

# Screening Guar (*Cyamopsis tetragonoloba* L.) Genotypes for Salt Tolerance using Physiological Indicators and Yield Performance

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## ABSTRACT

Guar (*Cyamopsis tetragonoloba* L.) is an important industrial legume widely cultivated across arid and semi-arid regions. To identify salt-tolerant genotypes, the fifteen guar genotypes originating from Iran, India, and Pakistan were evaluated under three salinity levels (0, 10, and 15 dS/m) in a factorial RCBD design. Salinity imposed highly significant reductions in physiological traits and caused phenological delays in guar genotypes. Under severe salt stress (15 dS/m), seed yield decreased across all genotypes. In response to 15 dS/m NaCl, the lowest yield reductions were recorded for the genotypes S6553, Saravan, S6260, and RGC-1031, at 16.3, 20.4, 21.2, and 21.4%, respectively, demonstrating relative tolerance to salt. In contrast, the largest decrease was observed in genotypes S-5885, S6566, and S-6560, by 31.4, 30.5, and 30.5%, respectively, confirming their relative sensitivity to salt stress. Although genotypes S6553, Saravan, S6260, and RGC-1031 exhibited the lowest yield reductions at 15 dS/m NaCl, overall, genotype RGC-986 consistently displayed superior performance across all salinity levels, while genotype Pishen was the most adversely affected, exhibiting sharp declines in physiological performance and delayed phenology. Inclusive, the study demonstrated substantial genetic variability in guar responses to salinity and highlighted key physiological traits, such as chlorophyll retention, osmotic adjustment, membrane stability, and growth maintenance, as essential determinants of tolerance. RGC-986 genotype, identified as salt-tolerant, represents valuable candidates for breeding programs and for sustainable guar cultivation in salinity-affected regions.

**Keywords:** Legume, Galactomannan, Variation, Salt tolerance

## INTRODUCTION

*Cyamopsis tetragonoloba* (L.) Taub has substantial economic value owing to the by-product galactomannan extracted from seeds, which is used in several industries, including food and cosmetics [1-6, 50]. It has recently been demonstrated that guar exhibits a variable response to salinity stress. For example, in a field experiment, different genotypes exhibited substantial differences in agronomic characters in response to salinity, with Matador and PI 268229 showing higher shoot and root biomass salt tolerance indices than PI 340261 and PI 537281 [7]. Salinity stress poses a significant threat to crop production, especially in arid and semi-arid regions [8, 9]. Salt stress (SS) clearly restricts the growth and development of vegetation, resulting in considerable yield loss [10, 11]. Many reports have confirmed that salt stress adversely affects the growth and metabolism of medicinal industrial plants [12, 13, 51]. The adverse effects of salinity on plant growth are primarily due to an increase in osmotic potential and the toxic accumulation of sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions, which disrupt the physiological functions of the plasma membrane, such as water and nutrient absorption, and cause ion imbalance [14, 15]. Selecting salt-tolerant genotypes can be an effective way to cultivate legumes in saline soils [16]. We conducted the present study to identify superior genotypes tolerant of salt stress and to develop guar varieties for cultivation in saline soils.

## MATERIALS AND METHODS

### Field Site Description, Plant Materials and Research Design

In a factorial farm trial (RCBD) at Ardakan University, Yazd, Iran, fifteen guar genotypes from India, Pakistan, and Iran (Table 1) were exposed to 0, 10, and 15 dS/m salinity. The local Iranian guar seeds were prepared and authenticated by the Medicinal Plant Research Institute, Ardakan University, Iran and other seeds were supplied by the Rajasthan Agricultural Research Center, India. The study was conducted in 2023 as a factorial experiment using a randomized complete block design (RCBD) with three replications (n=3). The seeds were sown on July 15 in 3 m × 4 m plots at a density of 8 seeds m<sup>-2</sup> (30 × 50 cm row spacing).

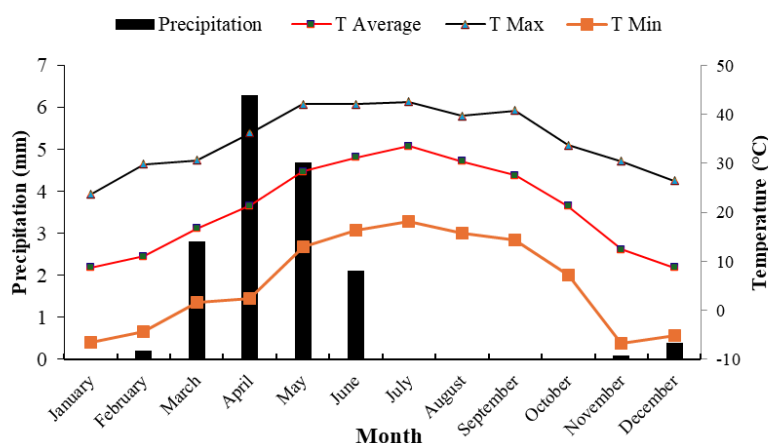
The main physicochemical properties of the field soil were presented in Table 2. Before sowing, fertilization was carried out at a total rate of 40 kg/ha N and 50 kg/ha P<sub>2</sub>O<sub>5</sub>. Water was applied through a trickle (drip) irrigation system. A total of four irrigation events were conducted throughout the plants' growth period. Salinity treatments were imposed by dissolving sodium chloride (NaCl) in the irrigation water to achieve the target electrical conductivity levels (0, 10, and 15 dS/m). Salinity stress was imposed at the two-leaf stage and maintained until harvest. Weed control was carried out manually by hoeing. The thermo-pluviometric diagram of the experimental field is shown in Figure 1. Harvest-related traits were measured when more than 70% of the plant pods had turned dry and brown, indicating physiological maturity. Harvest time was recorded for all treatments, and samples were subsequently collected for further analyses.

**Table 1** The specification of guar (*C. tetragonoloba* L.) genotypes

Genotype ID	Genotypes name	Origin of genotypes
1	RGC-986	Rajhestan, India
2	S6673	Bahawalpur, Pakistan
3	BR-99	Bahawalpur, Pakistan
4	S6566	Bahawalpur, Pakistan
5	S-6560	Rajhestan, India
6	S-5885	Bahawalpur, Pakistan
7	S-6581	Bahawalpur, Pakistan
8	Grembite	Sistan va Baluchistan, Iran
9	S6260	Bahawalpur, Pakistan
10	Saravan	Sistan va Baluchistan, Iran
11	S6553	Bahawalpur, Pakistan
12	S6486	Bahawalpur, Pakistan
13	RGC-1031	Bahawalpur, Pakistan
14	RGC-1066	Rajhestan, India
15	Pishen	Sistan va Baluchistan, Iran

**Table 2** The soil physical and chemical characteristics of the experimental field before planting

Texture	Clay (%)	Silt (%)	Sand (%)	K (mg/kg)	P (mg/kg)	N (%)	O.C (%)	pH	EC (dS/m)
Clay-silt	37	12	14	264	8.4	0.17	1.07	7.8	3.12

**Fig. 1** The precipitation and temperature values during the experiment period

### Measurement of Morphological, Yield, and Yield Components Parameters

Physiological and phenological characteristics were recorded from 10 randomly selected plant of each plot. The chlorophyll content (n=30 leaves) was measured with a SPAD-502 chlorophyll meter. Proline content was measured according to the method of Bates *et al.* [17]. The standard curve was illustrated according to Bates *et al.* [17]. The OD of plant samples and proline standard was read at 520 nm by BioTek Gen 5 software in a BioTek PowerWave XS2 Microplate Spectrophotometer, USA. Then, the OD of each sample entered the standard equation and was reported as  $\mu\text{g/g}$  FW [17].

