than root sensitivity to salinity. Twenty-four genotypes were classified based on salt tolerance index for each trait. Tissue ion analysis revealed that roots accumulated over 10-fold higher Na than leaves, demonstrating that guar effectively regulated the root-to-shoot movement of Na^+. However, higher Cl concentrations in leaves than roots indicated less regulatory control of Cl^- movement. Based on the morphological traits and tissue ion analysis, six genotypes (PI 164486, PI 253186, PI 26152, PI 158125, PI 179926, and PI 263698) with different responses to salinity were selected for gene expression analysis. Expression patterns of different genes showed that a complex network of component traits, including Na^+ exclusion, Cl^- exclusion, and tissue tolerance, regulate salinity tolerance in guar. Hence, the genetic information about different component traits will benefit guar breeders in developing new varieties that are more tolerant to salinity than current ones.

Abbreviations: *AKT1*, *Arabidopsis inward rectifying K^+ transporter 1*; *ALMT9*, *Aluminum-Activated Malate Transporter 9*; *CCC*, *Cation/Cl^- cotransporter*; *CLCc*, *Chloride channel c*; *CLCg*, *Chloride channel g*; EC, Electrical conductivity; EF-1a, Elongation factor-1 alpha; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; *HKT1*, *High-affinity potassium transporter 1*; *NHX1*, *Na^+ /H^+ exchanger 1*; *SLAH3*, *Slow-type anion channel associated homolog 3*; *SOS1*, *Salt Overly Sensitive 1*; *SOS2*, *Salt Overly Sensitive 2*; SRA, Sequence Read Archive; STI, Salt tolerance index.

1 | INTRODUCTION

Guar (*Cyamopsis tetragonoloba* (L.) Taub.) is a low-input, drought-tolerant legume crop primarily grown in semi-arid regions of South Asia, particularly in India and Pakistan. Being a legume crop, guar can improve soil quality by symbiotic nitrogen fixation (Thapa et al., 2018). Guar has a variety of purposes that include the use of the whole plant as forage, the pods as a vegetable, and the seeds for several commercial applications (Mahdipour-Afra et al., 2021). The endosperm that constitutes about half of the guar seed is processed to produce guar gum, a galactomannan polysaccharide (Kays et al., 2006). Guar gum is used as a stabilizer, emulsifier, and thickening agent in various food products. It is widely used in various commercial industries such as textile, paper, cosmetics, pharmaceutical, food processing, oil and gas mining, and petroleum (Mudgil et al., 2014). Guar gum makes fracturing fluid thicker, carrying sand into fractured rock. The presence of sand keeps the fracture open, which allows the flow of gas/oil to the wellbore. The development of anionic and cationic derivatives of guar gum facilitates oil and gas well stimulation via hydraulic fracturing (Mudgil et al., 2014).

Currently, India is the largest producer of guar (~80%), followed by Pakistan (10%) (Mudgil et al., 2014; Singh, 2014). However, several other countries, such as the United States, Brazil, Australia, South Africa, and China, are trying to increase their guar production (Mudgil et al., 2014). The United States is the largest importer of guar products worldwide, and its demand is met through imports from India (Sharma et al., 2018). The American Southwest is facing increasingly harsh weather conditions due to climate change (Summers et al., 2021). With the dwindling supply of available freshwater for agriculture, farmers continuously face the risks of reduced yield of crops or their complete loss, leading to significant revenue reductions. One alternative is the use of drought-tolerant crops that yield high-value products. Recent investigations point to guar as a viable crop in the American Southwest, where arid conditions, continuing drought, and economic value make guar an attractive crop (Singla et al., 2016a; Singla et al., 2016b; Summers et al., 2021).

One of the most important abiotic stresses faced by global agriculture is salinity, which is predicted to escalate in the coming decades due to climate change and the continuous use of underground water. As fresh water supplies dwindle, a major concern is the salinization of agricultural lands from the use of degraded or poor-quality waters. Currently, 33% of the irrigated agricultural land worldwide is salinized and is predicted to exceed 50% by 2050 (Ashraf, 2009). The southwestern United States is under continuous threat of increasing salinity, which is a major cause of reduction in crop yields (Summers et al., 2021). Various morphological, biochemical, and physiological factors are critical during salinity stress, challenging the characterization of plant salinity responses (Sandhu & Kaundal, 2018; Tripathi et al., 2021). Salinity affects plant growth in different ways, including interference with water uptake, imbalance of ion homeostasis that may lead to mineral nutrient deficiencies, reduced photosynthesis, and oxidative stress (Dias et al., 2016; Sandhu et al., 2017). Identifying salt-tolerant lines and understanding genetic mechanisms regulating salinity tolerance will be crucial in maintaining crop production in semi-arid regions.

Core Ideas

- Guar shoots are more sensitive to salinity than roots.
- Guar plants regulate the root-to-shoot movement of sodium efficiently, but not of chloride.
- Sodium exclusion, chloride exclusion, and tissue tolerance were important traits under salinity stress in guar.
- Guar genotypes varied for the component traits related to salinity tolerance mechanisms.

Guar is tolerant to abiotic stresses such as drought and heat (Abidi et al., 2015; Alshameri et al., 2020; Shrestha et al., 2022). However, not much is known about its salinity responses. In a salinity study involving three plant species, guar was more sensitive than *Sesbania* and *Kochia* under high-salinity irrigation with water of electrical conductivity (EC_{iw}) = 14 dS m^{-1} (Ghaffarian et al., 2020). However, guar has shown better salt tolerance than other legume crops such as soybean, cowpea, pigeon pea, black gram, and green gram (Keating & Fisher, 1985). A field study showed no effect of salinity on the seed yield of two cultivars of guar up to a soil-paste extract electrical conductivity (EC_e) of 8.8 dS m^{-1} in the root zone (Francois et al., 1990). However, over 8.8 dS m^{-1}, there was a 17% reduction in seed yield with every unit increase in salinity. Vegetative growth was more sensitive to salinity than seed yield, with a decline starting at an EC_e = 4.9 dS m^{-1} (Francois et al., 1990). Salinities over 8.5 dS m^{-1} delayed seed emergence, but there was no reduction in percent germination up to 18.8 dS m^{-1} (Francois et al., 1990). In the evaluation of 15 diverse guar genotypes, a direct correlation was found between the rooting system (root length and root biomass) and salinity tolerance (Ashraf et al., 2005). In a salinity study, irrigation with saline water with EC_{iw} of 9 dS m^{-1} resulted in an average reduction in yield by 63% compared to the control (Suthar et al., 2018). The comparison of seed germination and seedling growth also revealed diverse salinity responses of genotypes (Rasheed et al., 2015; Suthar et al., 2019). The salt tolerance index (STI) for shoot biomass, representing the performance ratio under salinity to control, was 1.35 and 1.58 for salt-tolerant genotypes and

TABLE 1 Composition of irrigation water

Treatment	EC_{iw} (dS m^{-1})	Ion concentration ($mmol_c$ L^{-1})							
		Cl^-	SO_4^{2-}	NO_3^-	PO_4^{3-}	Na^+	K^+	Ca^{2+}	Mg^{2+}
Control	1.46	1.41	1.44	5.39	1.5	1.88	6.6	3.35	2.1
Saline	13.65	128.4	27.32	5.39	1.5	106.9	6.6	29.6	23

0.21 and 0.20 for salt-sensitive genotypes (Sandhu et al., 2021). These authors reported that leaf Na concentration was 4 to 5.5-fold higher in salt-sensitive genotypes than in salt-tolerant ones, suggesting differences in abilities to control the root-to-shoot movement of Na. Although this study showed a significant genetic variation among genotypes, the study evaluated only four genotypes, which did not capture the extent of the genetic variation and the roles of different component traits in guar salinity tolerance. Hence, studies that evaluate several guar genotypes simultaneously and focus on understanding genetic mechanisms regulating salinity stress are still lacking.

In this investigation, we screened 24 guar genotypes for their salinity tolerance, determined how they regulated the uptake of different ions from the soil, and determined expression differences of various genes involved in salinity tolerance among different genotypes under control and salinity conditions.

