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# Evaluation of Salt-Tolerant Guar (*Cyamopsis tetragonoloba* L.) Genotypes Based on Physiological, Biochemical, and Yield-Related Traits

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## ABSTRACT

The 15 guar genotypes were exposed to different concentrations of salt (0, 10, and 15 dS $m^{-1}$ NaCl) in a factorial experiment (RCBD, r = 3) and different morpho-physiological, physiological, growth, and yield characteristics were assessed. The lowest seed gum (21.11%), carbohydrate (23.59%), protein content (24.20%), fat (2.21%), fiber (9.38%), digestible dry matter (37.90%), and yield (3865.50 kg/ha) were measured in plants treated with 15 dS/m salt; also, the highest ash content (5.08%), proline (82.88 μg/g FW), flavonoids (0.35%), phenols (2.37%), tannins (3.27%), and antioxidant enzyme activities (SOD 104.28, CAT 30.92, APX 74.80, and POD 40.20 units/mg protein) were observed in plants treated with 15 dS/m salt. Yield-related traits also differed significantly among genotypes ($p \leq 0.05$). The highest plant yield (8124.31 kg) was gained from RGC-986 genotype under control conditions (0 dS/m). Furthermore, RGC-986 demonstrated the highest 1000-seed weight (48.22 g), number of seeds per plant (395.6), and overall average plant yield of 6458.9 kg/ha under three salinity levels, indicating strong genetic potential for commercial cultivation. S6673 and S6260 followed with plant yields of 5632.7 kg/ha and 5829.2 kg/ha, respectively. Pishen recorded the lowest values for all yield-related parameters, including 1000-seed weight (32.22 g), seeds per plant (152.7), and yield (4565.8 kg/ha). In terms of seed quality, the genotypes RGC-986, S-5885, S-6581, Saravan, and RGC-1031 demonstrated better resilience to salt stress, maintaining seed quality traits, while genotypes BR-99, S-6560, and Grembite were more sensitive, exhibiting marked declines across these parameters. Overall, the RGC-986 genotype was the Top-Performing Genotype (excellence) with high biochemical performance, lowest tannin, and yield superiority. The PCA confirmed the results of the Duncan test and highlighted the superiority of RGC-986 as an elite genotype under experimental conditions.

## 1 | Introduction

Legumes provide the nutrient minerals, proteins and vitamins for a bulky part of people, especially in developing countries (Sichkar 2025). Intensification of soil salinity poses a significant risk to agronomic productions, especially in arid and semi-arid regions (Sharma et al. 2024; Yousif 2024). Salt stress (SS) harshly influences plant development, leading to considerable yield reduction of plants (Kopecká et al. 2023; Yadav et al. 2020). It induces both osmotic stress (resulting in reduced water and nutrient absorption and ionic imbalance) and triggers upsurge of reactive oxygen species (ROS; oxidative

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stress) (Sarker and Oba 2020), which disrupt normal physio-biochemical and molecular functions (Balasubramaniam et al. 2023). ROS induced by SS interfere with the absorption and metabolism of essential nutrients (Sachdev et al. 2021), destabilize protein-pigment complexes, and degrade chlorophyll molecules (Zhao et al. 2019), leading to decreased plant growth and productivity. Recent studies have shown that SS significantly reduces photosynthetic characteristics and growth in many plants, for example: *Satureja mutica* (Yousefi and Karamian 2025a, 2025b) and anise and fennel (Nouripour-Sisakht et al. 2022). Salt-induced oxidative stress activates signaling pathways involved in metabolic reprogramming, resulting in increased or decreased levels of secondary metabolites (Meftahizade et al. 2022; Ramakrishna and Ravishankar 2011), which has an imperative role in stress tolerance.

Guar [*Cyamopsis tetragonoloba* (L.) Taub.] well known as an industrial plant characterizing by a galactomannan compound (Gautam et al. 2024; Khurizadeh et al. 2024), which extracts from seed endosperm, extensively used in food and cosmetics industries (Chiofalo et al. 2018; Gresta et al. 2018). This plant, also consumption as a vegetable, animal feed, and green manure (Chiofalo et al. 2018).

SS interrupts the developing process, influences hormonal regulation, nitrogen fixation, and photosynthesis, triggering nutritional imbalance, resulting in inhibited growth of plants (Bouzroud et al. 2023). The identification of salinity-tolerant genotypes can be a comprehensive way to cultivate legumes in the salty soils (Atieno et al. 2021). Although guar genotypes are generally resistant to drought, their response to salinity stress considerably varies (Meftahizade et al. 2022; Sandhu et al. 2021). Guar plants exposed to 4.9 $dS\,m^{-1}$of NaCl showed reduced growth and seed yield (Francois et al. 1990). However, recent studies show tolerance to salt in the guar genotypes (Khator et al. 2020; Suthar et al. 2018; Suthar et al. 2025). For example, in a field experiment, different guar genotypes exhibited substantial differences in agronomical characters in response to salinity, so that the Matador and PI 268229 genotypes showed greater salt tolerance indices for shoot and root biomass compared with genotypes PI 340261 and PI 537281 (Sandhu et al. 2021). Although rare studies have investigated guar genotypes under SS, these studies have only investigated some aspects in limited genotypes, so it is necessary to study a wider range of genotypes under SS conditions in different environments.

The selection of salt tolerant genotypes can be a comprehensive way to cultivate legumes in the saline soils (Atieno et al. 2021). In a study under stress conditions, biological yield and harvest index were recommended as guar genotype selection criteria (Ghorbani et al. 2019).

In present study, we screened 15 cultivars of guar from different countries (Iran, India, and Pakistan) and discussed their responses salinity via morpho-physiological, biochemical, and yield-related responses to identify guar's salt tolerance genotypes. The results of this research highlight the kay data for cultivation of guar genotypes in the saline soils and will extended sustainable agriculture in adverse environments.

## 2 | Materials and Methods

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

Fifteen guar genotypes from India, Pakistan, and Iran (Table 1) exposed to 0, 10, and 15 dS/m salinity in a factorial farm trial (RCBD, R = 3) in research farm (32°14′ N,54° 45′ E,1074 m) of Ardkan University, Yazd, Iran during 2023. Guar seeds were planted at 30 × 50 cm row spacing. The main physicochemical properties of the field soil were presented in Table 2. Before sowing, fertilization was executed using a total net amount of 40 $kg\,ha^{-1}$ N and 50 $kg\,ha^{-1}$ $P_2O_5$. Water was applied through a trickle (drip) irrigation system. Weed control was done manually using hand hoeing. The thermopluviometric diagram of the experimental field was shown in Figure 1.

Based on the previous studies, at June 30, the seeds were sown with 5 cm depth in the soil and in a 50 × 30 cm row spacing, so that the distance between plants on the row was 30 cm and the distance between the rows is 50 cm. Each experimental plot of 5 × 2 m was considered to be a total of 10 $m^2$ and the planting method was flat. The plants were watered with drip irrigation method. During growth season, the five irrigation stages were performed as follows: Before sowing seeds, at the two-leaf stage, flowering stage, podding stage, and seed closure stages. In each irrigation stage, the 180 L water was used. The salinity levels were prepared using NaCl, and after the appearance of the cotyledons leaves, salinity stress was gradually applied. The application of salt treatments started from the vegetative growth stage and continued until the end of the plant growth stages. In each irrigation stage, the EC was calculated first, and then irrigation was done at different levels. For zero salinity level (control), the drinking water was used for irrigation. Figure 2 exhibits different vegetative growth stage of local guar genotype (Pishen) in the research farm.

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

| ID | Genotypes | Origin |
|---|---|---|
| 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 | 49 | 14 | 264 | 8.4 | 0.17 | 1.07 | 7.8 | 3.12 |

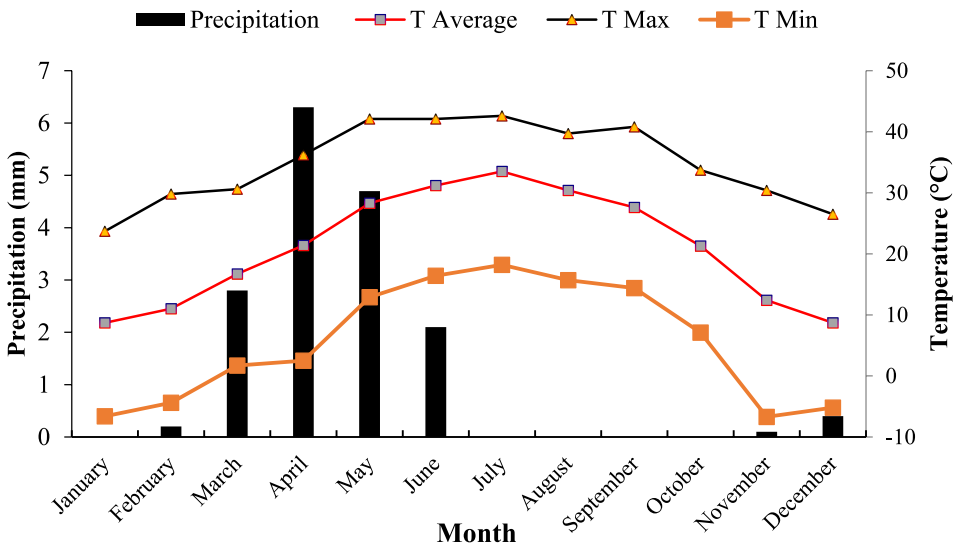


**FIGURE 1** | Thermopluviometric diagram at the experimental field (Ardakan, Yazd) during 2023.

**FIGURE 2** | The different vegetative growth stage of local guar genotype (Pishen) in the research farm.

## 2.2 | Measurement of Morphological, Yield, and Yield Component Parameters

The samples were taken on September 11. The last analysis, which is related to the root, was completed on October 7. The samples for morpho-physiological, physiological, and biochemical assays were collected from five plants randomly ($n = 10$) in each plot, while for yield estimation, the marginal plants were removed and the total plants of each plot were considered to measure seed and biomass yield. The seed-related traits (gum percentage, viscosity, etc.) have been assayed after the seeds ripen and mature. The activity of enzymes was measured at the beginning of the flowering stage, and the yield traits were measured at the end of the reproductive stage.