Malondialdehyde (MDA) content was determined as described by Hodges *et al.* [18], and  $\text{CO}_2$  concentration was measured based on Moss and Rawlins [19]. To determine the RWC (%), 30 young leaves were selected from each plant, separated, and immediately weighed (LFW) in the laboratory with a scale (Sartorius BP210D, Germany; 0.0001 g); then they were placed in DDW for 16 to 18 hours (for complete dehydration) in a laboratory environment with an approximate temperature of 22 °C. The leaf surface water was dried with filter paper, and the samples were reweighed (LTW). The leaves were placed in an oven at 70 °C for 48 hours, and the LDW measured. The means of LFW and LDW were calculated (mg). RWC calculated from the following formula [20].

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

### Data Analysis

Data were analyzed using R software (version 4.3.1). A two-way ANOVA was used to evaluate the effects of genotype, salinity, and their interaction. Tukey's HSD test was employed for mean comparisons when ANOVA results were significant.

## RESULTS AND DISCUSSION

Analysis of variance revealed that salt stress exerted a highly significant effect ( $p < 0.001$ ) on all physiological and phenological traits evaluated. Traits such as total chlorophyll content,  $\text{CO}_2$  concentration, relative water content, MDA accumulation, electrolyte leakage, proline content, root length, and early growth parameters showed strong reductions or increases under salt stress. Genotypic differences were also significant for most traits, including total chlorophyll, MDA, electrolyte leakage, root length, and all phenological and yield

traits, indicating substantial variability among genotypes in their inherent performance. Furthermore, the salt stress  $\times$  genotype interaction was significant for most variables.

### **Total Chlorophyll**

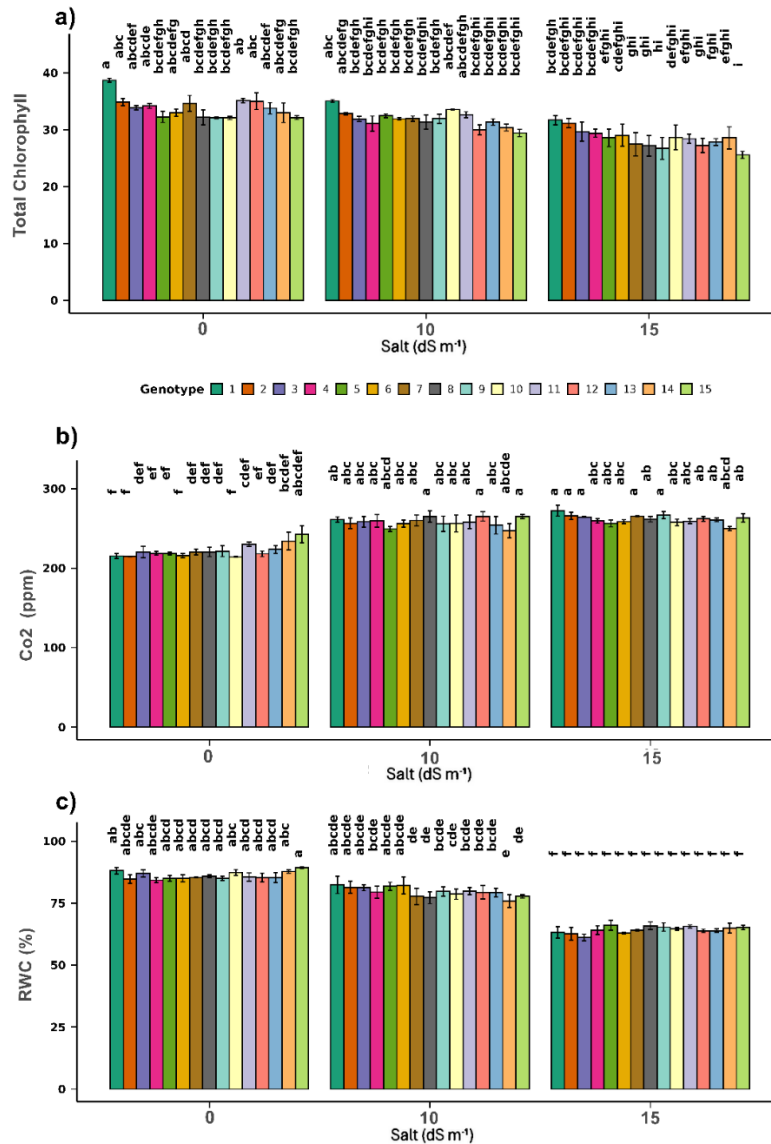
A clear and significant Salt  $\times$  Genotype interaction was observed for total chlorophyll, with values ranging from 38.7 (0 dS/m salt in genotype 1) to 25.57 (15 dS/m  $\times$  genotype 15) (Fig. 2a). Under non-saline conditions (0 dS/m), genotype 1 exhibited the highest chlorophyll content (38.7), representing the maximum observed across all treatments, followed closely by genotypes 11 and 12. Several other genotypes also maintained relatively high chlorophyll levels at this salt level, ranging between 34.2 and 35.0. At moderate salinity (10 dS/m), chlorophyll values decreased for all genotypes, but genotype 10 (33.56) and genotype 2 (32.83) preserved the highest values, indicating stronger pigment stability under increasing ionic stress. Under severe salinity (15 dS/m), chlorophyll reduction was most pronounced, and all genotypes showed their lowest values. Genotype 15 showed the lowest chlorophyll content (25.57), followed by genotypes 9, 8, 12, and 7 (27.2–27.5), indicating strong susceptibility to salinity. In contrast, genotype 1 remained among the highest performers even at the highest stress level (31.7), retaining substantially more chlorophyll than other genotypes. The strong interaction pattern reflects differential genotypic capacity to maintain pigment stability across stress levels, with genotypes 1, 2, 3, and 10 performing consistently better, while genotypes 12, 14, and 15 showed the sharpest declines in chlorophyll content under salinity.

### **CO<sub>2</sub> Concentration**

The interaction between salinity and genotype significantly affected CO<sub>2</sub> concentration, which ranged from a maximum of 272.33 ppm in genotype 1 under 15 dS/m NaCl to a minimum of 214.33 and 214.66 ppm in genotype 10 and 2 under non-stress condition, respectively (Fig. 2b). Under severe salinity (15 dS/m NaCl), the genotypes 1, 9, 2, 7, and 3 exhibited the highest CO<sub>2</sub> concentrations (265–272 ppm), suggesting an enhancement of internal CO<sub>2</sub> buildup under stress due to stomatal restriction (Fig. 2b). Under moderate salinity (10 dS/m NaCl), the genotypes 12, 15, and 8 (all 265 ppm of CO<sub>2</sub> concentration), also ranked among the top statistical group, indicating that moderate stress similarly increased CO<sub>2</sub> concentration in multiple genotypes. Under non-saline conditions, CO<sub>2</sub> concentrations were uniformly lower and more variable across genotypes, with the lowest values recorded for genotypes 6, 1, 2, and 10 (214–216 ppm). The lowest CO<sub>2</sub> values overall were observed under non-stress conditions, confirming that the CO<sub>2</sub> concentration increased with salinity levels. Collectively, the interaction data demonstrate that CO<sub>2</sub> accumulation increases strongly under salinity, with some genotypes, particularly 1, 2, 3, 7, 8, 9, and 15, showing the most pronounced increases.