2 | MATERIALS AND METHODS

2.1 | Plant material and salinity screening

Twenty-four genotypes, including Plant Introduction (PI) lines and some known cultivars, were used in this study. Ten seeds of each line were sown in lysimeters located at a US Salinity Laboratory (USDA-ARS) greenhouse (33.973265 latitude, −117.321158 W longitude). The study was arranged in a randomized complete block design. After germination, seedlings were thinned to six per line and grown with half-strength Hoagland's solution for 2 weeks. After 2 weeks, the high-salinity treatment (EC_w = 13.65 dS m^{-1}) was achieved by incrementing water salinity over 96 h to avoid osmotic shock (Table 1). Based on our previous study, irrigation water of EC = 13.65 dS m^{-1} resulted in an average reduction of 50% in shoot biomass and is suitable for discriminating salinity responses of various genotypes (Sandhu et al., 2021). Forty-eight hours after the final salt was added to the treatment, leaf and root samples were collected, frozen in liquid nitrogen, and stored at −80°C for genetic analysis. The remaining plants were grown for another 3 weeks. Then, the plants were harvested, the root and shoot tissues were separated, and samples were dried at 70°C for 96 h.

2.2 | Ion analyses

Oven-dried leaf and root tissues were ground to a fine powder and used for ion analyses. The chloride analysis was carried out with a mercuric thiocyanate reaction in the presence of ferric nitrate in an AQ300 discrete analyzer (EPA600/4-79-020, 1983). The concentrations for other ions were determined via nitric acid digestions by inductively coupled plasma optical emission spectrometry (3300DV, Perkin-Elmer Corp., Waltham, MA, USA).

2.3 | Expression analyses

Candidate genes for the expression analyses were selected based on their association with salt tolerance. Five genes involved in Na^+ transport, including efflux of Na^+ from roots (*Salt Overly Sensitive 1*; *SOS1* and *Salt Overly Sensitive 2*; *SOS2*), sequestration of Na^+ into vacuoles (*Na^+/H^+ exchanger 1*; *NHX1*), regulation of Na^+/K^+ homeostasis (*Arabidopsis inward rectifying K^+ Transporter* 1; *AKT1*), and retrieval of Na^+ from the xylem back into the roots (*High-affinity potassium transporter 1*; *HKT1*) were selected based on previously published information (Barragan et al., 2012; Ji et al., 2013; Ragel et al., 2019; Rubio et al., 1995). Five genes involved in Cl^- transport included genes involved in the sequestration of Cl^- into vacuoles (*Aluminum-Activated Malate Transporter 9*; *ALMT9*, *Chloride Channel c*; *CLCc*, and *Chloride Channel g*; *CLCg*), retrieval of Cl^- from the xylem back into the root (*Cation/Cl^- cotransporter*; *CCC*), and the movement of Cl^- from root to xylem (*Slow-type anion channel Associated Homolog 3*; *SLAH3*) (Colmenero-Flores et al., 2007; Jossier et al., 2010; Li et al., 2016; Li et al., 2017; Nguyen et al., 2016). The Arabidopsis sequence of each gene was obtained from The Arabidopsis Information Resource (TAIR; https://www.arabidopsis.org/). The Arabidopsis sequences were used to identify the corresponding soybean sequences. Then, the soybean sequences were analyzed in the NCBI Sequence Read Archive Nucleotide BLAST search against RNA-seq data from a previous study to obtain the corresponding guar sequences. Sequences with the highest homologies were used to design primers (Table S1). In addition, three housekeeping genes selected to be used for normalization of expression were guar elongation factor-1 alpha

(EF-1a), Actin 11 (Act11), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Jaiswal et al., 2019).

RNA extraction was performed using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and was treated with DNase I (Thermo Scientific, Waltham, MA, USA) to remove DNA contamination. RNA samples were then diluted to 10 ng/μL and underwent quantitative reverse transcription polymerase chain reaction (qRT-PCR) using the iTaq™ Universal SYBR® Green One-Step Kit in a BioRad CFX96 machine (Bio-Rad Laboratories, Hercules, CA, USA). The PCR reactions were carried out in 10 μL reactions containing 10 ng total RNA, 0.5 μM concentration of each primer, 5 μL of 2x one-step SYBR® Green Reaction mix, and 0.125 μL iScript™ Reverse Transcriptase enzyme. The protocol was as follows: 50°C for 10 min, 95°C for 1 min, followed by 40 cycles of 95°C for 10 s, 57°C for 30 s, 68°C for 30 s. All qRT-PCR reactions were performed using samples from three biological replicates and two technical replicates. Relative expression values were calculated using the comparative $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001). The relative gene expressions were determined according to the following formula:

$$\text{Relative expression}_{\text{sample(GOI)}} = [\text{RQ}_{\text{sample (GOI)}}] / [\text{RQ}_{\text{sample (ref 1)}} \times \text{RQ}_{\text{sample (ref 2)}} \times \cdots \text{RQ}_{\text{sample (ref n)}}]^{1/n}$$

where *RQ* represents the relative quantity of a sample, *ref* is the reference target in a run that includes one or more reference targets in a sample, and *GOI* is the gene of interest. The amplification specificity was tested using the melt-curve analysis by ramping up the temperature to 95°C for 10 s and lowering back to 65°C for 5 s followed by increments of 0.5°C/cycle up to 95°C.

2.4 | Statistical analysis

The STI, which indicates the tolerance of a plant to salt stress, was calculated for each trait by dividing the phenotypic value of a genotype in salt-treated tanks by the phenotypic value of the genotype in control tanks. The statistical comparisons were performed using one-way ANOVA and Student's *t*-test; *P* values ≤ 0.05 were considered significant.

3 | RESULTS AND DISCUSSION

3.1 | Evaluation of salinity tolerance of guar genotypes

This study focused on evaluating 24 guar genotypes for their salinity tolerance and understanding physiological and genetic mechanisms regulating salinity tolerance in guar. Screening of guar genotypes at the seedling stage under irrigation water of control (EC = 1.46 dS m^{-1}) and high-salinity (EC = 13.65 dS m^{-1}) treatments in a greenhouse lysimeter system revealed significant differences among genotypes (Figure 1). Shoot length and shoot biomass showed a higher reduction under salinity compared to control than root length and root biomass (Figure 1). These observations suggest that guar shoots are more sensitive to salinity than roots and are better protected from excessive salinity exposure, as leaves are known to show ionic toxicity much earlier than the roots (Munns & Termaat, 1986). Plants can move photosynthates to stressed roots to maintain the osmotic balance, thereby providing higher tolerance to roots compared to leaves (Al-Niemi et al., 1992).

All genotypes showed a reduction in shoot length; however, the reduction was statistically significant in 16 of the 24 genotypes (Figure 1a). On the other hand, the root length was statistically the same under control and salinity for all genotypes, except three (Figure 1b). The STI that represents performance under salt relative to the performance under the control condition is a better parameter for evaluating the salt tolerance of a particular genotype because it compares the same genotype to itself under contrasting salinity conditions (Sandhu et al., 2017; Sandhu & Kaundal, 2018). Based on STI for shoot length, PI 253187 was the top performer with an STI of 0.53, closely followed by PI 179926 (STI = 0.53) and PI 250360 (STI = 0.51) (Figure 1a). Conversely, PI 593048 (STI = 0.20), PI 338745 (STI = 0.23), and PI 253182 (STI = 0.27) were the three genotypes with the lowest STI for shoot length. Based on STI for root length, PI 671848 (STI = 1.22), PI 263698 (STI = 1.21), and PI 180434 (STI = 1.21) were the top performers, and PI 593048 (STI = 0.20), PI 253182 (STI = 0.61), and PI 164486 (STI = 0.69) were the worst performers (Figure 1b). The STI for shoot length varied from 0.20 to 0.53, suggesting that the severity of salinity treatment was adequately high for genotype comparison and led to a 47% reduction in height in the best-performing line (Figure 1a). Interestingly, most lines did not show a significant reduction in root length under salinity compared to the control (Figure 1b). PI 593048, PI 228745, PI 253182, and PI 164486 had low STI both for shoot and root length, with PI 593048 as the worst performer for both traits (Figure 1a,b).