## 2.3 | Determination of Phytochemical Parameters

Total phenolic content was assessed based on the method developed by Papoti and Tsimidou (2009). The plant extraction was performed from 500 mg dried materials using 20 mL of methanol (with a distilled water ratio of 8:2 v/v) under 10 min of room temperature ultrasonic. The total phenolic content (g gallic acid/100 g DW) was measured at 765 nm (UV 2600, Shimadzu Corporation, Kyoto, Japan). Based on Lin and Tang (2007), the flavonoid content was measured at 415 nm (Lin and Tang 2007). The total antioxidant was estimated using the DPPH assay, as described by Gil et al. (2000) at 517 nm, and the antioxidant activity was calculated using Equation (1).

$$\%\,\text{Inhibition} = \left[\frac{(AB - AA)}{AB}\right] \times 100 \quad (1)$$

AB displays the blank absorption (t = 0 min); while AA represents the absorption of extract (t = 30 min). The tannin content was measured at the 765 nm (Price et al. 1978). The seed gum was measured based on the method described by McCleary (1981), which was slightly modified (Gresta et al. 2009). Protein content was evaluated through the micro Kjeldhal method (Stuart 1936) by multiplying the nitrogen content in factor of 6.25 (Dubetz and Wells 1968). The crude fat measurement was measured using a Soxhlet apparatus (AOAC 1970). Finally, the gum viscosity was measured through the use of a Brookfield viscometer (T = 37°C ± 10°C, Spindle No. 64, 60 rpm) (Connor and Cock 1981).

## 2.4 | Photosynthetic Parameters

### 2.4.1 | SPAD and Photosystem II Maximal Quantum Yield ($F_V/F_M$)

The photosynthetic parameters were measured in the vegetative stage, nearly before starting flowering. The chlorophyll content ($n = 30$ leaves) was measured with a SPAD-502 chlorophyll meter. The maximum quantum yield of photosystem II (Fv/Fm) was estimated using the OJIP protocol using a Fluorpen FP 100-MAX, Photon Systems Instruments, Drasov, Czech Republic (Habibi et al. 2024).

## 2.5 | Biochemical Assays

Superoxide dismutase (SOD) activity was measured based on the method of Dhindsa et al. (1981). The catalase activity was evaluated at 240 nm for 3 min (Dazy et al. 2008) and ascorbate peroxidase (APX) activity (Asada 1992).

## 2.6 | Data Analysis

Data were processed to ANOVA and Duncan's Multiple Range by ABM SPSS Software's Ver. 27, Minitab software Ver. 20, and R software (Ver. 4.3.1), and the graphs were designed.

# 3 | Results

The ANOVA results demonstrated that SS significantly affected all studied morpho-physiological, physio-biochemical, and yield traits in guar genotypes including key parameters such as seed gum content, carbohydrate, protein, flavonoid, antioxidant, phenol, tannin, viscosity, ash, fiber, digestibility (DMD), antioxidant enzymes (SOD, CAT, APX, POD), chlorophyll, carotenoids, proline, seed weight, seed number, and overall plant yield ($p < 0.01$), with the 15 dS/m treatment resulting in the most substantial changes across characteristics. Genotypic differences were also significant for several traits including protein, fat, antioxidant, phenol, tannin, ash, fiber, digestibility, chlorophyll, carotenoids, seed weight, seed number, and yield at the level of 1% and carbohydrate content, CAT, and POD at the level of 5%, however some traits were not significantly affected by genotype including Fo, Fm Fv/Fm, proline, SOD and APX. The interaction between salinity and genotype was mostly non-significant for many traits, except seed protein content at the level of 1% and seed/plant number and fiber percent ($p < 0.05$).

## 3.1 | The Effects of SS

The effect of increasing salinity levels (10 and 15 dS/m) on guar genotypes significantly influenced their physio-biochemical and yield characteristics. Seed gum content, carbohydrate, protein, fat, fiber, dry matter digestibility (DMD), and yield parameters (1000 seed weight, seeds per plant, and plant yield) significantly reduced with increasing salt concentration, with the highest values observed at 0 dS/m and the lowest at 15 dS/m. Conversely, ash content, proline accumulation, flavonoids, phenols, tannins, and antioxidant enzyme activities (SOD, CAT, APX, POD) increased significantly under SS, peaking at 15 dS/m. Chlorophyll content and carotenoids declined significantly as salinity increased, correlating with reduced photosynthetic efficiency, although Fv/Fm values showed a slight increase at salinity, indicating adaptive responses. Viscosity of guar gum markedly decreased under SS, reflecting reduced gum quality. These results demonstrate that while SS adversely affects growth, yield, and biochemical composition, guar genotypes respond by enhancing antioxidative defenses and secondary metabolite production, highlighting potential mechanisms of salt tolerance. The highest values for seed gum (28.17%), carbohydrate (29.28%), protein content (29.12%), and fat (2.59%) were observed in the control plants (Table 3), while the highest proline (82.9 µg/g FW), flavonoid (0.35% DM), antioxidant (51.03%), phenol (2.37%), and tannin (3.27%) were measured in the plants treated with 15 dS/m NaCl (Table 3). The highest fiber (11.71%), DMD (46.96%), and highest viscosity (2644.93 cps) were evaluated in the control plants (Table 3). However, the lowest values for ash content (4.03%) and lowest antioxidant enzyme activities (SOD 65.01, CAT 25.62, APX 66.36, and POD 34.95 Units/mg protein) were observed in the control plants (Table 3). In addition, the lowest seed gum (21.11%), carbohydrate (23.59%), protein content (24.20%), and fat (2.21%) were measured in plants treated with 15 dS/m NaCl (Table 3); however, the highest antioxidant enzyme activities (SOD 104.28, CAT 30.92, APX 74.80, and POD 40.20 Units/mg protein) were observed in plants treated with 15 dS/m salt (Table 3). Highest chlorophyll (33.81 value), carotenoids (2.12 mg/g FW),

**TABLE 3** | The effect of salt levels on different physiological, biochemical, physico-biochemical, and yield characteristics of guar genotypes (Duncan Multiples Range Test, $\alpha \leq 0.5$).

| NaCl (dS/m) | Seed gum (%) | Carbohydrate (%) | Protein content (%) | Fat (%) | Flavonoid (% DM) | Antioxidant (%) | Phenol (%) | Tannin (%) | Viscosity (cps) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 28.17$^{a}$ | 29.28$^{a}$ | 29.12$^{a}$ | 2.59$^{a}$ | 0.25$^{c}$ | 45.10$^{c}$ | 1.04$^{c}$ | 2.43$^{c}$ | 2644.93$^{a}$ |
| 5 | 25.63$^{b}$ | 27.02$^{b}$ | 27.60$^{b}$ | 2.48$^{a}$ | 0.31$^{b}$ | 48.35$^{b}$ | 1.55$^{b}$ | 2.81$^{b}$ | 1906.31$^{b}$ |
| 10 | 21.11$^{c}$ | 23.59$^{c}$ | 24.20$^{a}$ | 2.21$^{b}$ | 0.35$^{a}$ | 51.03$^{a}$ | 2.37$^{a}$ | 3.27$^{a}$ | 1602.89$^{c}$ |

| NaCl (dS/m) | Ash (%) | Fiber (%) | DMD (%) | SOD (Units/mg protein) | CAT (Units/mg protein) | APX (Units/mg protein) | POD (Units/mg protein) | Total Chl. (SPAD value) | Carotenoids (mg/g FW) |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 4.03$^{b}$ | 11.71$^{a}$ | 46.96$^{a}$ | 65.01$^{c}$ | 25.62$^{c}$ | 66.36$^{c}$ | 34.95$^{c}$ | 33.81$^{a}$ | 2.12$^{a}$ |
| 5 | 5.07$^{a}$ | 10.40$^{b}$ | 43.63$^{b}$ | 75.82$^{b}$ | 28.32$^{b}$ | 72.49$^{b}$ | 37.26$^{b}$ | 31.87$^{b}$ | 1.59$^{b}$ |
| 10 | 5.08$^{a}$ | 9.38$^{c}$ | 37.90$^{c}$ | 104.28$^{a}$ | 30.92$^{a}$ | 74.80$^{a}$ | 40.20$^{a}$ | 28.49$^{c}$ | 1.25$^{c}$ |

| NaCl (dS/m) | Fo (mol/m$^{-2}$/s) | Fm (mmol m$^{-2}$/s) | Fv/Fm (value) | Proline (μg/g FW) | 1000 seed weight (g) | Seed/Plant (n) | Plant yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| 0 | 0.122$^{a}$ | 0.786$^{a}$ | 0.841$^{c}$ | 72.52$^{c}$ | 42.1$^{a}$ | 274.98$^{a}$ | 6222.91$^{a}$ |
| 5 | 0.080$^{b}$ | 0.742$^{b}$ | 0.891b | 77.24$^{b}$ | 38.2$^{b}$ | 22.71$^{b}$ | 5736.77$^{b}$ |
| 10 | 0.074$^{b}$ | 0.819$^{a}$ | 0.907$^{a}$ | 82.88$^{a}$ | 32.54$^{c}$ | 171.18$^{c}$ | 3865.50$^{c}$ |

*Note:* The common letters indicate non-significant differences.

