DOI: 10.53992/njns.v10i1.246 NUST Journal of Natural Sciences, Vol. 10, Issue 1, 2025

Research Article

Comparative studies on biochemical, antioxidants and yield characters on salt-resistant and salt-sensitive pea genotypes

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Abstract

Salinity is a vibrant environmental dynamic that has a detrimental impact on crop productivity. This experiment was initiated to compare the biochemical, antioxidant, and yield attributes of salt-resistant and sensitive pea (*Pisum sativum* L.) genotypes under saline environments. Four pea genotypes were cultivated in a two-factorial pot experiment based on completely randomized design and exposed to four distinct levels of salinity; control, 2.5, 5.0, 7.0, and 10 dS m^{-1} NaCl, $MgSO_4$, $CaCl_2$, and Na_2SO_4 in order to study the salt sensitivity of pea. The findings demonstrated that under salt stress, pea production and growth decreased. With applied salt stress, both genotypes displayed notable genetic variation. In terms of biochemical, antioxidant, and yield attributes, the salt-tolerant genotype of pea namely samrena zard unmistakably demonstrated the best results in comparison to the rest of the tested genotypes. The following enzymatic, biochemical, and yield-related traits of the tested pea genotypes showed a substantial difference: two genotypes were found and exposed to salt stress: one was salt-sensitive (ambasidar) and the other was salt-tolerant (samrena zard).

Keywords: Pea, Genotype, Biochemical, Antioxidants and Yield.

Article History: **Received:** 19 Sep 2024, **Revised:** 14 Mar 2025, **Accepted**: 15 Mar 2025, **Published:** 30 Apr 2025.

Introduction

Among vegetable crops grown in winter, the pea (*Pisum sativum* L.) holds the most imperative position in Pakistan. It is a legume plant with the third highest economic value after common beans and soya and belongs to the Fabaceae family [1]. In Pakistan, peas produced 105 thousand metric tons of green pods on 15,800 hectares of cultivable land. The production of peas is relatively low in comparison to the outputs of several other nations [2]. Peas are a good source of

vitamins A, B, and C because they include 27.8% protein, 42.65% carbohydrates, and essential minerals including sodium, phosphorus, potassium, iron, and other elements. Pea seeds are used to cure a number of fungal diseases because of their therapeutic properties [3]. Abiotic strains like heat, drought, and salinity are some of the many variables influencing pea production in Pakistan and globally [4]. One of the prevalent difficulties facing worldwide agriculture is salinity, which restricts plant productivity [5]. Numerous attempts have been made to enhance the tolerance of several crop species to salinity by conventional breeding and genetic modification [6]. However, choosing and identifying salt-tolerant varieties is the most crucial technique to lessen the influence of salinity on crop productivity [7]. Additionally, foliar-feeding plants macro, micronutrients, and bioregulators aid in situations where salt stress limits the uptake of specific vital minerals through the root system [8] also encounter salinity by reducing its effect on plants' tolerance level impact of salinity i.e. ascorbic acid (Vitamin, C) [9,10] proline [11,12] and glycine betaine [13, 14]. Reactive oxygen species buildup results in oxidative impairment that oxidizes the lipids as well as proteins and eventually kills the plant cell [15]. The antioxidant protection system of cell entails enzymes like ascorbate peroxidase, super oxidase dismutase, catalase, and peroxidase as well as antioxidants of non-enzymatic category like corbate, glutathione, and ά-tocopherol [16, 17,18].

Materials and Methods

Planting materials and growth environments

Seeds of four pea genotypes were acquired from the Ayyub Agriculture Research Institute, Faisalabad, Pakistan. The plastic containers filled with sandy loam soil were used to grow the seeds. The four plants per pot were upheld after the emergence of 1st true shoots (approximately fifteen days after sprouting), and they were irrigated according to their needs. After 20 days of seedling, the plants were given Hoagland solution (half strength 0.5) [19]. One month after seeding, salt treatments began. Salt concentrations of 2.5 dS m^{-1} were gradually increased every two days till the desired concentration was obtained to avoid osmotic shock. As per treatment we use four pots, and every pot containing four plants was regarded as a repetition.

Measurement of biochemical parameters

The technique of [20] was employed to define the proline contents. Applying the methodology of [21] the quantity of glycine betaine was determined by using the fresh leaf of about 1 g in distilled water (10 mL). The standard curve was used to calculate the glycine betaine concentrations. Distilled water was used to produce the blank.