There was significant variation in the performance of different genotypes under control and salinity with respect to biomass (Figure 1c,d). Although most genotypes showed less biomass under salinity than control, only three were statistically lower for shoot biomass and one for root biomass (Figure 1c,d). Based on STI for shoot biomass, PI 253187 (STI = 1.17), PI 263698 (STI = 0.85), and PI 179926 (STI = 0.0.79) were the top performers, whereas PI 164486 (STI = 0.26), PI 158126 (STI = 0.27), and PI 593048

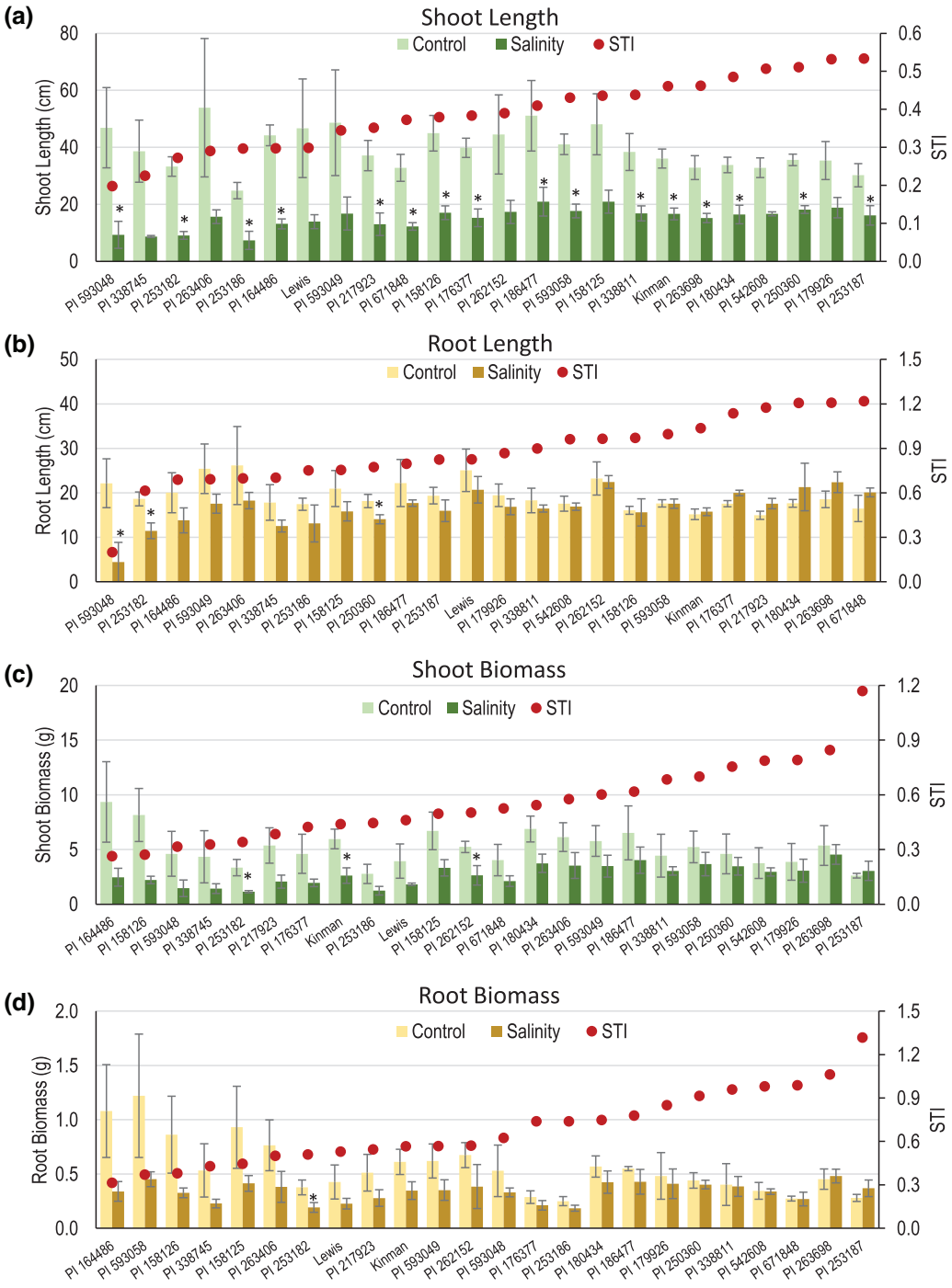

FIGURE 1 Performance of 24 guar genotypes under control and salinity conditions: (a) shoot length; (b) root length; (c) shoot biomass; (d) root biomass. Asterisks indicate significant differences between the control and saline conditions ($P \leq 0.05$) ($n = 3$). Error bars represent standard errors. STI, salt tolerance index

(STI = 0.32) were the bottom performers (Figure 1c). While, based on STI for root biomass, PI 253187 (STI = 1.32), PI 263698 (STI = 1.06), and PI 671848 (STI = 0.99) were the top-performing genotypes and PI 164486 (STI = 0.31), PI 593058 (STI = 0.37), and PI 158126 (STI = 0.38) were the poorest performers (Figure 1d). The best-performing genotype for both shoot and root biomass under salinity treatment was PI 253187, followed by PI 263698 (Figure 1c,d). Most of the genotypes that did not perform well under salinity also had low shoot biomass STI values (Figure 1c). Genotypes varied in shoot biomass STI from 0.26 to 1.17 and root biomass STI from 0.31 to 1.32. PI 164486, PI 158126, and PI 338745 had low STI for both shoot and root biomass (Figure 1c,d). The reductions in plant shoot and root biomass under

salinity are mainly due to osmotic stress or ionic stress, or both (Sandhu & Kaundal, 2018; Sandhu et al., 2020b). In a previous study involving 12 alfalfa genotypes, a maximum reduction of 61% in shoot biomass was observed under EC_{iw} of 16.6 dS m^{-1} compared to the control (Sandhu et al., 2017). In woodland strawberries, there was up to a 59% reduction in shoot biomass and a 47% reduction in root biomass among eight genotypes treated with EC_{iw} of 8 dS m^{-1} (Sandhu et al., 2019). As shoots are more sensitive to salinity, the reduction in shoot biomass is almost always more than the reduction in root biomass.

3.2 | Tissue ion analysis

Ion toxicity is the most important factor that limits plant growth and development under salinity stress (Sandhu & Kaundal, 2018). Excessive accumulation of ions such as Na^+ and Cl^- is toxic to plants. Plants use various molecular mechanisms to exclude toxic ions and prevent them from reaching leaves (van Zelm et al., 2020). Efficient deployment of these mechanisms may distinguish salt-tolerant plants from salt-sensitive ones. Hence, tissue ion concentration can be used as an effective trait in discriminating plants based on their salinity tolerance.

Our ion analysis indicated that the leaf Na concentrations were significantly higher under salinity than in control in all genotypes except five (Figure 2a). Different genotypes showed wide variation in leaf Na concentration under saline conditions that ranged from 181.7 to 3185.5 mmol kg^{-1} (Figure 2a). Under saline conditions, Lewis (181.7 mmol kg^{-1}) accumulated the least amount of Na in leaves, followed by PI 180434 (182.4 mmol kg^{-1}) and Kinman (190.8 mmol kg^{-1}) (Figure 2a). On the other hand, PI 262152 (3185.5 mmol kg^{-1}), PI 176377 (2472.2 mmol kg^{-1}), and PI 593048 (1018.5 mmol kg^{-1}) were the top three genotypes for high leaf Na accumulation under salinity. PI 262152 had a 247-fold increase in leaf Na concentration under salinity than control (Figure 2a).

In roots, genotypes with the lowest leaf Na accumulation under salinity were PI 593058, PI 593049, and PI 338745, with the leaf Na concentrations of 763.8, 779.1, and 780.4 mmol kg^{-1}, respectively (Figure 2b). In contrast, PI 262152 (1278.1 mmol kg^{-1}), PI 176377 (1153.1 mmol kg^{-1}), and PI 164486 (1052.4 mmol kg^{-1}) accumulated maximum root Na. PI 262152 had a 4.4-fold increase in Na concentration under salinity than control.