1000 seed weight (42.1 g), seeds per plant (274.98), and plant yield (6222.91 kg/ha) were observed in the salt control plants (Table 3). In response to 10 dS/m salt, plant yield decreased to 5736.77 kg/ha, and seed gum, carbohydrate, and protein content reduced compared with control but higher than its value in plants treated with 15 dS/m. Also, ash content (5.07%), proline content, phenolic compounds (flavonoid, phenol, tannin), and antioxidant enzyme activities increased in plants treated with 10 dS/m compared with control. However, viscosity and fiber dropped significantly (1906.31 cps and 10.40%) (Table 3). In response to 15 dS/m salt (high SS), the values of total chlorophyll and carotenoids decreased compared with control and plants treated with 10 dS/m salt, while chlorophyll fluorescence parameters Fv/Fm improved slightly compared with control, indicating some photosystem II efficiency adaptation (Table 3). SS negatively impacted yield and nutritional quality (gum, carbohydrates, protein) of guar and induced antioxidant enzyme activity and accumulation of secondary metabolites like flavonoids, phenols, and proline, likely as protective stress responses. The viscosity and fiber content of plants were decreased under SS, affecting the quality of guar gum. Although 10 dS/m salinity caused growth decline, some genotypes showed partial tolerance, while high SS (15 dS/m) severely reduced growth and yield in all genotypes.

## 3.2 | Effects of Genotypes Variation on Studied Variables

An analysis of variance revealed significant differences ($p \leq 0.05$) among guar (*C. tetragonoloba* L.) genotypes for all yield-related and physio-biochemical traits evaluated. Significant variation ($p \leq 0.05$) was also observed in enzymatic antioxidant activity (CAT, POD, and APX) and pigment content (chlorophyll and carotenoid). However, no significant differences were observed among genotypes for chlorophyll fluorescence parameters (Fo, Fm, and Fv/Fm), indicating uniformly healthy photosynthetic efficiency across genotypes. In addition, proline content, a marker of stress tolerance no-varied among genotypes.

Genotype RGC-986 exhibited the highest seed gum content (28.60%), significantly outperforming all other genotypes (Table 4). It also had the greatest carbohydrate (28.34%), protein content (29.11%), fat (3.00%) (Table 4), antioxidant activity (51.72%), flavonoid content (0.33% dry matter), and along with the highest phenol content (2.27%) and the lowest tannin concentration (2.56%) (Table 4). In contrast, genotype Pishen recorded the lowest seed gum (22.69%) and protein content (25.63%), despite having one of the highest flavonoid values (0.33%), and the highest proline accumulation (80.62 μg/g FW) (Table 4), which was followed closely by S6566 (79.47 μg/g FW) and RGC-986 (79.13 μg/g FW). Despite its high proline levels, Pishen did not demonstrate corresponding improvements in yield or biochemical performance. Several genotypes, including S6673 and BR-99, showed intermediate values across most biochemical parameters.

The highest fiber (11.38%) and DMD (47.41%), the lowest ash content (4.28%), and highest viscosity (2662.2 cps) were obtained from RGC-986, while the lowest values of fiber (10.02%) and DMD (40.66%), the highest ash content (5.08%), and lowest

**TABLE 4** | The mean comparisons of seed gum and biochemical variables between guar genotypes (Duncan's test, $\alpha \leq 0.05$).

| | Mean ± SE | | | | | | |
|---|---|---|---|---|---|---|---|
| **Genotypes** | **Seed gum (%)** | **Carbohydrate (%)** | **Protein content (%)** | **Fat (%)** | **Proline (μg FW-1)** | **Antioxidant (%)** | **Flavonoid (% DM)** |
| RGC-986 | $28.60 \pm 1.25^{a}$ | $28.34 \pm 0.92^{a}$ | $29.11 \pm 0.93^{a}$ | $3.00 \pm 0.11^{a}$ | $79.13 \pm 2.14^{a}$ | $51.7 \pm 21.11^{a}$ | $0.33 \pm 0.02^{a}$ |
| S6673 | $26.33 \pm 1.16^{b}$ | $26.38 \pm 1.08^{b}$ | $27.34 \pm 0.73^{ab}$ | $2.39 \pm 0.08^{ab}$ | $77.91 \pm 2.20^{a}$ | $48.57 \pm 1.03^{abc}$ | $0.30 \pm 0.01^{b}$ |
| BR-99 | $26.00 \pm 1.39^{bc}$ | $27.03 \pm 0.95^{ab}$ | $27.01 \pm 0.73^{ab}$ | $2.51 \pm 0.07^{b}$ | $78.24 \pm 2.29^{a}$ | $49.44 \pm 1.11^{ab}$ | $0.30 \pm 0.01^{b}$ |
| S6566 | $24.63 \pm 1.34^{bcde}$ | $26.43 \pm 1.09^{b}$ | $26.51 \pm 0.87^{ab}$ | $2.51 \pm 0.14^{b}$ | $79.47 \pm 2.19^{a}$ | $48.39 \pm 1.03^{bc}$ | $0.28 \pm 0.01^{b}$ |
| S-6560 | $24.59 \pm 1.44^{bcde}$ | $26.77 \pm 0.67^{ab}$ | $26.64 \pm 1.12^{ab}$ | $2.51 \pm 0.13^{b}$ | $78.89 \pm 2.29^{a}$ | $49.33 \pm 1.14^{ab}$ | $0.29 \pm 0.01^{b}$ |
| S-5885 | $24.63 \pm 1.13^{bcde}$ | $25.64 \pm 1.04^{b}$ | $27.19 \pm 1.28^{ab}$ | $2.51 \pm 0.13^{b}$ | $77.81 \pm 2.55^{a}$ | $47.84 \pm 1.19^{bc}$ | $0.29 \pm 0.01^{b}$ |
| S-6581 | $24.91 \pm 1.03^{bcd}$ | $26.88 \pm 1.218^{ab}$ | $26.59 \pm 1.12^{ab}$ | $2.38 \pm 0.19^{bc}$ | $76.93 \pm 2.42^{a}$ | $48.83 \pm 1.46^{abc}$ | $0.29 \pm 0.01^{b}$ |
| Grembite | $24.62 \pm 0.99^{bcde}$ | $26.42 \pm 1.21^{b}$ | $26.80 \pm 0.98^{ab}$ | $2.42 \pm 0.16^{bc}$ | $77.04 \pm 2.49^{a}$ | $48.58 \pm 1.25^{abc}$ | $0.30 \pm 0.01^{b}$ |
| S6260 | $25.20 \pm 0.94b^{cd}$ | $26.58 \pm 1.03^{ab}$ | $26.54 \pm 0.75^{ab}$ | $2.37 \pm 0.12^{bc}$ | $76.73 \pm 2.50^{a}$ | $48.24 \pm 1.30^{bc}$ | $0.29 \pm 0.01^{b}$ |
| Saravan | $25.08 \pm 0.90^{bcd}$ | $26.82 \pm 1.07^{ab}$ | $26.53 \pm 0.80^{ab}$ | $2.38 \pm 0.12^{bc}$ | $75.47 \pm 1.99^{a}$ | $48.16 \pm 1.14^{bc}$ | $0.30 \pm 0.01^{b}$ |
| S6553 | $24.89 \pm 0.91^{bcd}$ | $26.74 \pm 0.94^{ab}$ | $27.00 \pm 0.94^{ab}$ | $2.27 \pm 0.08^{bc}$ | $76.54 \pm 1.67^{a}$ | $48.88 \pm 1.12^{abc}$ | $0.31 \pm 0.01^{b}$ |
| S6486 | $24.81 \pm 1.16^{bcd}$ | $25.98 \pm 0.68^{b}$ | $26.50 \pm 0.81^{ab}$ | $2.34 \pm 0.07^{bc}$ | $76.27 \pm 2.22^{a}$ | $47.50 \pm 0.98^{bcd}$ | $0.30 \pm 0.01^{b}$ |
| RGC-1031 | $24.31 \pm 0.97^{cde}$ | $26.50 \pm 1.07^{ab}$ | $25.93 \pm 0.34^{b}$ | $2.38 \pm 0.07^{bc}$ | $77.79 \pm 1.95^{a}$ | $45.74 \pm 0.88^{cd}$ | $0.31 \pm 0.01^{b}$ |
| RGC-1066 | $23.26 \pm 1.00^{ce}$ | $27.36 \pm 0.97^{ab}$ | $26.04 \pm 0.43^{ab}$ | $2.32 \pm 0.07^{bc}$ | $75.87 \pm 1.60^{a}$ | $46.88 \pm 0.60^{bcd}$ | $0.30 \pm 0.01^{b}$ |
| Pishen | $22.69 \pm 0.91^{e}$ | $25.53 \pm 0.57^{b}$ | $25.63 \pm 0.47^{b}$ | $2.13 \pm 0.06^{c}$ | $80.62 \pm 1.65^{a}$ | $44.30 \pm 0.8^{d}$ | $0.33 \pm 0.01^{a}$ |

*Note:* The common letters indicate no significant difference between means.