Measurement of antioxidants

Fresh leaf or root samples (0.5 g from each treatment were ground in an ice-cooled tissue grinder with 5 mL of 50 mM phosphate buffer (pH 7.8) for the estimation of antioxidant enzyme activities. To estimate antioxidant enzyme activities, fresh leaf or root samples (0.5 g from each treatment) were ground and kept cool in ice with 5 mL of phosphate buffer having a pH of 7.8. The homogeneous was centrifuged for 20 minutes at 15,000 × g at 4°C and the following enzymes' activities were ascertained using the obtained supernatant. The activity of SOD was analyzed by assessing its efficiency to prevent the photoreduction of nitrobule tetrazolium, in accordance with the procedure reported by [22]. CAT and POD activities were then determined using the modified methods of [23]. For CAT, the reaction solution

comprises 0.1 mL of enzyme extract, phosphate buffer (pH of 7.0) at 50 mM, and H_2O_2, at 5.9 mM and for a total of 3 mL.

The absorbance was measured every 20 seconds at 240 nm. One unit of CAT activity is defined as a modification of 0.01-unit absorbance per minute. The POD reaction solution for 3 mL contained enzyme extract (0.1 mL), guaiacol (20 mM), phosphate buffer (50 mM having pH 5.0), and H_2O_2 (40 mM). Every 20 seconds, the absorbance of the response solutions was measured at 470 nm. The shift of 0.01 in absorbance per minute is termed as one unit activity of POD. The activity of each enzyme was expressed in relation to the protein content.

Yield

Three randomly chosen plants from each replication of each treatment were counted to determine the pods number per plant and seeds per pod. A digital venire caliper was used to measure the pod's size in centimeters. An electric balance was used to measure the pod weight in grams.

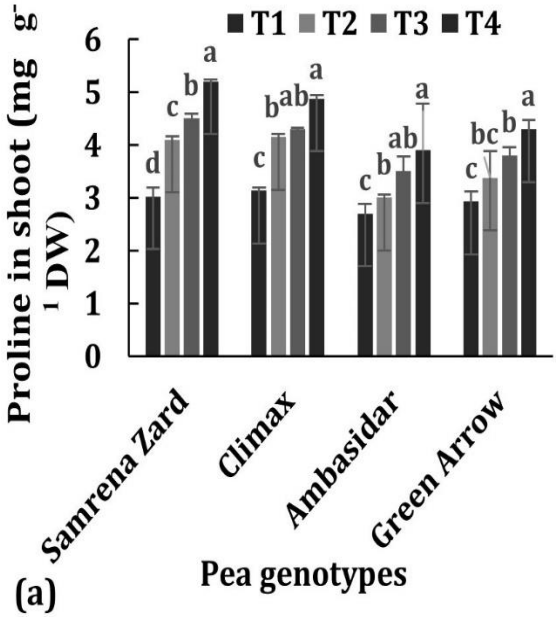
Statistical analysis

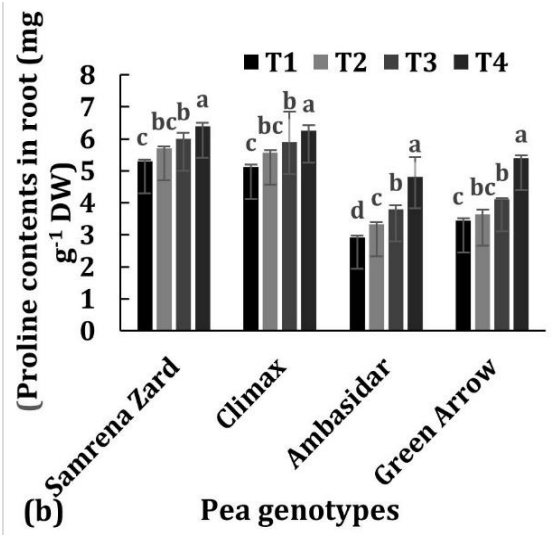
The factorial structure was used along with two components (genotypes and salinity) under a completely randomized design (CRD). To emphasize the relevance of variance among the treatments, the data's significance was evaluated using ANOVA at a 5% significance level.