The leaf-to-root ratio for Na concentration varied from 0.03 to 0.12 under the control condition (Figure 2a,b). The average leaf Na concentration for all genotypes was 10-fold less than the root Na concentrations under the control condition (Figure 2a,b). These observations suggest that under low salinity conditions, mechanisms involved in Na^+ exclusion are very effective and restrict Na^+ in the roots. As guar is exposed to high salinity, a large amount of Na^+ moves from roots to leaves. Under salinity, leaf Na concentration ranged from 0.21 to 2.49 relative to the root Na concentration. Although leaf Na concentrations were higher under salinity compared to control, the Na concentrations were still lower in leaves than roots in most genotypes, suggesting an efficient regulatory mechanism controlling the movement of Na^+ from root to shoot (Figure 2a,b). However, three genotypes (PI 262152, PI 176377, and PI 593048) had higher Na in leaves than roots, which also explains their STI for shoot biomass of 0.5 or less (Figures 1c and 2a,b). PI 262152 accumulated the highest Na concentration in leaves and the roots, suggesting that this genotype took up more Na^+ from the soil and/or there was less efflux from root to soil.

For Cl, all genotypes displayed high leaf concentrations under salinity than the control (Figure 2c). Three genotypes with the least leaf Cl concentrations under salinity were Lewis (904.1 mmol kg^{-1}), Kinman (935.3 mmol kg^{-1}), and PI 158125 (1196.3 mmol kg^{-1}). Conversely, PI 262152 (3685.8 mmol kg^{-1}), PI 176377 (3066.3 mmol kg^{-1}), and PI 253182 (1981.3 mmol kg^{-1}) were the three top leaf Cl accumulating genotypes (Figure 2c). There was a 10.1-fold (Lewis) to 57-fold (PI 262152) increase in leaf-Cl concentration under salinity compared to the control (Figure 2c). Like leaves, the root Cl concentrations were also significantly higher under salinity than under control (Figure 2d). PI 262152 accumulated the highest concentration of Cl in roots (688.38 mmol kg^{-1}) under salinity (Figure 2d), which was over 15-fold higher than in control (43.6 mmol kg^{-1}).

On average, Na and Cl concentrations in leaves were respectively 30.9-fold and 22.7-fold higher under salinity than in control (Figure 2a,c), while in roots, increases in Na and Cl concentrations were, respectively, 3.3-fold and 5.0-fold higher under salinity than under control (Figure 2b,d).

Under salinity stress, PI 176377, PI 217923, PI 262406, and PI 262152 were the four top genotypes for high leaf-Cl concentration compared to roots (Figure 2c). In these four genotypes, the ratio of tissue Cl in leaves versus roots varied from 5.35 to 6.67 (Figure 2c,d). PI 164486, which showed relatively low Na concentration in the leaves compared to roots, was among the genotypes with high tissue Cl concentration in leaves versus roots (Figure 2a–d). For all genotypes, the average Cl concentration in leaves and roots was similar under the control condition; however, it was 4.55-fold higher in the leaf than in the root under salinity (Figure 2c,d). These observations indicate that Cl moves more freely, and there is not much regulation of Cl^- movement from root to shoot in guar.

Potassium is an important nutrient that plays various roles in plants related to osmotic balance, homeostasis, cell expansion, regulation of membrane electric potential, and enzymatic activation (Ragel et al., 2019). K content in plants is negatively affected under salinity; K^+ absorption by roots

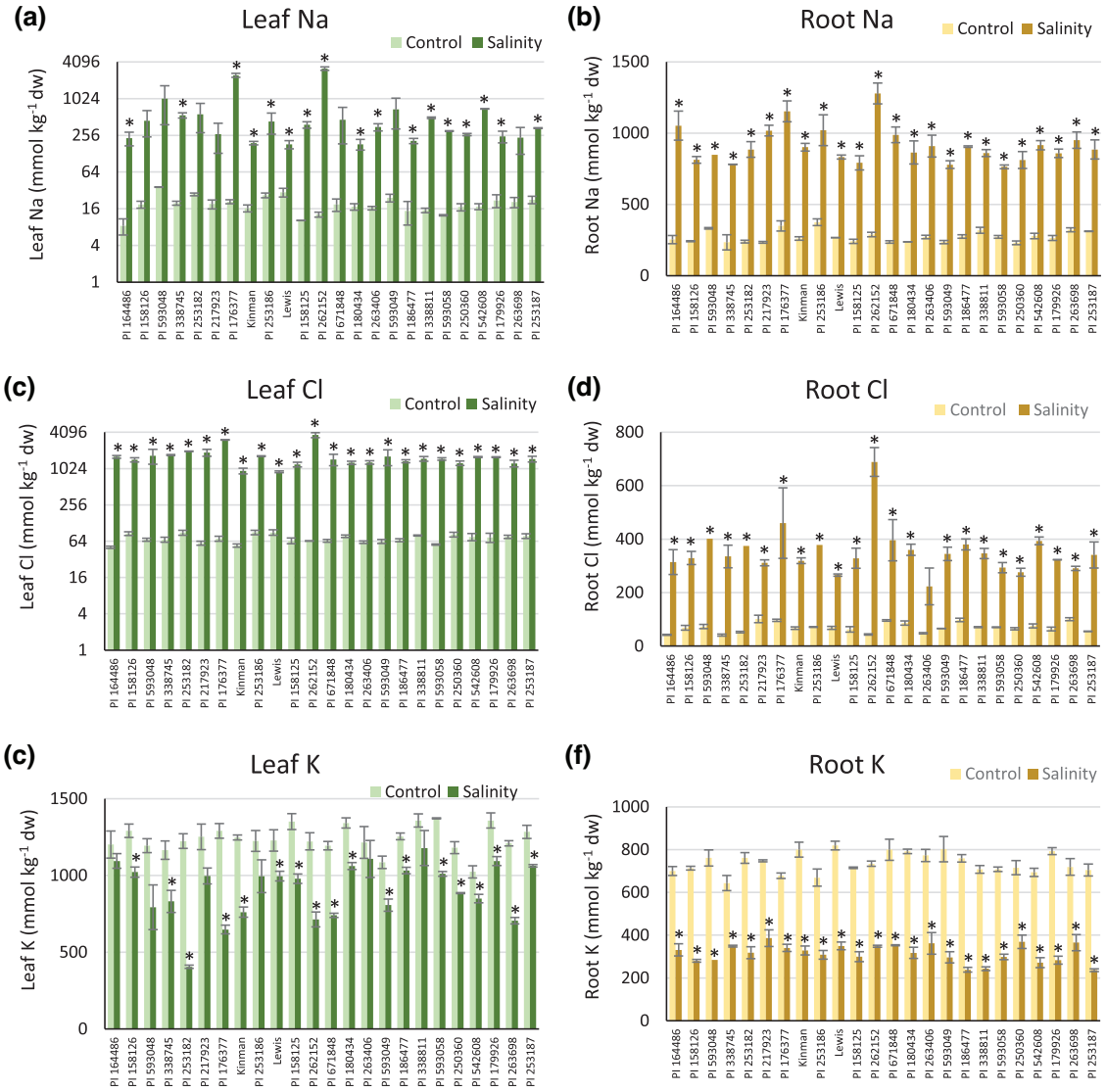

FIGURE 2 Tissue ion concentrations of the 24 guar genotypes irrigated with control and saline irrigation waters: (a) leaf Na concentrations; (b) root Na concentrations; (c) leaf Cl concentrations; (d) root Cl concentrations; (e) leaf K concentrations; (f) root K concentrations. Asterisks indicate significant differences between the control and saline conditions ($P \leq 0.05$) ($n = 3$). Error bars represent standard errors

is reduced, and increased Na^+ concentration in the cell leads to K^+ efflux (Almeida et al., 2017). Hence, K tissue concentration is an important parameter in determining the salinity tolerance of a genotype. Most genotypes had significantly lower K concentrations under salinity than the control (Figure 2e,f). There was an average 27% reduction in leaf K concentrations, while in roots, that reduction was 57% under salinity compared to under control. Under salinity, the average leaf-K concentrations varied from 405.1 mmol kg^{-1} for PI 253182 to 1178.0 mmol kg^{-1} for PI 338811. Genotypes that maintained a higher ratio of leaf K concentration under salinity to control (K_s/K_c) also had higher biomass STI (Figures 1c and 2e,f). For instance, PI 253187, PI 179926, and PI 542608 with K_s/K_c values of 0.83, 0.81, and 0.83 (Figure 2e,f) had average shoot biomass STI of 1.17, 0.79, and 0.79, respectively (Figure 1c). It was demonstrated earlier in guar and *Prunus* that the leaf K_s/K_c ratio is a better indicator of salinity tolerance of a genotype under salinity in comparison to leaf K concentration under salinity (Sandhu et al., 2020a; Sandhu et al., 2021).