**TABLE 5** | The mean comparisons of biochemical and physico-biochemical variables between guar genotypes (Duncan's test, $\alpha \leq 0.05$).

| | Mean ± SE | | | | | |
|---|---|---|---|---|---|---|
| **Genotypes** | **Phenol (%)** | **Tannin (%)** | **Fiber (%)** | **DMD (%)** | **Ash (%)** | **Viscosity (cps)** |
| RGC-986 | 2.27 ± 0.34a | 2.56 ± 0.15c | 11.38 ± 0.43a | 47.41 ± 1.34a | 4.28 ± 0.16d | 2662.2 ± 206.9a |
| S6673 | 1.64 ± 0.18b | 2.82 ± 0.17ab | 10.45 ± 0.43bc | 43.93 ± 1.11b | 4.61 ± 0.18c | 2283.8 ± 225.0b |
| BR-99 | 1.67 ± 0.19b | 2.90 ± 0.13ab | 10.43 ± 0.40bcd | 43.50 ± 1.45bc | 4.72 ± 0.18bc | 2150.0 ± 224.1ab |
| S6566 | 1.60 ± 0.18b | 2.81 ± 0.10ab | 10.76 ± 0.34b | 42.78 ± 1.47bcd | 4.80 ± 0.18bc | 2113.3 ± 192.7bcd |
| S-6560 | 1.74 ± 0.24b | 2.81 ± 0.14ab | 10.53 ± 0.41bc | 42.80 ± 1.77bcd | 4.85 ± 0.18abc | 2092.2 ± 147.2bcd |
| S-5885 | 1.69 ± 0.19b | 2.87 ± 0.13ab | 10.36 ± 0.44bcd | 42.23 ± 1.74bcd | 4.87 ± 0.204ab | 2066.7 ± 150.7bcd |
| S-6581 | 1.66 ± 0.26b | 2.90 ± 0.12ab | 10.45 ± 0.32bc | 42.84 ± 1.69bcd | 4.70 ± 0.17ab | 2041.1 ± 192.1bcd |
| Grembite | 1.65 ± 0.22b | 2.75 ± 0.12b | 10.30 ± 0.30 cd | 42.45 ± 1.69bcd | 4.69 ± 0.23ab | 2003.3 ± 161.3 cd |
| S6260 | 1.65 ± 0.22b | 2.86 ± 0.09ab | 10.39 ± 0.32bcd | 42.96 ± 1.75bcd | 4.64 ± 0.19ab | 1994.6 ± 166.1 cd |
| Saravan | 1.50 ± 0.20b | 2.75 ± 0.10b | 10.63 ± 0.32bc | 43.33 ± 1.85bc | 4.70 ± 0.20ab | 1991.1 ± 143.4 cd |
| S6553 | 1.55 ± 0.22b | 2.91 ± 0.12ab | 10.39 ± 0.37bcd | 43.32 ± 1.42bc | 4.78 ± 0.19ab | 1972.2 ± 150.7 cd |
| S6486 | 1.50 ± 0.19b | 2.91 ± 0.13ab | 10.54 ± 0.38bc | 42.32 ± 1.69bcd | 4.69 ± 0.16ab | 1977.8 ± 148.5 cd |
| RGC-1031 | 1.61 ± 0.20b | 2.86 ± 0.14ab | 10.43 ± 0.33bcd | 41.18 ± 1.52 cd | 4.66 ± 0.20ab | 1933.3 ± 184.7 cd |
| RGC-1066 | 1.47 ± 0.20b | 2.87 ± 0.15ab | 10.40 ± 0.35bcd | 40.66 ± 1.31d | 4.83 ± 0.20ab | 1836.7 ± 206.61de |
| Pishen | 1.53 ± 0.13b | 2.96 ± 0.18a | 10.02 ± 0.23c | 40.72 ± 1.40d | 5.08 ± 0.16a | 1652.3 ± 180.9e |

*Note:* The common letters indicate no significant difference between means.

viscosity (1652.3 cps) were observed in the genotype Pishen (Table 5).

RGC-986 showed consistently high activity levels of SOD (79.48 Units/mg protein), catalase (CAT; 28.96 Units/mg protein), APX (71.39 Units/mg protein), and peroxidase (POD; 37.05 Units/mg protein) (Figure 3a,b), suggesting superior oxidative stress tolerance. In terms of pigment content, RGC-986 had the highest carotenoids (2.00 mg/g fresh weight) and total chlorophyll (35.15 SPAD value) (Figure 3c,d), while Pishen recorded the lowest carotenoid content (1.38 mg/g FW) and chlorophyll (29.04 SPAD), further confirming its overall lower physiological performance. Yield-related traits also differed significantly among genotypes ($p \leq 0.05$). RGC-986 demonstrated the highest 1000-seed weight (48.22 g), while Pishen recorded the lowest value of 1000-seed weight (32.22 g) (Figure 3c).

Number of seeds per plant (395.6 n), and overall plant yield (6458.9 kg/ha) (Figure 4a,b), indicating strong genetic potential for commercial cultivation. S6673 and S6260 followed with plant yields of 5632.7 and 5829.2 kg/ha, respectively. Pishen recorded the lowest values for yield-related parameters, including seeds per plant (152.7) and yield (4565.8 kg/ha) (Figure 4a,b).

The interaction effect of genotype × salinity was significant only for protein content (**), fiber content (*), seed per plant (**), and biomass yield (*) (Figure 5a–d); however, the non-significant variations in the physiological, physio-biochemical, and biochemical characteristics were observed between guar genotypes in response to salinity. Overall, in control, genotypes RGC-986 and S6673 exhibited the highest seed gum (32%–33%) and fat content, while genotypes Saravan, S6486, and RGC-1066 maintained relatively higher seed gum under moderate SS (10 dS/m). Carbohydrate content was consistently higher in genotypes RGC-986, S-6581, and RGC-1031 across all stress levels, whereas genotypes BR-99, Grembite, and Pishen showed the lowest values under high SS (15 dS/m). Protein content declined in all genotypes with increasing salinity, but genotypes RGC-986, S-5885, and Saravan retained comparatively higher levels, indicating greater tolerance. Conversely, genotypes S-6560, Grembite, and RGC-1066 pronounced declines, suggesting higher sensitivity. Fat content varied least in response to SS but remained highest in genotypes RGC-986 and S6673. The genotypes RGC-986, S-5885, S-6581, Saravan, and RGC-1031 demonstrated better resilience to SS, maintaining seed quality traits, while genotypes BR-99, S-6560, and Grembite were more sensitive, exhibiting marked declines across these parameters. Although all genotypes exhibited elevated phytochemical levels under SS, genotypes RGC-986, BR-99, and Pishen generally displayed the highest increases across these parameters, indicating enhanced stress response. Under control conditions (0 dS/m), flavonoid levels ranged from 0.22% to 0.28% DM, with minimal variation among genotypes. However, at 15 dS/m, flavonoid content markedly increased, ranging from 0.30% to 0.40% DM. Genotypes RGC-986, RGC-1066, and Pishen exhibited the highest flavonoid accumulation under severe SS, suggesting superior adaptive capacity through enhanced secondary metabolite production. Phenol content showed a strong positive response to SS. At 0 dS/m, phenol levels were low and relatively uniform (1.0%–1.3%). Upon exposure to 15 dS/m salinity, phenol content more than doubled, reaching values between 2.0% and 3.3%. Notably, genotype RGC-986 displayed the highest phenol accumulation, followed by genotypes BR-99 and Pishen. Tannin content also increased significantly with

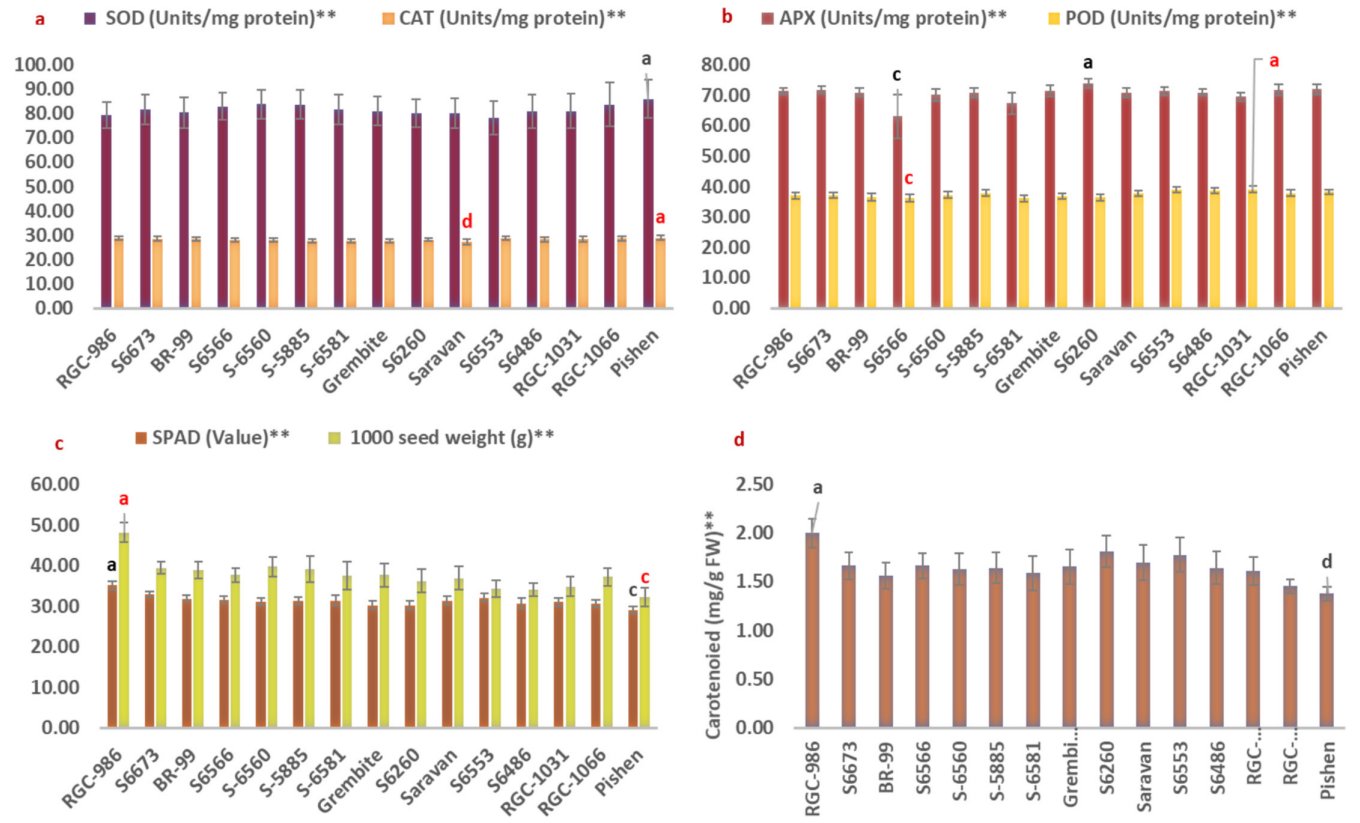


**FIGURE 3** | The mean comparison between different guar genotypes cultivated under different levels of salinity for SOD and CAT activities (a), APX and POD activities (b), carotenoid content (c); in addition, SPAD value and 1000 seed weight (d) (Duncan's Multiples Range Test [$\alpha = 0.05$]).