### **Relative Water Content (RWC)**

RWC displayed a strong and highly structured salt  $\times$  genotype interaction, with values ranging from a maximum of 89.3% which observed in genotype 15 under non-saline condition to a minimum of 61.1% (observed under severe salinity in genotype 3) (Fig. 2c). Under non-stress condition (0 dS/m NaCl), all genotypes maintained high RWC, generally above 84%, so that in genotype 15 achieved to the maximum value (89.3%), followed closely by genotypes 1, 14, 10, and 3. At moderate salinity conditions (10 dS/m NaCl), RWC declined in all genotypes, falling to 75.8–82.3% for most genotypes. Under this condition, the genotypes 1, 2, and 3 retained the highest hydration (82.3%, 82.2%, and 81.7%, respectively, while genotype 14 showed the largest decline within this group (75.83%). Under severe NaCl stress (15 dS/m NaCl), RWC decreased sharply to 61–66%, representing the lowest hydration levels. At 15 dS/m NaCl, genotypes 3, 2, 6, and 1 exhibited the minimum RWC values (61–63%), demonstrating high susceptibility to severe salt stress. Conversely, genotypes 5, 8, 11, 9, and 15 maintained relatively higher RWC values (65–66%) at 15 dS/m NaCl, indicating superior osmotic adjustment and water retention capacity. The interaction pattern demonstrated that while RWC values decreased substantially across all genotypes under 0 to 15 dS/m NaCl, genotypes 5, 8, 9, 11, and 15 maintained water status more effectively under severe salt stress.



**Fig. 2** The effect of 0, 10, and 15 dS/m NaCl on Total chlorophyll content (a), CO<sub>2</sub> content (b), and relative water content (c) of different guar genotypes (Tukey test,  $\alpha=0.05$ ,  $n=30$ ).

### MDA Concentration

MDA content, an indicator of lipid peroxidation and oxidative stress, increased sharply with higher salinity, displaying the strong stress-dependent responses (Fig. 3a). Under control conditions, most genotypes maintained low MDA values (2.0–2.5 mol/g FW), with genotype 10 showing the lowest oxidative damage and genotype 7 presenting slightly higher values. Under moderate stress (10 dS/m NaCl), MDA increased substantially across genotypes. Genotype 6 exhibited the highest MDA accumulation in response to 10 dS/m NaCl, whereas genotypes 1, 3, and 5 showed relatively lower increases, suggesting better oxidative protection mechanisms stress (Fig. 3a). Severe salinity (15 dS/m NaCl), induced the highest MDA levels overall, with several genotypes, particularly 6, 7, 10, and 12, exceeding 3.8–4.2 mol/g FW, which represented the maximum oxidative damage. Meanwhile, genotypes 1, 3, and 8 maintained the lowest MDA concentrations under severe salt stress (Fig. 3a).

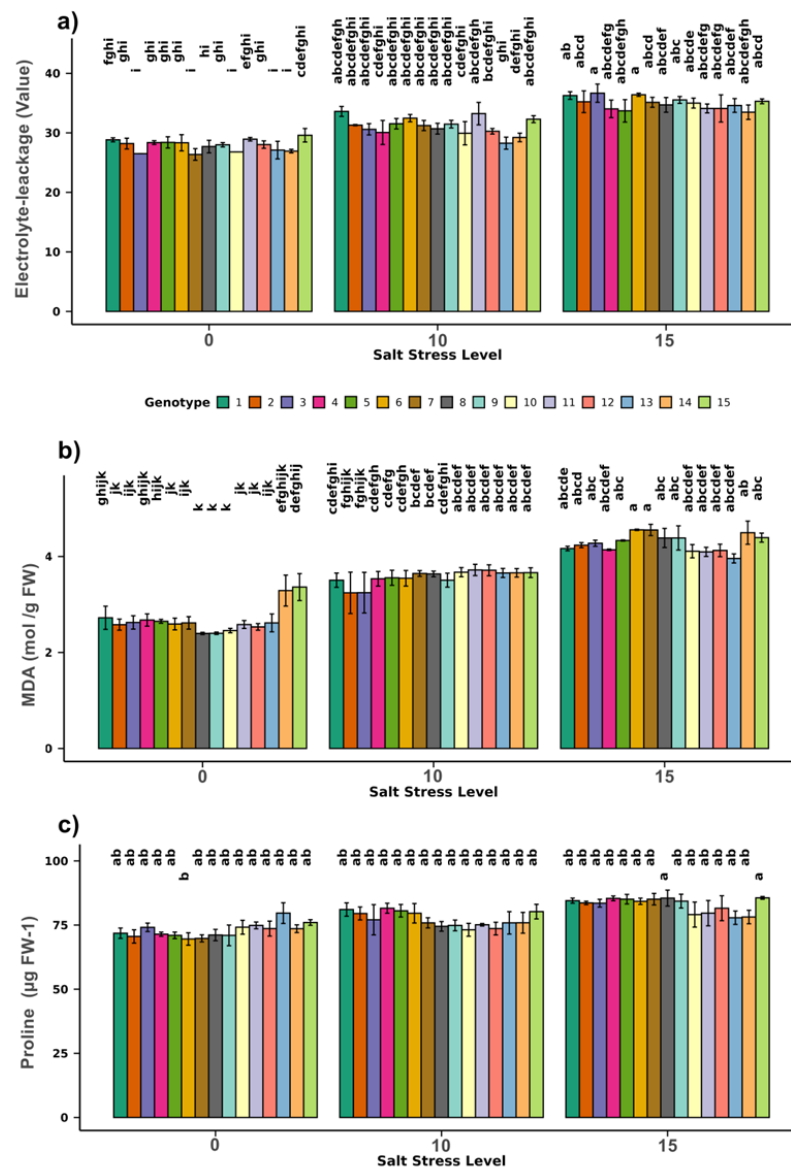
### Electrolyte Leakage

A similar pattern was found for electrolyte leakage, where genotypes exposed to 15 dS/m NaCl, particularly genotypes 3, 6, 1, and 9, showed the highest leakage rates, confirming substantial membrane injury at severe saline conditions. Conversely, the genotypes 13, 14, 10, 3, and 7 displayed the lowest leakage values under control conditions (Fig. 3b). Under low stress (10 dS/m NaCl), leakage rose noticeably across all genotypes, with the highest values (33–35 units) in genotypes 7, 10, and 12, while genotypes 1 and 3 continued to exhibit the lowest values. Under the highest stress condition (15 dS/m NaCl), electrolyte leakage reached its highest values, ranging from approximately 33 to 38 units. The highest leakage was recorded in genotypes 7, 10, and 12, indicating heightened membrane sensitivity, whereas genotypes 1, 3, and 5 retained comparatively lower leakage despite the severe stress.