3.3 | Expression analyses

The expression analysis of 10 genes involved in salinity tolerance was carried out on six genotypes (PI 164486, PI 253186, PI 26152, PI 158125, PI 179926, and PI 263698) selected based on the evaluation of 24 genotypes for shoot biomass STI, leaf Na accumulation, leaf Cl accumulation, and K_s/K_c. Of the 10 selected genes, 5 were involved in Na transport,

including *AKT1*, *HKT1*, *NHX1*, *SOS1*, and *SOS2* (Figure 3), and 5 were involved in Cl transport, which included *ALMT9*, *CCC*, *CLCc*, *CLCg*, *SLAH3* (Figure 4).

Among the genes involved in Na^+ transport, *AKT1* plays an important role in Na^+ and K^+ homeostasis in plants (Ragel et al., 2019). PI 158125 had upregulation of *AKT1* under salinity compared to control in the roots and leaves (Figure 3a,b). *AKT1* expression in leaves was significantly higher in salt-tolerant genotypes (PI 263698 and PI 179926) compared to salt-sensitive ones (PI 164486 and PI 253186) (Figure 3b). Additionally, *AKT1* expression was upregulated in PI 263698 leaves but downregulated in PI 164486 and PI 253186 (Figure 3b). Furthermore, *AKT1* was also upregulated in PI 158125 roots under salinity compared to the control, whereas other genotypes did not show any significant change under salinity. The higher expression of *AKT1* in salt-tolerant genotypes compared to salt-sensitive ones and the induction of *AKT1* under salinity compared to control suggest a critical role of *AKT1* in salinity tolerance in guar.

HKT1 is known to retrieve Na^+ from the xylem back into the roots, thereby restricting the root-to-shoot movement of Na^+ (Kaundal et al., 2019; Rubio et al., 1995). Downregulation of *HKT1* in roots of PI 253186 may explain its low performance under salinity (Figure 3c). Also, the upregulation of *HKT1* in leaves of PI 164486 and PI 263698 may be the reason for their relatively low leaf and high root Na concentrations (Figures 2a,b and 3d).

NHX1, a Na^+/H^+ exchanger known to sequester Na^+ in vacuoles (Barragan et al., 2012), was downregulated in roots of PI 253186 and leaves of PI 164486, justifying their poor performance under salinity, based on STI for shoot biomass (Figure 3e,f).

SOS1 and *SOS2* are components of the salt overly sensitive pathway in plants that regulates the efflux of Na^+ from root to soil (Ji et al., 2013). *SOS1* was induced in the roots of PI 158125 and PI 263698 under salinity compared to the control (Figure 3h). However, it was repressed in the leaves of PI 164486 and PI 179926 under salinity compared to the control (Figure 3h). *SOS2* was upregulated in PI 263698 roots under salinity compared to the control (Figure 3i). In leaves, *SOS2* was repressed in PI 164486 but induced in PI 179926 under salinity compared to the control (Figure 3j). Upregulation of *SOS1* and *SOS2* under salinity compared to control in PI 263698 roots may explain its low leaf Na concentration (Figure 3g,i).

Among the genes involved in Cl^- transport, *ALMT9* is known to be involved in the sequestration of Cl^- in root vacuoles (Li et al., 2017). Although *ALMT9* tended to be upregulated under salinity compared to control in roots, the differences were not significant (Figure 4a). *ALMT9* was downregulated in PI 253186 leaves but upregulated in PI 262152 leaves under salinity compared to the control (Figure 4b). Although PI 262152 had the highest leaf Cl concentration, it showed medium biomass STI, suggesting efficient partitioning of Cl^- into the vacuoles (Figure 2c). On the other hand, PI 253186 had a medium leaf Cl concentration, but the vacuole partitioning was probably inefficient based on the downregulation of *ALMT9* (Figure 4b). Hence, it showed a low STI for biomass (Figure 1c).

CCC is involved in retrieving Cl^- back from the xylem into the root (Colmenero-Flores et al., 2007). The genotypes PI 164486 and PI 253186 showed significant downregulation of *CCC* in leaves, whereas PI 262152 had significant upregulation in leaves under salinity compared to the control (Figure 4d). *CCC* was downregulated in roots of PI 253186 (salt-sensitive) but upregulated in roots of PI 263698 (salt-tolerant) under salinity compared to control, indicating a critical role of *CCC* during salinity stress in guar (Figure 4c).

CLCc is involved in the sequestration of Cl^- in root and leaf vacuoles, and *CLCg* sequesters Cl^- in vacuoles of leaf mesophyll cells (Jossier et al., 2010; Nguyen et al., 2016). Although there was no significant differential expression of *CLCc* in roots (Figure 4e), *CLCc* displayed a significant differential expression between salinity and control in PI 253186 leaves (Figure 4f). Downregulation of *CLCc* also indicated the inability of PI 253186 to partition Cl^- into the leaf vacuole (Figure 4f), leading to the sensitivity of this genotype to salinity stress. The *CLCg* gene was repressed in PI 253186 roots (Figure 4g) and PI 164486 and PI 253186 leaves (Figure 4h) under salinity compared to control, which may be the reason for the poor performance of both PI 164486 and PI 253186, based on STI for shoot biomass (Figure 1).

SLAH3 is involved in the movement of Cl^- from the roots to the xylem (Li et al., 2016). Upregulation of *SLAH3* in PI 164486 and PI 262152 in roots may have increased the loading of Cl^- to the xylem of roots, leading to their salt sensitivity (Figure 4i). These observations are in agreement with the observation that PI 262152 had the highest leaf Cl concentration among all 24 genotypes (Figure 2c). On the other hand, the significant and considerable downregulation of this gene in PI 263698 leaves may explain its low leaf Cl concentration and concomitant salt tolerance (Figure 4j).

Transporters play crucial roles in salinity tolerance in guar (Acharya et al., 2022; Tanwar et al., 2017). The comparisons of selected genes involved in Na^+ and Cl^- transport displayed some striking differences among genotypes varying in salinity tolerance. PI 164486, one of the salt-sensitive genotypes, displayed repression of four genes involved in Na^+ transport (*AKT1*, *NHX1*, *SOS1*, and *SOS2*) and two genes involved in Cl^- transport (*CCC* and *CLCg*) in leaves under salinity compared to control (Figures 3 and 4). Even the two salt-tolerant genotypes (PI 263698 and PI 179926) showed significant differences in gene expressions (*AKT1, HKT1, SOS1, SOS2, CCC, SLAH3*), suggesting the involvement of different component traits of salinity tolerance mechanisms in these guar genotypes.