SS across genotypes. Control plants exhibited tannin content between 2.0% and 2.6%, which rose to 3.0%–3.6% at 10 dS/m. Genotypes RGC-986, BR-99, and Pishen consistently maintained elevated tannin levels under both moderate and high SS, indicating their potential to accumulate defense-related compounds that mitigate oxidative damage. Antioxidant activity followed a similar pattern, increasing with salinity. Under non-stress conditions, antioxidant capacity ranged from 42% to 48%, increasing to 47%–55% under 15 dS/m. The highest antioxidant activities under SS were observed in genotypes RGC-986, BR-99, and S-6581. These genotypes likely possess enhanced enzymatic or non-enzymatic antioxidant mechanisms, contributing to improved stress tolerance. Genotypes RGC-986, BR-99, and Pishen consistently showed non-significant superior increases in flavonoid, phenol, tannin, and antioxidant contents, suggesting a robust adaptive response to salinity. Conversely, genotypes such as S-6560, S6260, and S6486 exhibited comparatively lower phytochemical induction, indicating potential sensitivity to SS. Under control conditions (0 dS/m), viscosity ranged from approximately 2350 to 3500 cps, with genotype RGC-986 showing the highest viscosity. Exposure to moderate (10 dS/m) and high SS (15 dS/m) led to substantial reductions, with values dropping to 1400%–2400% and 1150%–2100%, respectively. Notably, genotypes S6673 and S-6581 exhibited relatively better viscosity retention under stress, suggesting a degree of tolerance in maintaining seed quality. In the control plants, ash content ranged from 3.7% to 4.4%, rising to 4.5%–5.4% at 5 and 15 dS/m. Genotypes RGC-1066 and Pishen consistently exhibited the highest ash content under SS, potentially reflecting salt accumulation or altered mineral metabolism. Under control conditions, fiber ranged from 11.3% to 13.0%, decreasing to approximately 10.0%–11.3% at 10 dS/m, and further to 8.7%–10.0% at 15 dS/m. Genotypes RGC-986 and Pishen showed higher fiber content under non-stress but experienced sharper declines under SS, whereas genotypes S6260 and S6486 maintained relatively stable fiber levels, indicating better fiber retention. Values of dry matter digestibility (DMD) ranged from about 43% to 52% under control conditions, decreasing to 42%–48% at 10 dS/m and further declining to 35%–42% at 15 dS/m. Genotypes RGC-986 and Saravan demonstrated the highest DMD under control, but all genotypes experienced similar proportional declines under SS. Overall, the results defined, while in all genotypes showed decreased viscosity, fiber, and DMD, and increased ash content with rising salinity, genotypic variation in the magnitude of these changes suggests differential adaptive responses. Genotypes S6673 and S-6581 appear to maintain better viscosity, genotypes RGC-1066 and Pishen accumulate more ash, and genotypes S6260 and S6486 retain fiber more effectively under stress. These traits are critical for seed quality and digestibility and provide valuable targets for breeding salt-tolerant guar varieties. SOD activity ranged from approximately 63 to 68 U/mg protein across genotypes. At moderate (10 dS/m) and high (15 dS/m) SS, SOD activity increased substantially, with values rising to 70–80 and 95–115 U/mg protein, respectively. Genotypes Pishen, RGC-1066, and S620 showed the highest SOD activity under severe SS, indicating enhanced ROS scavenging capacity. Under control conditions, CAT activity ranged between 25 and 27 U/mg protein, increasing to 28–32 U/mg protein at 15 dS/m. Genotypes Pishen, BR-99, and RGC-1066 exhibited the highest CAT activities under

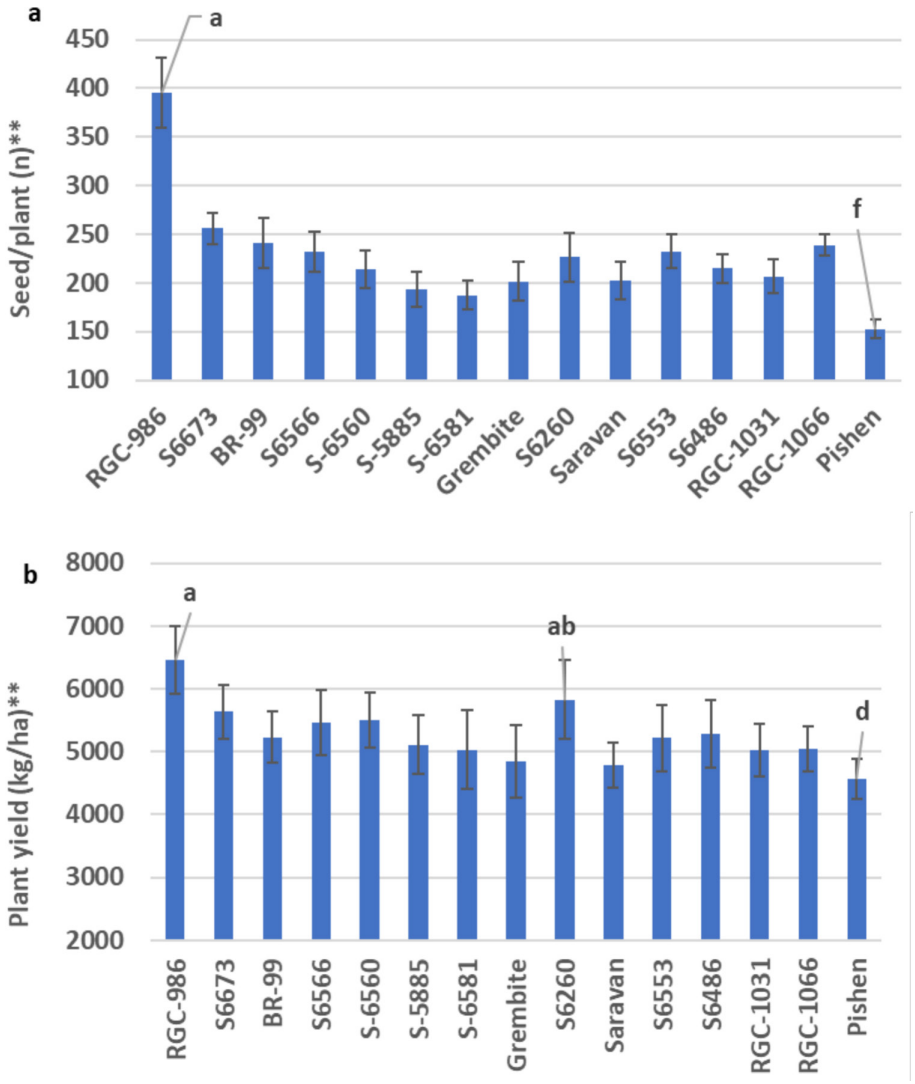


**FIGURE 4** | The mean comparison between different guar genotypes cultivated under different levels of salinity for seed number per plant (a) and plant yield (b) (Duncan's Multiples Range Test [$\alpha = 0.05$]).

SS, suggesting effective breakdown of hydrogen peroxide. Also, APX activity ranged from 63 to 68 U/mg protein, increasing to 70–77 U/mg protein at 15 dS/m. Genotypes S-5885, Grenbte, and Pishen showed the greatest APX activity under high salinity, indicating efficient detoxification of peroxides. Furthermore, POD activity followed a similar trend, with control values ranging from 32 to 37 U/mg protein, increasing to 36–43 U/mg protein at 15 dS/m. Genotypes Grenbte, S-620, and RGC-986 demonstrated the highest POD activity under SS, reflecting enhanced cell wall reinforcement and ROS scavenging. Under control conditions (0 dS/m), total Chl. ranged from 30 to 40 mg/g FW, with genotypes RGC-986 and Pishen exhibiting the highest levels. At moderate SS (10 dS/m), Chl. a decreased moderately to 25–35 mg/g FW, and under severe stress (15 dS/m), it further declined to 20–30 mg/g FW. This reduction indicates salt-induced impairment of photosynthetic pigment synthesis or stability. Also, under control conditions, carotenoids ranged from 2.0 to 3.0 mg/g FW, with genotypes RGC-986 and S6673 showing higher values. At 15 dS/m, carotenoid content declined to 1.5–2.5 mg/g FW. The maintenance of carotenoids relative to chlorophyll suggests their role in photoprotection under SS.