### Proline Acclimation

Proline content also increased markedly under high salinity, with the greatest accumulation occurring in genotypes 15, 8, 4, 7, and 5 at 15 dS/m NaCl (Fig. 3c). In the control plants, proline values ranged mainly from 70 to 90  $\mu\text{g}/\text{FW}$ , with genotypes 1 and 5 presenting slightly higher accumulation, while genotypes 12 and 14 exhibited the lowest values. In response to 10 dS/m NaCl, most genotypes showed a modest increase, with concentrations ranging from 85 to 95  $\mu\text{g}/\text{FW}$ . Genotypes 1, 3, 5, and 7 recorded the highest proline amounts, whereas

11 and 14 accumulated the least. Under the highest stress level (15 dS/m NaCl), proline content increased further, reaching the maximum values (90–100 µg/FW). The largest increases were observed in genotypes 1, 5, 7, and 10, which markedly outperformed others in osmolyte accumulation, whereas the genotypes 11, 12, and 14 continued to exhibit comparatively lower concentrations (Fig. 3c).



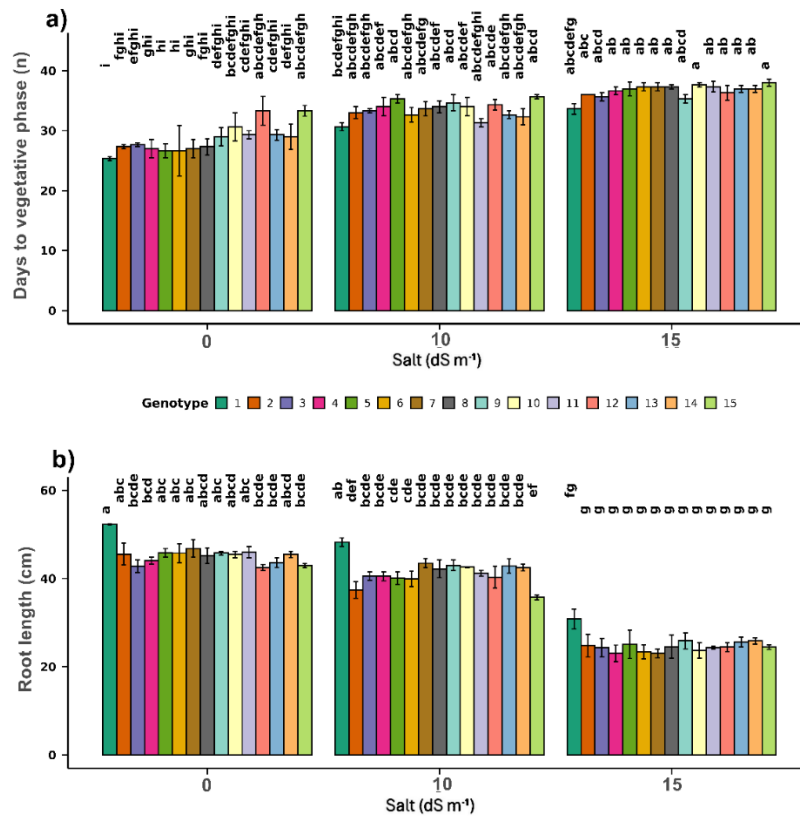
**Fig. 3** The effect of 0, 10, and 15 dS/m NaCl on electrolyte-leakage (a), MDA content (b) and proline content (c) of different guar genotypes (Tukey test,  $\alpha=0.05$ ,  $n=30$ ).

### Days to Vegetative Phase

Time to reach the vegetative phase also varied as a function of salt  $\times$  genotype interaction. The longest developmental duration was observed for genotype 15 under the effect of 15 dS/m NaCl (38.0 days) (Fig. 4a). In contrast, the shortest time to vegetative phase was observed in control plants of genotype 1, which completed this stage in 25.3 days. A clear pattern emerged in which most genotypes exposed to high salinity (15 dS/m NaCl) exhibited significantly delayed vegetative development, while control plants and genotypes grown under lower salinity (10 dS/m NaCl) reached the vegetative stage earlier (Fig. 4a).

### Root Length

The longest roots were measured for genotype 1 under non-stress conditions (52.3 cm). In contrast, the shortest roots were observed across multiple sensitive genotypes under high salinity, with the lowest value (23.0 cm) recorded for genotypes 4 and 7 under application of 15 dS/m NaCl (Fig 4b).



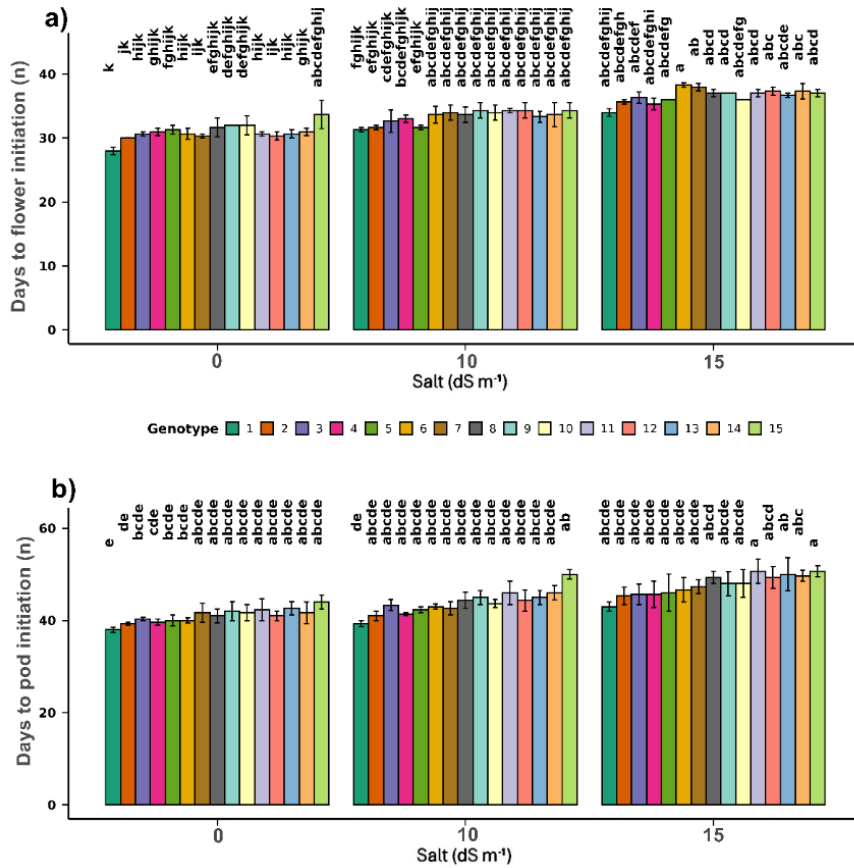
**Fig. 4** The effect of 0, 10, and 15 dS/m NaCl on days to vegetative phase (a) and root length (b) of different guar genotypes (Tukey test,  $\alpha=0.05$ ,  $n=30$ ).

#### Days to Flower Initiation

The longest time to flower initiation (38.3 days) occurred in genotype 6 in response to 15 dS/m NaCl, and in the next step for several genotypes, including 7, 12, 14, and 11 exposed to the 15 dS/m NaCl (Fig. 5a). In contrast, the earliest flowering was recorded in genotype 1 under non-stress conditions (28.0 days). Overall, most genotypes subjected to 15 dS/m NaCl clustered in the upper groups (a–f), whereas control plants and genotypes grown at salinity 10 dS/m NaCl were predominantly placed in the lower groups (g–k). This pattern demonstrates that increasing salinity leads to a progressive delay in the onset of flowering, with genotypic differences strongly influencing the magnitude of this response.