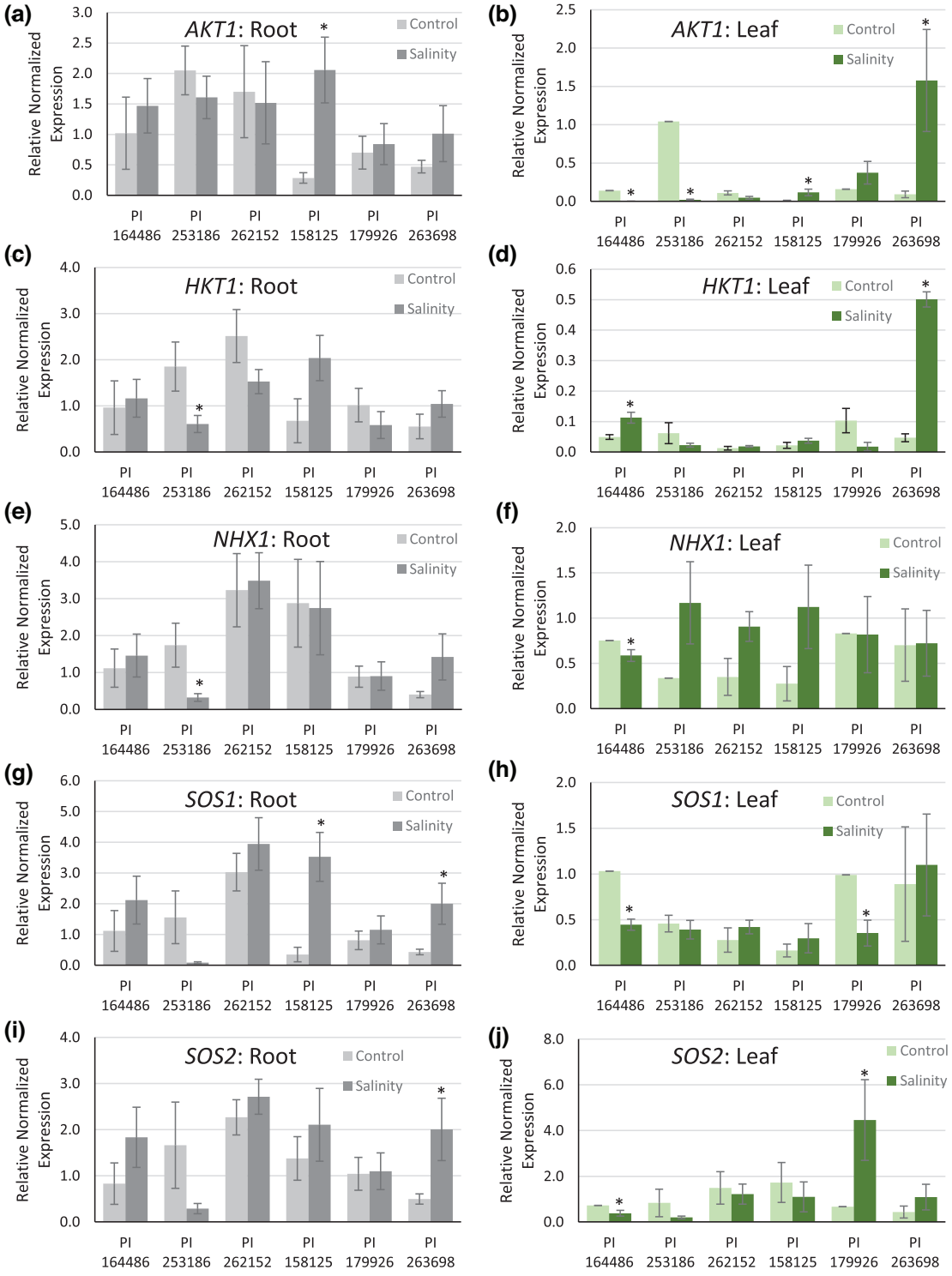

FIGURE 3 Expression of genes involved in Na^+ transport in the roots and leaves of the 24 guar genotypes irrigated with control and saline irrigation waters ($n = 3$). (a) *Arabidopsis inward rectifying K+ transporter 1* (*AKT1*) expression in roots. (b) *AKT1* expression in leaves. (c) *High-affinity potassium transporter 1* (*HKT1*) expression in roots. (d) *HKT1* expression in leaves. (e) Na+/H+ exchanger 1 (*NHX1*) expression in roots. (f) *NHX1* expression in leaves. (g) *Salt Overly Sensitive 1* (*SOS1*) expression in roots. (h) *SOS1* expression in leaves. (i) *Salt Overly Sensitive 2* (*SOS2*) expression in roots. (j) *SOS2* expression in leaves. Asterisks indicate significant differences between the control and saline conditions ($P \leq 0.05$) ($n = 3$). Error bars represent standard errors

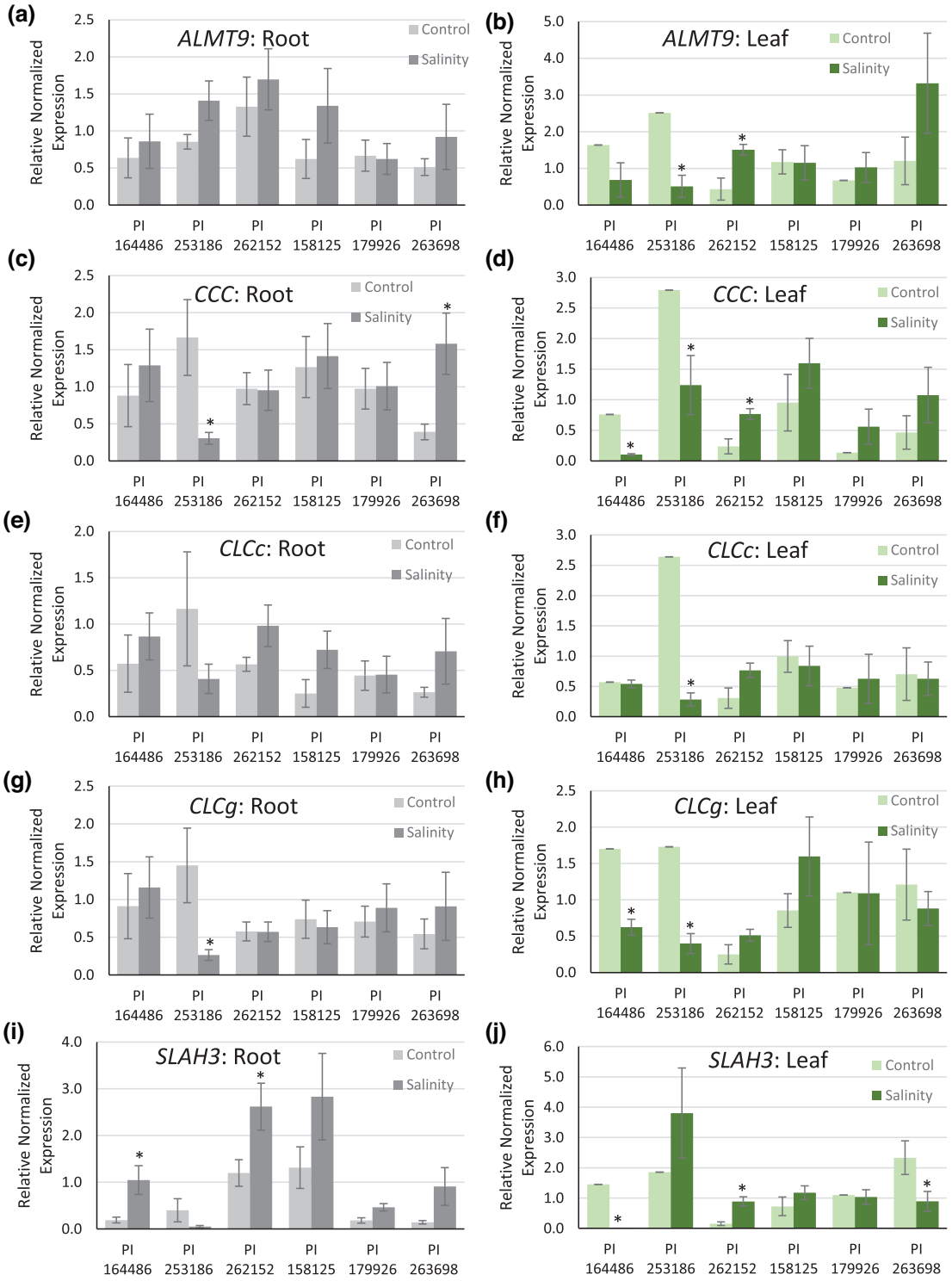

FIGURE 4 Expression of genes involved in Cl^- transport in the roots and leaves of the 24 guar genotypes irrigated with control and saline irrigation waters ($n = 3$). (a) *Aluminum-Activated Malate Transporter 9* (*ALTM9*) expression in roots. (b) *ALMT9* expression in leaves. (c) *Cation/Cl– cotransporter CCC*) expression in roots. (d) *CCC* expression in leaves. (e) *Chloride channel c* (*CLCc*) expression in roots. (f) *CLCc* expression in leaves. (g) *Chloride channel g* (*CLCg*) expression in roots. (h) *CLCg* expression in leaves. (i) *Slow-type anion channel associated homolog 3* (*SLAH3*) expression in roots. (j) *SLAH3* expression in leaves. Asterisks indicate significant differences between the control and saline conditions ($P \leq 0.05$) ($n = 3$). Error bars represent standard errors

This study led to the identification of several guar genotypes (such as PI 253187, PI 263698, and PI 179926) that can be cultivated in regions of the United States afflicted by salinity. The ion analysis and expression studies characterized genotypes based on the component traits of the salinity tolerance mechanisms. The new information conveyed here can be utilized by guar breeders to develop new salt-tolerant varieties with multiple component traits for salinity tolerance.