Under control conditions, proline levels (an important osmoprotectant) were low (approximately 60–70 μg/g FW). At 15 dS/m, proline content rose to 80–95 μg/g FW, with genotypes Pishen, RGC-986, and RGC-1066 showing the highest accumulation. This elevation in proline likely reflects an adaptive response to osmotic stress, helping maintain cell turgor and protect cellular structures. In control plants, seed weights ranged from 4.0 to 6.0 g, dropping to 3.0–4.5 g at 10 dS/m and further declining to 2.0–3.5 g at 15 dS/m. Genotypes RGC-986 exhibited higher seed weights under all conditions, while genotypes such as S5885 and S6581 were more adversely affected. Seed number per plant followed a similar trend. Under non-stress conditions, seed number ranged from 300 to 600 (n/plant). This was reduced to 200–450 n/plant under 10 dS/m and declined sharply to 100–300 n/plant at 15 dS/m (Figure 5c). Genotype RGC-986 again performed best, maintaining the highest seed yields under both moderate and severe SS. Furthermore, under control conditions (0 dS/m), genotypes RGC-986 recorded the highest plant yields (8124.31 kg) (Figure 5d). At moderate SS (10 dS/m), a noticeable reduction in plant yield was observed for some genotypes. Yield values dropped by approximately 15%–30%, with ranges shifting

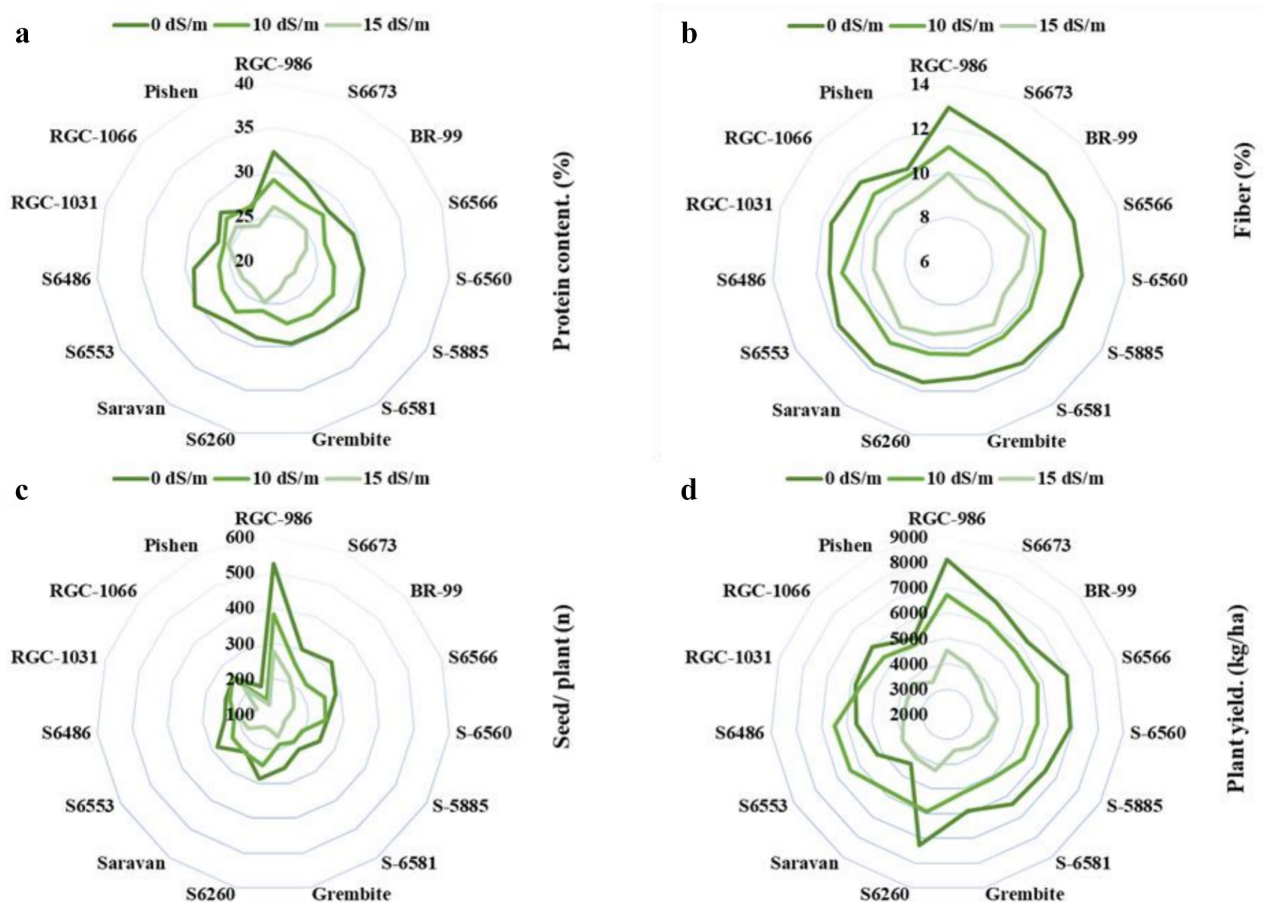


**FIGURE 5** | The interaction effect of genotypes × salinity for protein content (a), fiber percent (b), seed/plant (c), and plant yield (d) (Duncan's Multiples Range Test [α = 0.05]).

between 4500 and 7000 kg/ha. However, the genotype RGC-986 showed relatively better performance. Furthermore, under high SS (15 dS/m), plant yields were further reduced across all genotypes, ranging from approximately 3000–5000 kg/ha. Despite this decline, RGC-986 consistently maintained the highest yield under all salinity levels. In contrast, genotype Grenbite was the most sensitive to salinity, showing sharp yield declines under increasing SS. Overall, the results demonstrated that while all genotypes experienced yield reduction under salinity, the extent varied significantly. RGC-986 emerged as the most salt-tolerant genotype, exhibiting superior yield retention even under high salinity. These findings highlight its potential for cultivation in salt-affected regions and their value as parental lines in breeding programs aimed at improving salt tolerance in guar.

### 3.3 | Pearson's Correlation Estimation

Pearson correlation analysis (Figure 6) revealed strong positive associations among seed biochemical, physiological, and yield-related traits. Seed gum content showed a strong positive and significant correlation with carbohydrate (r = 0.633), protein (r = 0.889), fat (r = 0.845), flavonoid (r = 0.823), and phenol content (r = 0.801), indicating coordinated accumulation of primary metabolites and secondary compounds. Flavonoids were significantly correlated with phenols (r = 0.913), fiber (r = 0.887), viscosity (r = 0.910), and 1000-seed weight (r = 0.944), suggesting their close linkage with seed quality and physical properties. Digestible dry matter (DMD) exhibited very strong positive and significant correlations with seed gum (r = 0.962), protein (r = 0.909), fat (r = 0.878), phenol (r = 0.822), fiber (r = 0.821), total chlorophyll (r = 0.889), and viscosity (r = 0.928), highlighting its strong dependence on both biochemical composition and physiological status. Yield-related traits, including seed per plant and plant yield, were positively and meaningfully correlated with DMD (r = 0.841 and 0.788, respectively), fiber (r = 0.868 and 0.763), total chlorophyll (r = 0.883 and 0.745), and viscosity (r = 0.871 and 0.835), indicating that enhanced biochemical and photosynthetic traits contribute to higher productivity. In contrast, tannin and ash contents showed strong noteworthy negative correlations with most quality and yield traits, including seed gum (r = −0.714 and −0.889), DMD (r = −0.760 and −0.811), fiber (r = −0.832 and −0.785), total chlorophyll (r = −0.692 and −0.782), and viscosity (r = −0.773 and −0.825), suggesting their antagonistic role in seed quality and yield performance.

### 3.4 | Principal Component Analysis

Principal component analysis (PCA) (Table 6, Figure 7) revealed that the first two principal components (PC1 and PC2) explained a substantial proportion of the total variability among traits, accounting for 54% and 14% of the variance, respectively, with a cumulative variance of 68%. PC1 was primarily associated with

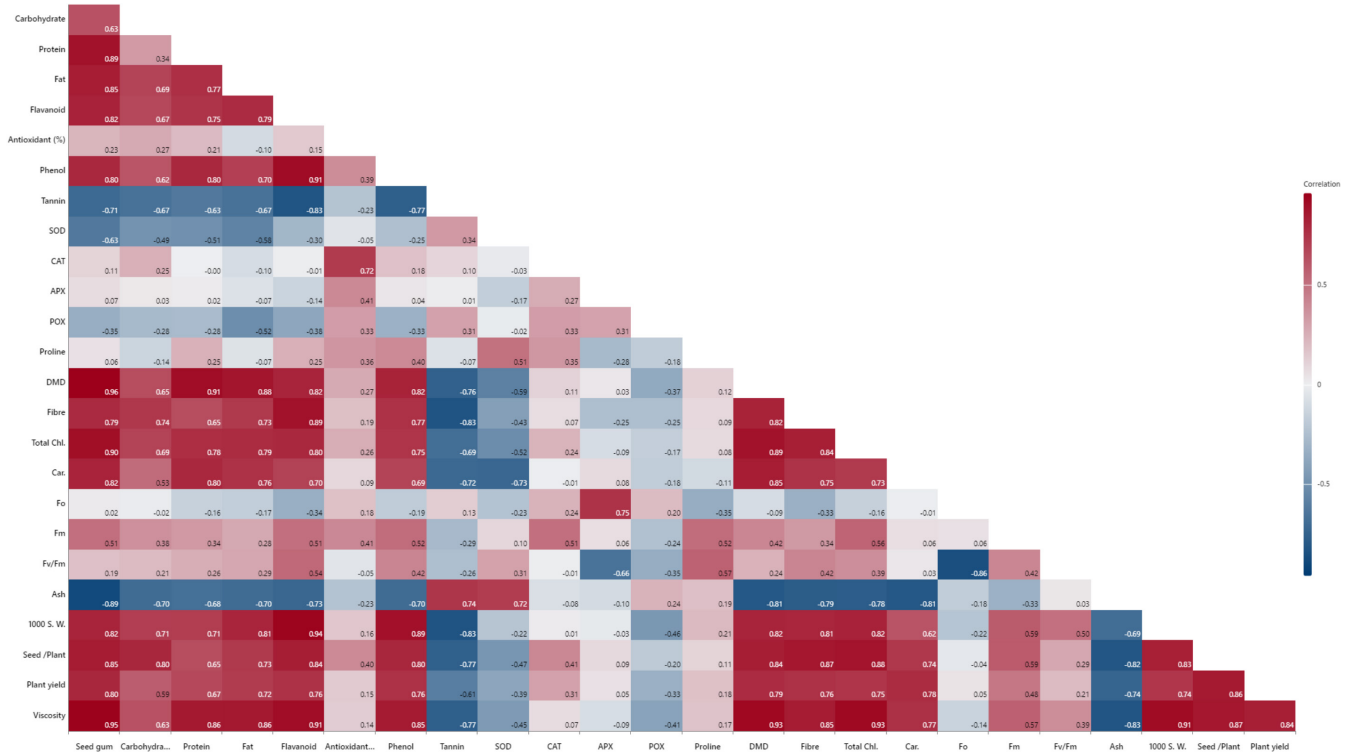


**FIGURE 6** | The correlogram of correlation between variables of guar genotypes cultivated under different salinity levels.