#### Days to Pod Initiation (n)

A similarly wide range of variation was observed for days to pod initiation under the salt  $\times$  genotype interaction. The greatest delay occurred in treatments with genotype 11 under severe salt stress (50.7 days). The shortest duration to pod initiation was found in genotype 1 under non-stress conditions (38.0 days) (Fig. 5b). In general, the results indicate that higher salinity consistently delays pod initiation, and that genotypic variation plays an important role in determining the extent of this delay.



**Fig. 5** The effect of 0, 10, and 15 dS/m NaCl on days to flowering (a) and days to pod initiation (b) of different guar genotypes (Tukey test,  $\alpha=0.05$ ,  $n=30$ ).

### Days to 50% Podding

The longest duration of days to 50% podding (72.3 days) was recorded for genotype 15 under application of 15 dS/m NaCl, followed by genotype 15 under application of 10 dS/m NaCl (66.7 days) and the genotypes of 11, 12, 10, and 14 under application of 15 dS/m NaCl (Fig. 6a). In contrast, the shortest time to 50% podding (50.3 days) observed for genotype 1 in the non-stress condition. Overall, all genotypes exposed to high salinity (15 dS/m NaCl) exhibited a pronounced delay in pod development. These results highlight the strong inhibitory effect of salinity on pod development.

### Days to Maturity (n)

Days to maturity also differed among the salt  $\times$  genotype combinations. The longest maturity period (131.3 days) was observed in genotype 15 under 15 dS/m NaCl, followed by genotypes 7, 8, 3, and 4 in 15 dS/m NaCl, reflecting significant delays in crop completion under high salt stress (Fig. 6b). The shortest time to maturity (95.7 days) occurred in genotype 1 under salt control conditions. Overall, the results clearly show that increased salinity prolongs the time to maturity, and that genotypes differ substantially in their responsiveness to salt stress during the late stages of development.

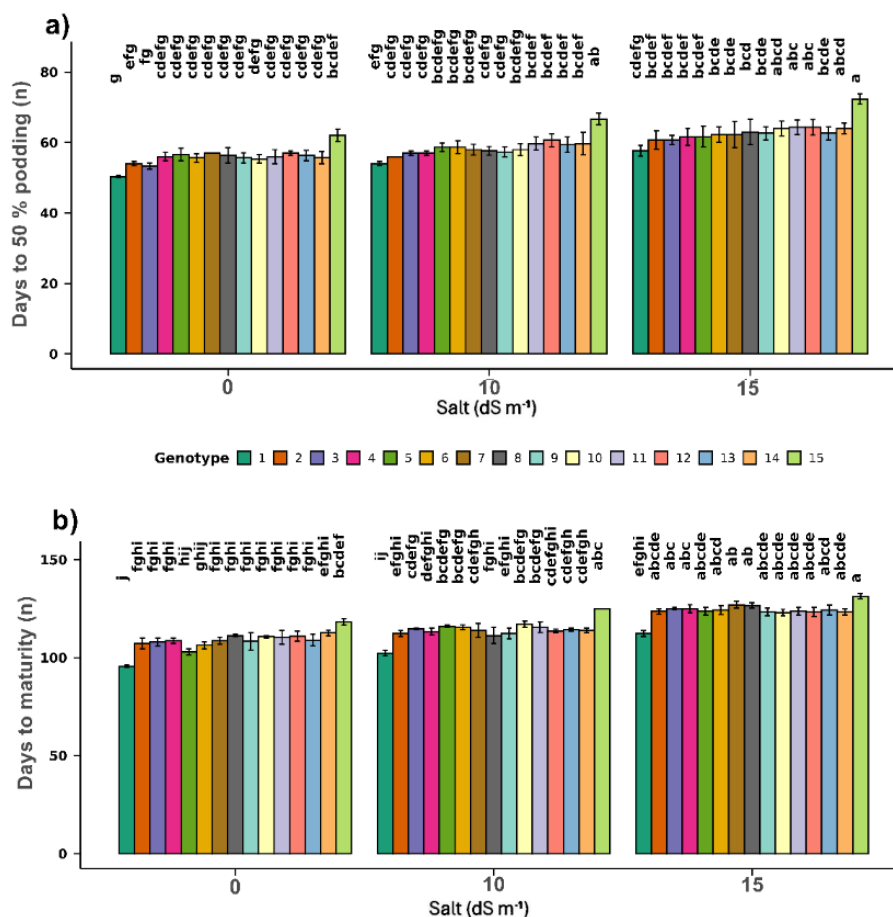


Fig. 6 The effect of 0, 10, and 15 dS/m NaCl on days to 50% podding (a) and days to maturity (b) of different guar genotypes (Tukey test,  $\alpha=0.05$ ,  $n=30$ ).

### The Reduction of Seed Yield under Stress Condition

Under severe salt stress (15 dS/m), seed yield decreased across all genotypes. The lowest reductions were recorded for the genotypes 11, 10, 9, and 13 by 16.3, 20.4, 21.2, and 21.4% respectively, demonstrating relative tolerant genotypes to salt, whereas the highest decrease was observed in genotypes 6, 4, and 5 by 31.4, 30.5, and 30.5%, respectively, which confirmed the relative sensitivity to salt stress (Fig. 7).