AUTHOR CONTRIBUTIONS

Devinder Sandhu: Conceptualization; Formal analysis; Funding acquisition; Project administration; Supervision; Visualization; Writing – original draft; Writing – review & editing. **Andrew Pallete**: Formal analysis; Investigation; Methodology; Visualization; Writing – review & editing. **Maria William**: Formal analysis; Investigation; Writing – review & editing. **Jorge Ferreira**: Formal analysis; Writing – review & editing. **Amita Kaundal**: Formal analysis; Writing – review & editing. **Kulbhushan Grover**: Conceptualization; Funding acquisition; Project administration, Supervision; Writing – review & editing.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Abidi, N., Liyanage, S., Auld, D., Norman, L., Grover, K., Augadi, S., Singla, S., & Trostle, C. (2015). Challenges and opportunities for increasing guar production in the United States to Support unconventional oil and gas production. In V. Uddameri, A. Morse, & K. J. Tindle (Eds.), *Hydraulic Fracturing Impacts and Technologies* (pp. 207–225). CRC Press.

Acharya, B. R., Sandhu, D., Dueñas, C., Ferreira, J. F. S., & Grover, K. K. (2022). Deciphering molecular mechanisms involved in salinity tolerance in guar (*Cyamopsis tetragonoloba* (L.) Taub.) using transcriptome analyses. *Plants*, *11*(3), 291. https://doi.org/10.3390/plants11030291

Al-Niemi, T. S., Campbell, W. F., & Rumbaugh, M. D. (1992). Response of alfalfa cultivars to salinity during germination and post-germination growth. *Crop Science*, *32*(4), 976–980. https://doi.org/10.2135/cropsci1992.0011183x003200040029x

Almeida, D. M., Oliveira, M. M., & Saibo, N. J. M. (2017). Regulation of Na^+ and K^+ homeostasis in plants: Towards improved salt stress tolerance in crop plants. *Genetics and Molecular Biology*, *40*(1 suppl 1), 326–345. https://doi.org/10.1590/1678-4685-GMB-2016-0106

Alshameri, A., Al-Qurainy, F., Gaafar, A. R., Khan, S., Nadeem, M., & Alansi, S. (2020). Identification of heat-responsive genes in guar [*Cyamopsis tetragonoloba* (L.) Taub]. *International Journal of Genomics*, *2020*, 3126592. https://doi.org/10.1155/2020/3126592

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnology - Advanced*, *27*(1), 84–93. https://doi.org/10.1016/j.biotechadv.2008.09.003

Ashraf, M. Y., Akhtar, K., Sarwar, G., & Ashraf, M. (2005). Role of the rooting system in salt tolerance potential of different guar accessions. *Agronomy for Sustainable Development*, *25*(2), 243–249. https://doi.org/10.1051/agro:2005019

Barragan, V., Leidi, E. O., Andres, Z., Rubio, L., De Luca, A., Fernandez, J. A., Cubero, B., & Pardo, J. M. (2012). Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor and stomatal function in Arabidopsis. *Plant Cell*, *24*(3), 1127–1142. https://doi.org/10.1105/tpc.111.095273

Colmenero-Flores, J. M., Martinez, G., Gamba, G., Vazquez, N., Iglesias, D. J., Brumos, J., & Talon, M. (2007). Identification and functional characterization of cation-chloride cotransporters in plants. *The Plant Journal*, *50*(2), 278–292. https://doi.org/10.1111/j.1365-313X.2007.03048.x

Dias, N. S., Ferreira, J. F. S., Liu, X., & Suarez, D. L. (2016). Jerusalem artichoke (*Helianthus tuberosus*, L.) maintains high inulin, tuber yield, and antioxidant capacity under moderately-saline irrigation waters. *Industrial Crops and Products*, *94*, 1009–1024. https://doi.org/10.1016/j.indcrop.2016.09.029

EPA600/4-79-020 (Ed.). (1983). Method 325.2. Chloride by Automated Colorimetry. *Environmental Protection Agency*. http://www.caslab.com/EPA-Methods/PDF/EPA-Method-3252.pdf

Francois, L. E., Donovan, T. J., & Maas, E. V. (1990). Salinity effects on emergence, vegetative growth, and seed yield of guar. *Agronomy Journal*, *82*(3), 587–592. https://doi.org/10.2134/agronj1990.00021962008200030030x

Ghaffarian, M. R., Yadavi, A., Movahhedi Dehnavi, M., Dabbagh Mohammadi Nassab, A., & Salehi, M. (2020). Improvement of physiological indices and biological yield by intercropping of Kochia (*Kochia scoparia*), Sesbania (*Sesbania aculeata*) and Guar (*Cyamopsis tetragonoliba*) under the salinity stress of irrigation water. *Physiology and Molecular Biology of Plants*, *26*(7), 1319–1330. https://doi.org/10.1007/s12298-020-00833-y

Jaiswal, P. S., Kaur, N., & Randhawa, G. S. (2019). Identification of reference genes for qRT-PCR gene expression studies during seed development and under abiotic stresses in *Cyamopsis tetragonoloba*. *Crop Science*, *59*(1), 252–265. https://doi.org/10.2135/cropsci2018.05.0313

Ji, H., Pardo, J. M., Batelli, G., Van Oosten, M. J., Bressan, R. A., & Li, X. (2013). The Salt Overly Sensitive (SOS) pathway: Established and emerging roles. *Molecular Plant*, *6*(2), 275–286. https://doi.org/10.1093/mp/sst017

Jossier, M., Kroniewicz, L., Dalmas, F., Le Thiec, D., Ephritikhine, G., Thomine, S., Barbier-Brygoo, H., Vavasseur, A., Filleur, S., &

Leonhardt, N. (2010). The Arabidopsis vacuolar anion transporter, AtCLCc, is involved in the regulation of stomatal movements and contributes to salt tolerance. *The Plant Journal*, *64*(4), 563–576. https://doi.org/10.1111/j.1365-313X.2010.04352.x

Kaundal, A., Sandhu, D., Dueñas, M., & Ferreira, J. F. S. (2019). Expression of the high-affinity K^+ *transporter 1 (PpHKT1)* gene from almond rootstock 'Nemaguard' improved salt tolerance of transgenic Arabidopsis. *PLoS ONE*, *14*(3), e0214473. https://doi.org/10.1371/journal.pone.0214473

Kays, S. E., Morris, J. B., & Kim, Y. (2006). Total and soluble dietary fiber variation in *Cyamopsis tetragonoloba* (L.) Taub. (guar) genotypes. *Journal of Food Quality*, *29*(4), 383–391. https://doi.org/10.1111/j.1745-4557.2006.00080.x

Keating, B., & Fisher, M. (1985). Comparative tolerance of tropical grain legumes to salinity. *Australian Journal of Agricultural Research*, *36*(3), 373–383. https://doi.org/10.1071/AR9850373

Li, B., Byrt, C., Qiu, J., Baumann, U., Hrmova, M., Evrard, A., Johnson, A. A., Birnbaum, K. D., Mayo, G. M., Jha, D., Henderson, S. W., Tester, M., Gilliham, M., & Roy, S. J. (2016). Identification of a stelar-localized transport protein that facilitates root-to-shoot transfer of chloride in Arabidopsis. *Plant Physiology*, *170*(2), 1014–1029. https://doi.org/10.1104/pp.15.01163