**TABLE 6** | The contributions of variables in components, Eigenvalues, and variance proportion of PC1 and PC2.

| Variable | PC1 | PC2 | Variable | PC1 | PC2 |
|---|---|---|---|---|---|
| Seed gum | 0.258 | 0.085 | DMD | 0.258 | 0.047 |
| Carbohydrate | 0.203 | 0.076 | Fiber | 0.245 | −0.062 |
| Protein | 0.227 | 0.003 | Total Chl. | 0.25 | 0.008 |
| Fat | 0.236 | −0.002 | Car. | 0.224 | 0.148 |
| Flavonoid | 0.252 | −0.124 | Fo | −0.039 | 0.446 |
| Antioxidant | 0.068 | 0.141 | Fm | 0.141 | −0.099 |
| Phenol | 0.243 | −0.067 | Fv/fm | 0.098 | −0.468 |
| Tannin | −0.226 | 0.005 | Ash | −0.233 | −0.19 |
| SOD | −0.14 | −0.32 | 1000 S. W. | 0.249 | −0.103 |
| CAT | 0.039 | 0.12 | Seed/Plant | 0.252 | 0.063 |
| APX | −0.009 | 0.409 | Plant yield | 0.232 | 0.047 |
| POD | −0.101 | 0.213 | Viscosity | 0.264 | −0.035 |
| Proline | 0.04 | −0.328 | | | |
| Eigenvalue | 13.57 | 3.52 | Eigenvalue | 13.57 | 3.52 |
| Proportion of V. | 0.54 | 0.14 | Proportion of V. | 0.54 | 0.14 |
| Cumulative V. | 0.54 | 0.68 | Cumulative V. | 0.54 | 0.68 |

seed quality, biochemical composition, and yield-related traits, showing high positive loadings for viscosity (0.264), seed gum (0.258), DMD (0.258), flavonoid (0.252), seed per plant (0.252), total chlorophyll (0.250), 1000-seed weight (0.249), fiber (0.245), phenol (0.243), fat (0.236), plant yield (0.232), protein (0.227), and carbohydrate (0.203). In contrast, tannin (−0.226) and ash (−0.233) exhibited strong negative loadings on PC1, indicating their inverse relationship with seed quality and productivity traits. PC2 was mainly influenced by physiological and antioxidant-related variables, with strong positive contributions

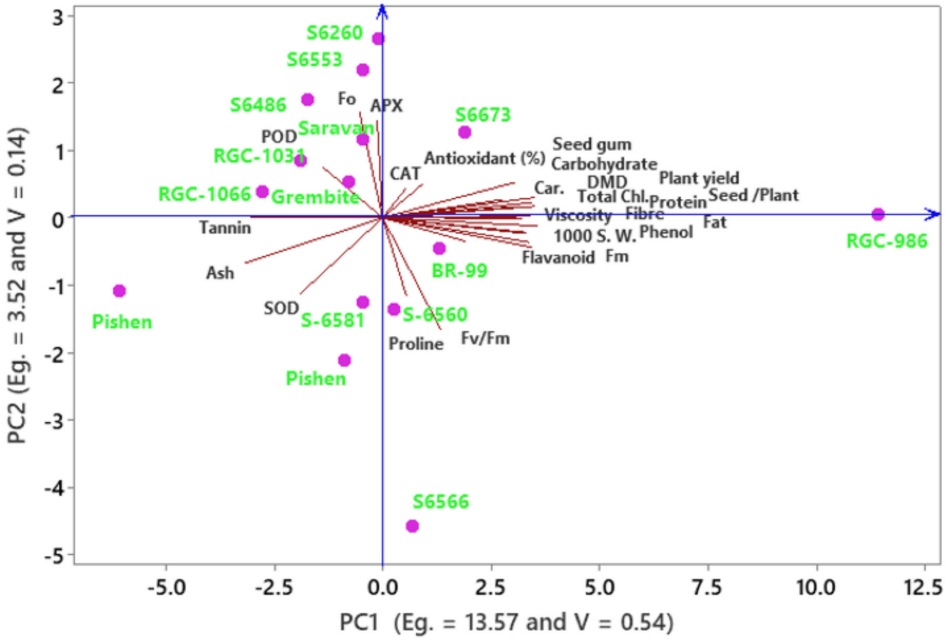


**FIGURE 7** | The diagram of principal components analysis for guar genotypes cultivated under different salinity levels.

from Fo (0.446), APX (0.409), POD (0.213), antioxidant activity (0.141), and CAT (0.120), whereas Fv/fm (−0.468), SOD (−0.320), and proline (−0.328) showed strong negative loadings. These results suggest that PC1 represents a gradient of enhanced biochemical composition, seed physical properties, and yield performance, while PC2 reflects variation in photosynthetic efficiency and antioxidant defense mechanisms.

The PCA biplot based on PC1 and PC2 further clarified the relationships among genotypes and measured traits. PC1, explaining 54% of the total variance, clearly separated genotypes associated with superior seed quality, biochemical composition, and yield traits from those characterized by higher anti-nutritional or stress-related attributes. Traits such as seed gum, carbohydrate, protein, fat, flavonoid, phenol, DMD, fiber, total chlorophyll, 1000-seed weight, seed per plant, plant yield, and viscosity clustered closely and loaded positively along PC1, indicating their strong interrelationships and collective contribution to improved productivity. Genotype RGC-986 was positioned far along the positive side of PC1, suggesting its strong association with these desirable quality and yield attributes. In contrast, tannin, ash, and SOD loaded negatively on PC1 and were positioned opposite to yield and quality traits, highlighting their antagonistic relationship. PC2, accounting for 14% of the variance, primarily reflected variation in physiological and antioxidant responses, with Fo, APX, POD, CAT, and antioxidant activity contributing positively, while Fv/Fm, proline, and SOD contributed negatively. Genotypes such as S6260, S6553, and S6486 were associated with higher antioxidant and enzyme activity along PC2, whereas genotypes like S6566 and Pishen were aligned with negative PC2 values, indicating greater association with stress-related traits.

## 4 | Discussion

Guar genotypes gained different yield value and yield traits under non stress and SS conditions and exhibited significant variation across multiple morpho-physiological, biochemical, and agronomic traits under increasing salinity levels (10 and 15 dS/m). As salinity increased, a general decline was observed in yield-related parameters such as seed yield, 100-seed weight, seed weight per plant, fat content, fiber, digestible dry matter (DMD), gum content, and viscosity. Notably, RGC-986 consistently maintained superior performance across these traits under all salinity levels, indicating strong inherent salt tolerance. These findings highlight its potential for cultivation in salt-affected regions and its value as parental line in breeding plans to improving tolerance to salinity in guar. In contrast, genotype of Grenbite showed substantial reductions in yield and related traits under SS, highlighting its sensitivity. Suthar et al. (2025) assessed and considered the 25 guar genotypes as tolerant, slightly tolerant, moderately sensitive, and sensitive based on STI values (Suthar et al. 2025). In a study, some guar cultivar (Matador and PI 268229) were classified as salt-tolerant, while PI 340261 and PI 537281 as salt-sensitive (Sandhu et al. 2021). In research of Ma et al. (2025), salinity reduced faba bean growth, root development, yield, and biomass (Ma et al. 2025). The high salinity (9 dS $m^{-1}$) reduced plant height of S-6005, S-8885, and 2/1 guar genotypes by 20%, and decreases branch numbers of BR 90 (Suthar et al. 2018). Furthermore, seed yields of all guar genotypes treated with 9 dS $m^{-1}$ NaCl significantly has reduced compared to control (Suthar et al. 2018). Extreme temperatures likely intensified salinity stress by increasing leaf water deficit and limiting water uptake. Heat × salinity also enhances ROS production, causing oxidative damage to membranes, photosynthetic machinery, and carbohydrate metabolism. These interacting stresses likely explain the stronger reductions in photosynthesis, growth, and gum yield under high salinity, highlighting the importance of considering Heat × Salinity interactions in arid environments. Suthar et al. (2018) considered BR-99, S-5608, 2/1, S-5615, and S-8885 genotypes as salt tolerant (Suthar et al. 2018). Severe salinity has reduced chlorophyll, carbohydrates, and phenol contents of guar, while encouraged antioxidant activity (Meftahizade et al. 2022). Acharya et al. (2022)

stated that genes involved in stress-signaling pathways and transporters have important roles in guar salinity tolerance (Acharya et al. 2022). Furthermore, in line with recently publications (Acharya et al. 2022; Deepika and Dhingra 2014), our findings showed that the NaCl significantly decrease guar gum content and viscosity. Previous studies indicate that the reduction in guar gum viscosity under SS is primarily associated with structural changes in galactomannan polymers, particularly alterations in the mannose-to-galactose (M/G) ratio and molecular weight. Several studies have shown that environmental stresses, including salinity, can modify carbohydrate metabolism in guar seeds, leading to changes in galactose substitution along the mannan backbone. A higher M/G ratio (lower galactose content) reduces chain branching and intermolecular interactions, resulting in lower hydration capacity and viscosity (Mudgil et al. 2014). In guar specifically, reduced galactose substitution has been linked to decreased gum viscosity and poorer rheological properties (Mudgil et al. 2014).