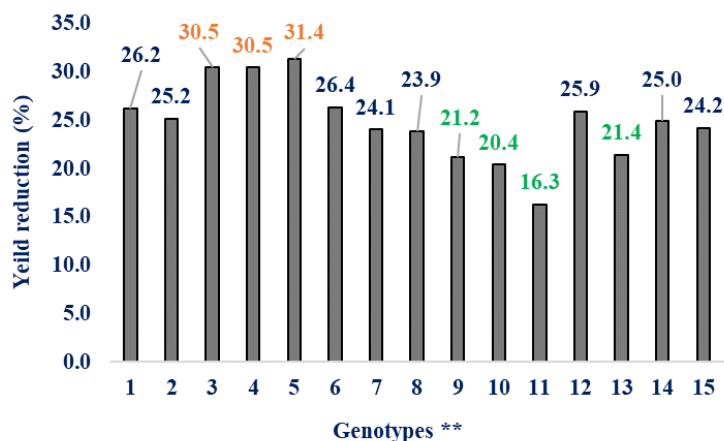
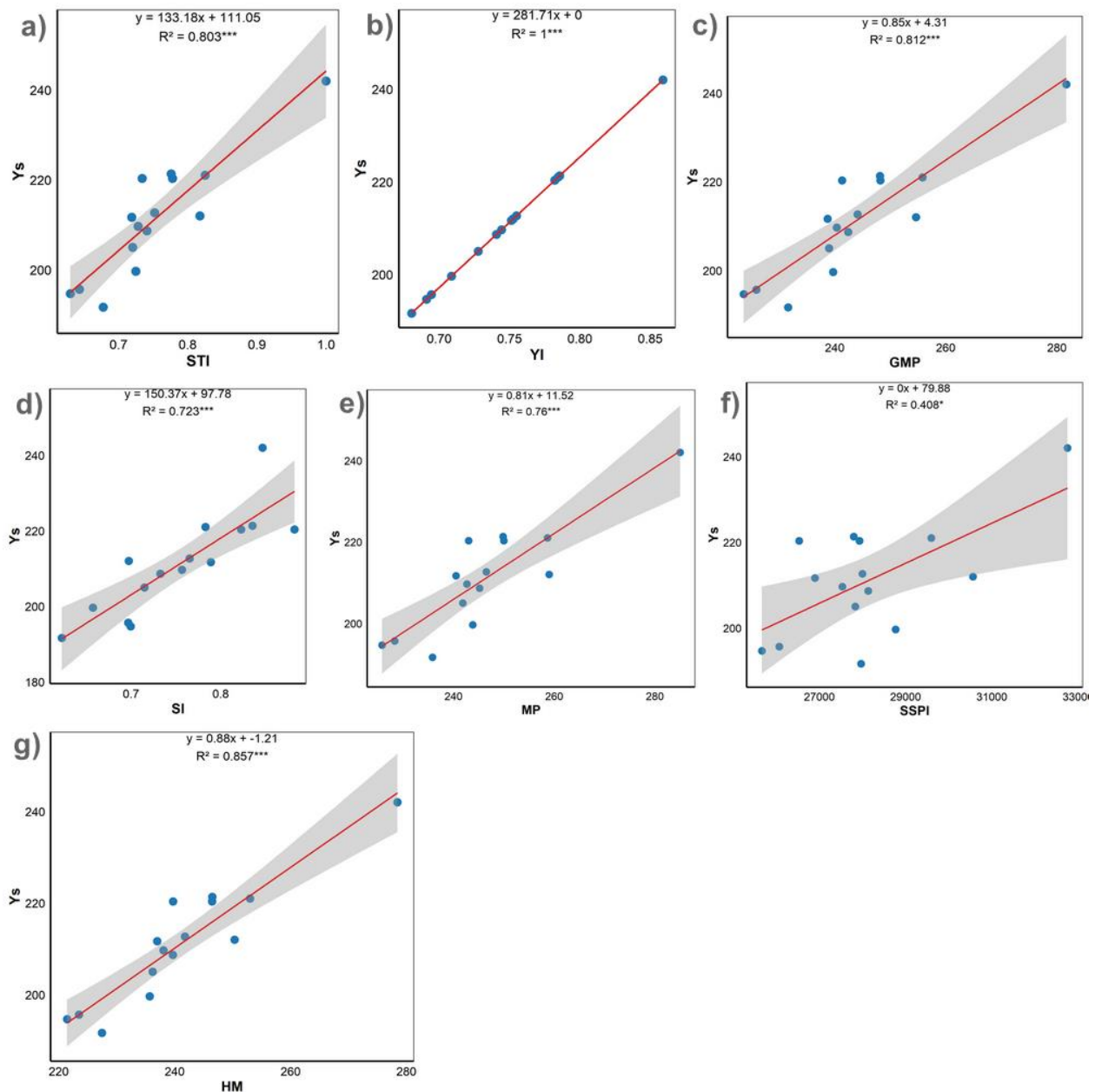


Fig. 7 Yield reduction (%) of different guar genotypes under severe salt stress (15 dS/m) compared with non-stress conditions (Tukey's test,  $\alpha = 0.05$ ,  $n = 30$ ).

### Regressions under Saline Conditions

Under stress conditions, the seed yield ( $Y_s$ ) showed strong and highly significant positive linear associations with STI ( $R^2 = 0.803$ ), YI ( $R^2 = 1.00$ ), GMP ( $R^2 = 0.812$ ), SI ( $R^2 = 0.723$ ), MP ( $R^2 = 0.760$ ), and HM ( $R^2 = 0.857$ ), indicating that genotypes with higher values for these indices generally produced higher yields under salinity (Fig. 8 a–g). Among these, YI exhibited a nearly perfect linear relationship with  $Y_s$ , reflecting its direct dependence on stress-condition yield. ST-related indices such as STI, GMP, MP, and HM also demonstrated high explanatory power, suggesting their strong suitability for identifying salt-tolerant genotypes. In contrast, SSPI showed only a moderate but significant association with  $Y_s$  ( $R^2 = 0.408$ ), indicating weaker predictive ability than the other indices. Overall, the results

highlight that indices integrating both stress and non-stress performance (STI, GMP, MP, HM) are more robust indicators of salt-stress yield than SSPI.



**Fig. 8** The linear regressions between seed yield under salt stress conditions ( $Y_s$ ) of guar genotypes as the dependent variable and stress tolerance indices (predictors).

## DISCUSSION

Substantial genetic variability exists in the magnitude of guar responses to salt stress. High salinity (15 dS/m) produced the greatest declines in chlorophyll content, RWC, leaf area, plant height, and root length, while markedly intensifying electrolyte leakage,  $\text{CO}_2$  accumulation, and proline synthesis. The pronounced genotype  $\times$  salinity interactions indicate differential adaptive capacities among cultivars. Genotype 1 consistently outperformed all others by maintaining higher pigment stability, superior water status, minimal oxidative damage, and earlier phenological progression. In contrast, genotypes 14 and 15 showed drastic reductions in physiological traits and showed severe phenological delays under salinity, classifying them as highly sensitive to salt stress. These findings are consistent with the well-established model of salt injury in plants, in which excessive  $\text{Na}^+$  and  $\text{Cl}^-$  disrupt ionic homeostasis, induce osmotic stress, and promote oxidative damage [21–25]. Mechanistically, the observed declines in chlorophyll content, relative water content (RWC), and growth reflect salt-induced osmotic restriction and impaired photosynthetic machinery [26–27].

Salinity reduces water uptake by lowering soil water potential, leading to cellular dehydration and compromised turgor, which directly limits leaf expansion and biomass accumulation [22, 28–31]. These processes align with recent reviews emphasizing that salt stress negatively affects plant water relations and photosynthesis, leading to reduced crop productivity [32, 33]. In line with present findings, increasing salinity levels caused significant reductions in relative water content, chlorophyll, and carotenoid content, while drastically enhances the antioxidant activities, proline, electrolyte-leakage, and MDA content in medicinal plants [34]. Also, in a previous study, levels of stress led to a significant reduction in grain yield and harvest index of guar [35].

In guar, the strong genotype  $\times$  salinity interactions observed indicate substantial genetic variation in stress response strategies. Similar genotype-dependent variation in growth and performance has been reported in other guar collections, where genotypes that maintained higher root length and biomass under salinity exhibited superior tolerance [36]. In a study conducted under saline conditions, a wide genotypic variation in shoot and root length, fresh and dry weight was observed across 25 guar genotypes, and eight genotypes performed well at the highest level of irrigation water salinity (9.0 dS/m) and were considered salt-tolerant [36]. Relative water content and leaf proline are key determinants of leaf survival and overall plant metabolic activity under salinity conditions [37]. The capacity of certain genotypes in the current study (e.g., G1 and G3) to maintain higher chlorophyll and RWC under high salinity suggests effective osmotic adjustment and water-conservation mechanisms, supporting findings in other legumes, where tolerant cultivars preserve water status more effectively than sensitive ones [32].