Li, B., Tester, M., & Gilliham, M. (2017). Chloride on the move. *Trends in Plant Science*, *22*(3), 236–248. https://doi.org/10.1016/j.tplants.2016.12.004

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods (San Diego, Calif.)*, *25*(4), 402–408. https://doi.org/10.1006/meth.2001.1262

Mahdipour-Afra, M., AghaAlikhani, M., Abbasi, S., & Mokhtassi-Bidgoli, A. (2021). Growth, yield and quality of two guar (*Cyamopsis tetragonoloba* L.) ecotypes affected by sowing date and planting density in a semi-arid area. *PLoS ONE*, *16*(9), e0257692. https://doi.org/10.1371/journal.pone.0257692

Mudgil, D., Barak, S., & Khatkar, B. S. (2014). Guar gum: Processing, properties and food applications-A review. *Journal of Food Science and Technology*, *51*(3), 409–418. https://doi.org/10.1007/s13197-011-0522-x

Munns, R., & Termaat, A. (1986). Whole plant responses to salinity. *Australian Journal of Plant Physiology*, *13*, 143–160. https://doi.org/10.1071/PP9860143

Nguyen, C. T., Agorio, A., Jossier, M., Depre, S., Thomine, S., & Filleur, S. (2016). Characterization of the chloride channel-like, AtCLCg, involved in chloride tolerance in *Arabidopsis thaliana*. *Plant & Cell Physiology*, *57*(4), 764–775. https://doi.org/10.1093/pcp/pcv169

Ragel, P., Raddatz, N., Leidi, E. O., Quintero, F. J., & Pardo, J. M. (2019). Regulation of K^+ nutrition in plants. *Frontiers in Plant Science*, *10*, 281–281. https://doi.org/10.3389/fpls.2019.00281

Rasheed, M. J. Z., Ahmad, K., Ashraf, M., Qurainy, F. A., Khan, S., & Athar, H. U. R. (2015). Screening of diverse local germplasm of guar (*Cyamposis tetragonoloba* (L.) Taub.) for salt tolerance: A possible approach to utilize salt-affected soils. *Pakistan Journal of Botany*, *47*(5), 1721–1726. http://inis.iaea.org/search/search.aspx?orig_q=RN:47020611

Rubio, F., Gassmann, W., & Schroeder, J. I. (1995). Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. *Science*, *270*(5242), 1660–1663. https://doi.org/10.1126/science.270.5242.1660

Sandhu, D., Cornacchione, M. V., Ferreira, J. F., & Suarez, D. L. (2017). Variable salinity responses of 12 alfalfa genotypes and comparative expression analyses of salt-response genes. *Scientific Reports*, *7*, 42958. https://doi.org/10.1038/srep42958

Sandhu, D., & Kaundal, A. (2018). Dynamics of salt tolerance: Molecular perspectives. In S. S. Gosal & S. H. Wani (Eds.), *Biotechnologies of crop improvement, Volume 3: Genomic approaches* (pp. 25–40). Springer International Publishing. https://doi.org/10.1007/978-3-319-94746-4_2

Sandhu, D., Kaundal, A., Acharya, B. R., Forest, T., Pudussery, M. V., Liu, X., Ferreira, J. F. S., & Suarez, D. L. (2020a). Linking diverse salinity responses of 14 almond rootstocks with physiological, biochemical, and genetic determinants. *Scientific Reports*, *10*(1), 21087. https://doi.org/10.1038/s41598-020-78036-4

Sandhu, D., Pallete, A., Pudussery, M. V., & Grover, K. K. (2021). Contrasting responses of guar genotypes shed light on multiple component traits of salinity tolerance mechanisms. *Agronomy*, *11*(6), 1068. https://doi.org/10.3390/agronomy11061068

Sandhu, D., Pudussery, M. V., Ferreira, J. F. S., Liu, X., Pallete, A., Grover, K. K., & Hummer, K. (2019). Variable salinity responses and comparative gene expression in woodland strawberry genotypes. *Scientia Horticulturae*, *254*, 61–69. https://doi.org/10.1016/j.scienta.2019.04.071

Sandhu, D., Pudussery, M. V., Kumar, R., Pallete, A., Markley, P., Bridges, W. C., & Sekhon, R. S. (2020b). Characterization of natural genetic variation identifies multiple genes involved in salt tolerance in maize. *Functional & Integrative Genomics*, *20*(2), 261–275. https://doi.org/10.1007/s10142-019-00707-x

Sharma, G., Sharma, S., Kumar, A., Al-Muhtaseb, A. H., Naushad, M., Ghfar, A. A., Mola, G. T., & Stadler, F. J. (2018). Guar gum and its composites as potential materials for diverse applications: A review. *Carbohydrate Polymers*, *199*, 534–545. https://doi.org/10.1016/j.carbpol.2018.07.053

Shrestha, R., Adams, C. B., & Rajan, N. (2022). Does the drought tolerance of guar [*Cyamopsis tetragonoloba* (L.) Taub.] extend belowground to root nodules? *Journal of Agronomy and Crop Science*, *208*(5), 599–608. https://doi.org/10.1111/jac.12494

Singh, S. K. (2014). An analysis of guar crop in India. https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=An%20Analysis%20of%20Guar%20Crop%20in%20India_New%20Delhi_India_5-6-2014.pdf

Singla, S., Grover, K., Angadi, S. V., Begna, S., Schutte, B. J., & VanLeeuwen, D. (2016a). Growth and yield of guar (*Cyamopsis tetragonoloba* L.) genotypes under different planting dates in the semi-arid Southern High Plains. *Journal of Agronomy and Crop Science*, *7*, 1246–1258. https://doi.org/10.4236/ajps.2016.78120

Singla, S., Grover, K., Angadi, S. V., Schutte, B., & VanLeeuwen, D. (2016b). Guar stand establishment, physiology, and yield responses to planting date in southern New Mexico. *Agronomy Journal*, *108*(6), 2289–2300. https://doi.org/10.2134/agronj2016.04.0206

Summers, H. M., Sproul, E., Seavert, C., Angadi, S., Robbs, J., Khanal, S., Gutierrez, P., Teegerstrom, T., Zuniga Vazquez, D. A., Fan, N., & Quinn, J. C. (2021). Economic and environmental analyses of incorporating guar into the American southwest. *Agricultural Systems*, *191*, 103146. https://doi.org/10.1016/j.agsy.2021.103146

Suthar, J. D., Rajpar, I., Ganjegunte, G. K., & Shah, Z.-u.-h. (2018). Evaluation of guar (*Cyamopsis tetragonoloba* L.) genotypes performance under different irrigation water salinity levels: Growth parameters and

seed yield. *Industrial Crops and Products*, *123*, 247–253. https://doi.org/10.1016/j.indcrop.2018.06.084

Suthar, J. D., Rajpar, I., Ganjegunte, G. K., Shah, Z. - U. - H., Niu, G., & Grover, K. (2019). Germination, growth, and ion uptake of 15 guar accessions under elevated salinity. *Agrosystems, Geosciences & Environment*, *2*(1), 190020. https://doi.org/10.2134/age2019.03.0020

Tanwar, U. K., Pruthi, V., & Randhawa, G. S. (2017). RNA-Seq of guar (*Cyamopsis tetragonoloba*, L. Taub.) leaves: *De novo* transcriptome assembly, functional annotation and development of genomic resources. *Frontiers in Plant Science*, *8*, 91–91. https://doi.org/10.3389/fpls.2017.00091

Thapa, S., Adams, C. B., & Trostle, C. (2018). Root nodulation in guar: Effects of soils, Rhizobium inoculants, and guar varieties in a controlled environment. *Industrial Crops and Products*, *120*, 198–202. https://doi.org/10.1016/j.indcrop.2018.04.060

Tripathi, R. K., Aguirre, J. A., & Singh, J. (2021). Genome-wide analysis of *wall associated kinase (WAK)* gene family in barley. *Genomics*, *113*(1 Pt 2), 523–530. https://doi.org/10.1016/j.ygeno.2020.09.045

van Zelm, E., Zhang, Y., & Testerink, C. (2020). Salt tolerance mechanisms of plants. *Annual Review of Plant Biology*, *71*, 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

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