Salt-induced growth arrest has been reported in many plants of the legume family. Pitann et al. (2011) reported that SS significantly reduced the growth features of legumes (Pitann et al. 2011). Qados and Moftah (2014) emphasized that increased NaCl negatively affected plant height, leaf number, and leaf area of faba bean cultivars (Qados and Moftah 2014). Also, the detrimental effects of salinity have been reported in the growth and development of pea and chickpea plants (Yousef et al. 2020). In soybean, exposure to 150 mM NaCl for 7 days resulted in plant growth inhibition (Ning et al. 2018). increased NaCl concentration inhibited growth of mung bean seedlings (HanumanthaRao et al. 2016; Sehrawat et al. 2014). Salinity stress causes a decrease in soil water potential, impaired nutrient uptake and ionic balance, and altered photosynthetic enzymes (Loudari et al. 2022). These abnormalities caused by accumulation of $Na^+$ and $Cl^-$ led to chlorophyll degradation and photosynthesis and quantum yield reduction (Lu et al. 2023). Reduced photosynthesis and nutrients imbalance reduces the cell development and plant growth (Bouzroud et al. 2023; Ma et al. 2024).

In a study salinity stress had a moderately negative impact on biomass, plant height, leaf elongation, and prolonged time to flowering of two guar accessions, and BWP 5595 accession showed higher biomass and morpho-physiological values than accession no. 24320 in response to salinity (Al-Shameri et al. 2019). The 3.0, 6.0, and 9.0 $dS\,m^{-1}$ salinity has reduced shoot and root lengths, shoot and root biomass of 25 guar genotypes. These 25 guar genotypes were categorized into four groups based on salinity tolerance index (STI) and composite biomass of shoot and root, including tolerant (S-5981), moderately tolerant (S-5612, S-5881, S-5932, S-6003, S-6052, and S-6067), moderately sensitive, and sensitive genotypes (Suthar et al. 2025). Growth of nine chickpea genotypes with different backgrounds (BG 1103, DCP 92-3, S7, ICCV 10, BG 256, KWR 108, JG 16, K 850, and ICC 4463) was affected by salinity, but more reduction was shown by the genotypes BG 256, DCP 92-3, and ICC 4463 (Kaur et al. 2025).

Photosynthetic activities are a valuable indicator for studying the effects of stress on plants and identifying stress-tolerant plants. Overall, the results indicated that salinity negatively impacts photosynthetic pigments and chlorophyll fluorescence parameters of guar genotypes, with genotype-specific variations in tolerance. Genotype RGC-986 exhibited relatively higher pigment contents and fluorescence values under SS, suggesting better maintenance of the photosynthetic apparatus and potential salt tolerance. The decline in Fv/Fm and Fo was more pronounced in guar sensitive genotypes, suggesting greater damage to the photosynthetic apparatus. The correlation of these parameters with stress tolerance highlights their utility as early indicators of salinity-induced physiological impairment.

Chlorophyll content of nine chickpea genotypes reduced in response to 6 and 9 $dS\,m^{-1}$ salinity (Kaur et al. 2025). In a recent study, similar to the present finding, SS significantly impaired photosynthetic efficiency and reduced chlorophyll content in faba bean (Ma et al. 2025).

Plant exposure to SS induces a wide range of physiological changes. Several studies have emphasized the inhibitory effect of SS on photosynthesis. SS alters photosynthetic pigments, which affect photosynthetic activity (Nadeem et al. 2019). Ahmad et al. (2018) explained the change in plant photosynthetic activity by the limitation of $Mg^{2+}$ uptake and the change in photosynthetic pigments, mainly chlorophyll, by the activation of chlorophyllase enzymes (Ahmad et al. 2018). In addition, the excessive accumulation of ROSs (induced by SS) damages chloroplast membranes, inhibits Rubisco, and leads to stomatal closure, which ultimately leads to a significant reduction in plant growth and productivity (Bouzroud et al. 2023; Ikram et al. 2025; Manimaran et al. 2025). Also, excessive ROSs limits cellular metabolism by stimulating the oxidation of DNA, proteins and lipids, which can disrupt the normal cell metabolism and causes damage to cell membranes (Sarker and Oba 2020). Alqarawi et al. (2014) explained this dangerous effect to the detrimental effects of salinity stress on the composition of polyunsaturated fatty acids, which ultimately disrupts membrane function in legumes (Alqarawi et al. 2014).

Salinity induced significant increases in key biochemical stress markers of guar genotypes, including proline, phenol, flavonoid, and tannin content, particularly at 15 dS/m. NaCl (150 $mmol\,L^{-1}$) caused a marked elevation in soluble protein and sugar of faba bean plant (Ma et al. 2025). In two cultivars of basil, salinity increased leaf concentrations of the two major polyphenolic compounds, quercetin-rutinoside and rosmarinic acid (Scagel et al. 2019). In tolerant tomato cultivars, a high salt-mediated proline accumulation, high antioxidant enzyme activities, and differential patterns of gene expression were observed (Gharsallah et al. 2016).

Antioxidant enzyme activities of SOD, catalase, APX, and peroxidase increased under SS, reflecting activation of the plant's oxidative defense system. RGC-986 and Pishen exhibited the highest increases in SOD and proline levels under high salinity, aligning with their superior physiological resilience. These biochemical adjustments likely contributed to maintaining membrane stability, photosynthetic efficiency, and osmotic balance under SS. Mi et al. (2024) identified CAT and peroxidase (POD) activity in roots, POD and SOD activity in stems and leaves as the primary indicators for evaluating the salt tolerance of *Medicago sativa* during its growth period.

In a study, the antioxidative defense mechanism was quite active in the genotypes S7, ICCV 10, KWR 108, and check CSG 8962 of guar because higher values of antioxidative enzymes and a low increment in the content of hydrogen peroxide and malondialdehyde were noted in these genotypes (Kaur et al. 2025). The activities of POD and APX increased in *Astragallus membranaceus*, and the activity of CAT increased in *M. sativa* in response to 50–300 mmol/L NaCl (Mi et al. 2024).

In two legume species, CAT and POD activities exhibited similar increasing trends to SOD in response to salt, but with varying degrees of enhancement, and the change in SOD activity was not significant (Mi et al. 2024). Malakar and Chattopadhyay (2021) stated the activities of CAT, POD, and APX all increased to different extents under SS, suggesting the plant's ability to perform ion regionalization through self-regulation and the mutual influence of osmoregulatory substances, thereby alleviating high SS (Malakar and Chattopadhyay 2021). *Pisum sativum* treated with 300 mM of NaCl was shown to maintain nitrogenase activity at 24 h, but showed a strong decrease at 72 h together with cytochrome activity. On the contrary, in *Phaseolus vulgaris*, respiration rates were maintained by an enhanced antioxidant activity under salinity (Ortiz et al. 2025). Our findings highlight differential responses of guar genotypes to salinity, while some studied cultivars relatively tolerate salt; others were sensitive.

The simultaneous use of morpho-physiological, biochemical, and yield-related traits provides a comprehensive and reliable approach for screening salt-tolerant guar (*C. tetragonoloba*) genotypes. Morpho-physiological traits reflect the plant's capacity to maintain growth, water status, and photosynthetic efficiency under saline conditions, while biochemical traits reveal the underlying adaptive mechanisms, including osmotic adjustment, antioxidant defense, and ionic homeostasis. The integration of these traits enables early and accurate discrimination of tolerant genotypes that may not be identifiable through growth parameters alone. Importantly, the inclusion of yield-related traits validates physiological tolerance by linking stress resilience to agronomic performance, ensuring that selected genotypes sustain productivity under SS. This integrated, multi-trait strategy enhances selection efficiency, minimizes the risk of false positives, and supports the development of guar cultivars with stable yield performance in salt-affected environments. In addition, our observations clearly show that increased salinity prolongs the time to maturity, and that genotypes differ substantially in their responsiveness to SS during the late stages of development.

The PCA confirmed the results of the Duncan test and highlighted the superiority of RGC-986 as elite genotypes under experimental conditions. The highly significant correlations between variables highlighted the strong dependence between biochemical composition, physiological status, and yield-related traits, including seed per plant and plant yield.

## 5 | Conclusion

The integration of morpho-physiological, biochemical, and yield data revealed that salt tolerance in guar is a complex trait governed by a combination of productivity-related parameters and stress mitigation mechanisms. Genotypes such as RGC-986 and S6673 consistently performed well across multiple traits and salt levels, suggesting their potential as salt-tolerant cultivars or breeding parents. Key traits including proline accumulation, SOD activity, Fv/Fm, and yield attributes emerged as reliable indicators of SS resilience. These findings provide a valuable foundation for targeted selection and improvement of guar under saline conditions. Results confirmed that RGC-986 (Genotype 1) was the top-performing genotype (excellence) with high biochemical performance including highest seed gum (28.60%), carbohydrate (28.34%), fat content (3.0%), and protein (29.11%), and lowest tannin (2.56% important for taste/quality), and yield superiority including highest plant yield (6458.9 kg/ha), highest 1000 seed weight (48.22 g) and highest seed per plant (395.6), and also, the top photosynthetic performance (highest SPAD chlorophyll [35.15] and carotenoids [2.00 mg/g FW]), and stress resilience (high proline: 79.13 µg/g FW). Thus, we recommend the plantation of RGC-986 as a top yield and quality genotype and stress tolerant for industrial use.


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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data is provided within the manuscript.

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