Increases in proline content and in oxidative indicators, such as MDA and electrolyte leakage, reflect typical biochemical responses to ionic and osmotic stress. Proline accumulation under salinity is widely recognized as both an osmoprotectant and ROS scavenger that helps stabilize proteins and membranes [38, 39]. However, excessive oxidative damage (high MDA and leakage) in sensitive genotypes suggests that their antioxidant defenses were insufficient to mitigate ROS accumulation, consistent with broader plant stress physiology models [40–45]. Root traits emerged as particularly important in our study, with longer roots correlating strongly with yield under stress. Enhanced root growth likely supports improved water and ion acquisition. It contributes to ion exclusion, a key tolerance strategy identified in molecular studies of guar, in which transporter genes and salt-responsive pathways were differentially expressed between tolerant and sensitive genotypes [46–49].

Furthermore, the reductions in growth, chlorophyll content, and RWC observed at 15 dS/m are in agreement with previous studies on guar, where only tolerant genotypes maintained higher growth under saline irrigation [36]. In contrast, sensitive genotypes showed severe declines in physiological traits, emphasizing the role of genetic background in determining salinity tolerance. These patterns are consistent with the general model of salt stress in plants, where excessive  $\text{Na}^+$  and  $\text{Cl}^-$  disrupt osmotic balance, inhibit photosynthesis, and induce oxidative damage [22]. The superior performance of tolerant genotypes in maintaining water status and pigment stability suggests effective osmotic adjustment and stress mitigation mechanisms. Moreover, the observed increases in proline and membrane leakage align with known biochemical responses to salinity, where proline functions as an osmoprotectant and antioxidant, while high MDA and electrolyte leakage indicate oxidative injury in sensitive genotypes [39]. Together, these findings highlight that salt tolerance in guar involves coordinated regulation of water relations, photosynthesis, and antioxidant defenses.

## CONCLUSION

The study demonstrates that substantial genetic variation exists among guar genotypes in their ability to cope with salinity stress, with physiological traits such as chlorophyll stability, osmotic adjustment, membrane integrity, and growth performance serving as key determinants of tolerance. Genotypes exhibiting consistent resilience, particularly RGC-986, provide valuable genetic resources for breeding programs aimed at developing salt-tolerant cultivars. Incorporating these physiological and phenological traits into selection criteria can enhance the efficiency of breeding strategies and support sustainable cultivation practices in salinity-affected regions. Overall, the findings underscore the importance of integrating genotype evaluation with targeted physiological screening to improve guar productivity under challenging environmental conditions.

## Ethics Approval and Consent to Participate

This study is not a clinical trial and no human participants involved in this research.

## Consent for Publication

The authors declare their consent to the publication of this article.

## Data Availability

All data generated during this study are included in this article.

## Funding

There is no financial support.

## Authors' contributions

The authors conducted the experiments in collaboration and wrote the manuscript.

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## REFERENCES

1. Chiofalo B., Lo Presti V., D'Agata A., Rao R., Ceravolo G., Gresta F. Qualitative profile of degummed guar (*Cyamopsis tetragonoloba* L.) seeds grown in a Mediterranean area for use as animal feed. *Journal of Animal Physiology and Animal Nutrition*. 2018; 102(1): 260-7.
2. Gresta F., Avola G., Cannavò S., Santonoceto C. Morphological, biological, productive and qualitative characterization of 68 guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes. *Industrial Crops and Products*. 2018; 114: 98-107.
3. Sichkar V.I., Lavrova H. Nutritional value of grain legumes in relation for global food security. *Acta fytotechnica et zootechnica*. 2025; 28: 40-57

4. Gautam R., Verma A., Dwivedi S., Jhang T. Breeding guar [*Cyamopsis tetragonoloba* (L.) Taub]: A variety compendium of a multifaceted industrial crop for resource-constrained scenario in India. *Industrial Crops and Products*. 2024; 214: 118502.
5. Sharma S., Tyagi A., Ramakrishna G., Saxena S., Mithra A., Mehla H.R., Golui D., Sharma R., Gaikwad K. Comparative assessment of nutritional, industrial and agronomic valuable traits of underutilized guar genotypes. *Legume Research-An International Journal*. 2021; 1(11).
6. Meftahzade H., Baath G., Kaur R., Falakian M., Hatami M. Melatonin-mediated alleviation of soil salinity stress by modulation of redox reactions and phytochemical status in guar (*Cyamopsis tetragonoloba* L.). *Journal of Plant Growth Regulation*. 2022; 42:1-19.
7. Sandhu D., Pallete A., Puduserry M.V., Grover K.K. Contrasting responses of guar genotypes shed light on multiple component traits of salinity tolerance mechanisms. *Agronomy*. 2021; 11(6): 1068.
8. Sharma M., Sidhu A.K., Samota M.K., Shah P., Pandey M.K., Gangurde S.S. Technological advancements in the CRISPR toolbox for improving plant salt tolerance. *Discover Agriculture*. 2024; 2(1): 102.
9. Yousif S.A. Soil salinization impacts on land degradation and desertification phenomenon in An-Najaf Governorate, Iraq. In *Natural resources deterioration in MENA region: land degradation, soil erosion, and desertification*. 2024. (pp. 295-319). Cham: Springer International Publishing.
10. Kopecká R., Kameniarová M., Černý M., Brzobohatý B., Novák J., Abiotic stress in crop production. *International Journal of Molecular Sciences*. 2023; 24(7): 6603.
11. Yadav S., Modi P., Dave A., Vijapura A., Patel D., Patel M. Effect of abiotic stress on crops. *Sustainable crop production*. 2020; 3(17): 5-16.
12. Yousefi B., Karamian R. Effects of salicylic acid on photosynthetic pigments, osmolytes, and antioxidant enzyme activities in white savory (*Satureja mutica* Fisch.) exposed to various salt levels. *International Journal of Horticultural Science and Technology*. 2025; 12(1): 69-82.
13. Yousefi B., Karamian R. Effect of salinity stress and salicylic acid on morpho-physiological and growth characteristics *Satureja mutica* Fisch. & CA Mey. *Journal of Rangeland Science*. 2025; 15(1): 1-9.
14. Jamshidi A., Javanmard H.R. Evaluation of barley (*Hordeum vulgare* L.) genotypes for salinity tolerance under field conditions using the stress indices. *Ain Shams Engineering Journal*. 2018; 9(4): 2093-9.
15. Rasel M., Tahjib-Ul-Arif M., Hossain M.A., Hassan L., Farzana S., Brestic M. Screening of salt-tolerant rice landraces by seedling stage phenotyping and dissecting biochemical determinants of tolerance mechanism. *Journal of Plant Growth Regulation*. 2021; 40(5): 1853-68.
16. Atieno J., Colmer T.D., Taylor J., Li Y., Quealy J., Kotula L., Nicol D., Nguyen D.T., Brien C., Langridge P., Croser J. Novel salinity tolerance loci in chickpea identified in glasshouse and field environments. *Frontiers in Plant Science*. 2021; 12: 667910.
17. Bates L.S., Waldren R.P., Teare I.D. Rapid determination of free proline for water-stress studies. *Plant and soil*. 1973; 39(1): 205-7.
18. Hodges D.M., DeLong J.M., Forney C.F., Prange R.K. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*. 1999; 207(4): 604-11.
19. Moss D.N., Rawlins S.L. Concentration of carbon dioxide inside leaves. *Nature*. 1963; 197(4874): 1320-1.
20. Bian S., Jiang Y. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. *Scientia Horticulturae*. 2009; 120(2): 264-70.
21. Zhu J.K. Abiotic stress signaling and responses in plants. *Cell*. 2016; 167(2): 313-24.
22. Munns R., Tester M. Mechanisms of salinity tolerance. *Annual Review of Plant Biology*. 2008; 59: 651-81.
23. Alharbi K., Al-Osaimi A.A., Alghamdi B.A. Sodium chloride (NaCl)-induced physiological alteration and oxidative stress generation in *Pisum sativum* (L.): a toxicity assessment. *ACS omega*. 2022; 7(24): 20819-32.
24. Khalid S., Chaudhary N., Irum A., Aas M., Noor S., Munsha A., Toor S., Iqbal R., Hussain M., Farooq M. Impact of salt stress on plant growth and approaches for enhanced tolerance. *Biological and Clinical Sciences Research Journal*. 2024; 2024: 1356.
25. Atta K., Mondal S., Gorai S., Singh A.P., Kumari A., Ghosh T., Roy A., Hembram S., Gaikwad D.J., Mondal S., Bhattacharya S. Impacts of salinity stress on crop plants: improving salt tolerance through genetic and molecular dissection. *Frontiers in Plant Science*. 2023; 14: 1241736.
26. Roy S.J., Negrão S., Tester M. Salt resistant crop plants. *Current opinion in Biotechnology*. 2014; 26: 115-24.
27. Acosta-Motos J.R., Ortuño M.F., Bernal-Vicente A., Diaz-Vivancos P., Sanchez-Blanco M.J., Hernandez J.A. Plant responses to salt stress: adaptive mechanisms. *Agronomy*. 2017; 7(1): 18.
28. Parida A.K., Das A.B. Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and environmental safety*. 2005; 60(3): 324-49.
29. Dos Santos T.B., Ribas A.F., de Souza S.G., Budzinski I.G., Domingues D.S. Physiological responses to drought, salinity, and heat stress in plants: a review. *Stresses*. 2022; 2(1): 113-35.
30. Muhammad M., Waheed A., Wahab A., Majeed M., Nazim M., Liu Y.H., Li L., Li W.J. Soil salinity and drought tolerance: an evaluation of plant growth, productivity, microbial diversity, and amelioration strategies. *Plant Stress*. 2024; 11: 100319.
31. Lu Y., Fricke W. Salt stress—regulation of root water uptake in a whole-plant and diurnal context. *International Journal of Molecular Sciences*. 2023; 24(9): 8070.
32. Fu H., Yang Y. How plants tolerate salt stress. *Current Issues in Molecular Biology*. 2023; 45(7): 5914-34.
33. Rengasamy P. Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology*. 2010; 37(7): 613-20.
34. Yousefi B., Karam R. Nano-selenium reduced the adverse effects of salinity stress on *Satureja spicigera* (C. Koch) Boiss. *Journal of Medicinal Plants and By-Products*. 2024; 13(4): 971-81.
35. Ghorbani M., Ramazani S.H.R., Fallahi H., Mousavi Koochi S.M. Effect of drought stress and bio-fertilizer on yield and yield components of guar *Cyamopsis tetragonoloba* (L.) Taub. *Journal of Medicinal plants and By-products*. 2019; 8(1): 13-19.

36. Suthar J.D., Rajpar I., Ganjgunte G.K. Comparative study of early growth stages of 25 guar (*Cyamopsis tetragonoloba* L.) genotypes under elevated salinity. *Industrial Crops and Products*. 2018; 123: 164-72.
37. Rahmati H., Yousefi A., Ghaderi J. The Effect of NaCl stress on biochemical, physiological, photosynthetic and morpho-physiological indicators of the medicinal plant forest savory. *Journal of Medicinal plants and By-products*. 2025; 14(6): 614-9.
38. Al-Maskari B.J., Yaish M.W. Proline enhances cowpea resilience to salinity by maintaining water content, regulating sodium ions, and reducing hydrogen peroxide. *Discover Plants*. 2025; 2(1): 138.
39. Ashraf M.F., Foolad M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*. 2007; 59(2): 206-16.
40. Inal B., Çatkin S., Altıntaş S. Oxidative stress suppression in plant system and elevation of antioxidant gene expression under diverse stress situations. In *Role of Antioxidants in Mitigating Plant Stress*. 2025. (pp. 269-308). Academic Press.
41. Zhou R., Kong L., Yu X., Ottosen C.O., Zhao T., Jiang F., Wu Z. Oxidative damage and antioxidant mechanism in tomatoes responding to drought and heat stress. *Acta Physiologiae Plantarum*. 2019; 41(2): 20.
42. Jomova K., Alomar S.Y., Alwasel S.H., Nepovimova E., Kuca K., Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of Toxicology*. 2024; 98(5): 1323-67.
43. Sachdev S., Ansari S.A., Ansari M.I., Fujita M., Hasanuzzaman M. Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. *Antioxidants*. 2021; 10(2): 277.
44. Mishra N., Jiang C., Chen L., Paul A., Chatterjee A., Shen G. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. *Frontiers in Plant Science*. 2023; 14: 1110622.
45. Fathi A., Shiade S.R., Saleem A., Shohani F., Fazeli A., Riaz A., Zulfiqar U., Shabaan M., Ahmed I., Rahimi M. Reactive oxygen species (ROS) and antioxidant systems in enhancing plant resilience against abiotic stress. *International Journal of Agronomy*. 2025; 2025(1): 8834883.
46. Acharya B.R., Sandhu D., Dueñas C., Ferreira J.F., Grover K.K. Deciphering molecular mechanisms involved in salinity tolerance in guar (*Cyamopsis tetragonoloba* (L.) Taub.) using transcriptome analyses. *Plants*. 2022; 11(3): 291.
47. Sandhu D., Pallete A., William M., Ferreira J.F., Kaundal A., Grover K.K. Salinity responses in 24 guar genotypes are linked to multigenic regulation explaining the complexity of tolerance mechanisms in planta. *Crop Science*. 2023; 63(2): 585-97.
48. Acharya B.R., Reyes L.A., Mishra L., Ferreira J.F., Skaggs T.H., Sandhu D. Alfalfa MsSOS2 confers salinity tolerance by promoting lateral root growth and regulating Na<sup>+</sup>/K<sup>+</sup> homeostasis. *Scientific Reports*. 2025; 15(1): 43187.
49. Talarico E., Greco E., Guarasci F., Araniti F., Chiappetta A., Bruno L. Epigenetic regulation of salt stress responses in rice: mechanisms and prospects for enhancing tolerance. *Epigenomes*. 2025; 9(4): 46.
50. Maktabdaran M., Mahmoodi S., Eslami S.V., Ramazani S.H.R. Optimizing Planting Date and Density for Enhanced Seed Yield and Quality in *Cyamopsis tetragonoloba* L. *Journal of Medicinal Plants and By-products*. 2026; 15(1):53-61. doi: 10.22034/jmpb.2025.368972.1931.
51. Fahramand M., Khammari I., Sirousmehr A., Dahmardeh M., Zamanipour M. The Effect of Foliar Spraying of Ascorbic Acid and Glycine Betaine on some Morpho-physiological and Biochemical Characteristics of Carola (*Momordica charantia* L.) under Salt Stress. *Journal of Medicinal Plants and By-products*. 2026; 15(2): 243-248. doi: 10.22034/jmpb.2025.370044.2015