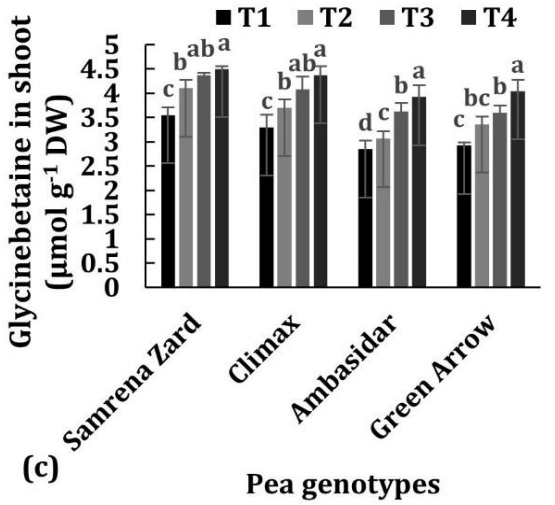
Results

Impact of salinity on biochemical parameters

A significant decline in the proline and glycine betaine contents in the root and shoot of all tested pea genotypes was observed under salinity. However, the highest proline content in shoots was recorded under 7.0 dS m^{-1}, followed by 5.0 and 2.5 dS m^{-1} (Figure.1b). The highest proline content in shoots showed the same pattern. Samrena zard (30.18%) and climax (27.25%) performed the best among all genotypes detected in terms of proline content in roots (Figure. 1b) exposed to salinity at 7.0 dS m^{-1} in contrast to ambasidar (24.13%) and green arrow (29.03%). In comparison to ambasidar (41.37%) and green arrow (47%) in shoots, the genotypes samrena zard (71.92%) and climax (55.5%) performed the best (Figure.1a). Control plants had the lowest amount of glycine betaine in their roots. A comparison of the means of the salt treatments revealed that the climax had the highest glycine betaine concentration in the roots, at 7.0 dSm^{-1} (34.88%), followed by ambasidar (20.68%) and green arrow (22.68%) (Figure. 1d). In contrast to ambasidar (25%) and green arrow (27.58%), samrena zard and (32.42%) had the greatest glycine betaine concentration in shoots at 7.0 dS m^{-1} (32.57%) seen in the climax (Figure.1c). Samrena zard and climax performed the best when compared to ambasidar and green arrow in terms of the amount of glycine betaine in roots and shoots exposed to 7.0 dS m^{-1} of stress, out of all the genotypes that were detected.







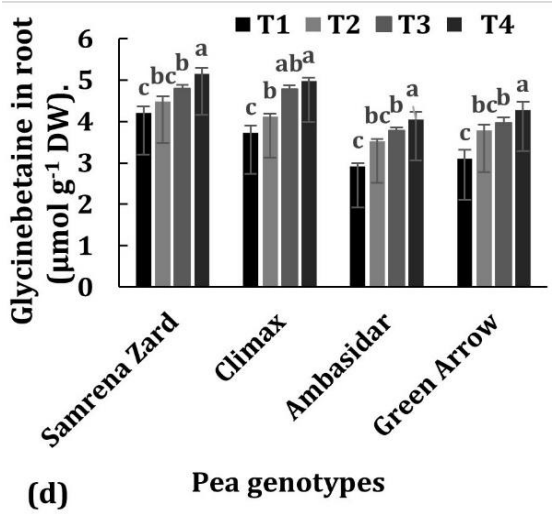
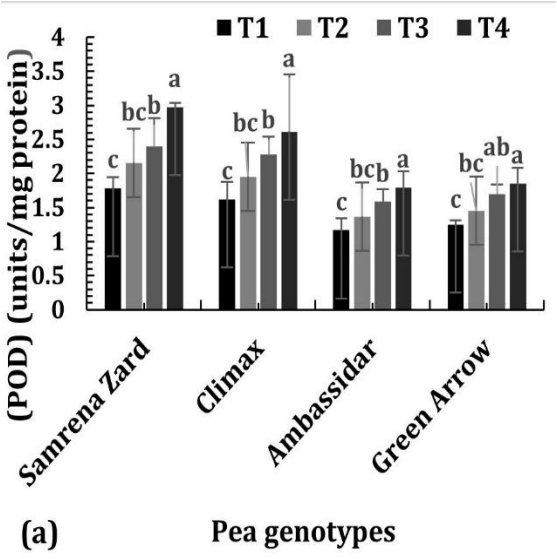
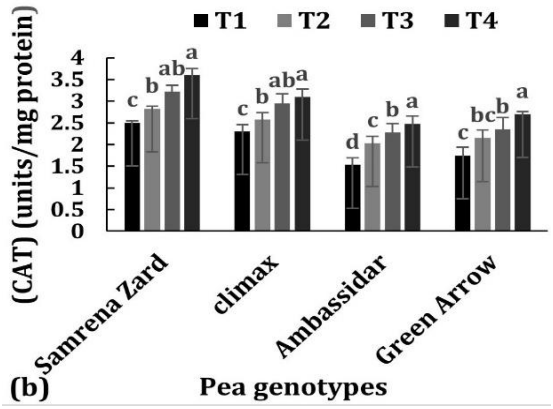


Figure.1 a,b,c & d: Effect of salts on Proline & Glycinebetaine contents in shoot and root of salt-tolerant (Samrena Zard and Meteor) and salt-sensitive (Ambasidar and Green arrow) pea genotypes under salts stress. Each value in the figure is the mean of four replicates and the vertical bars give the standard error (SE) of the mean. The least significance difference (LSD) test was used to evaluate the significance of differences between the treatments (T_1) control, (T_2) 2.5, (T_3) 5.0 and (T_4) 7.0.; dS m^{-1}.

Impact of salinity on antioxidant activities

All pea genotypes exhibited elevated antioxidant activity, including SOD, POD, and CAT, in saline conditions. The control had the highest SOD at 7.0 dS m^{-1} among the salt treatments, according to percentage reduction. Samrena zard (26.25%) and climax (23.63%) performed the best among all genotypes detected in relation to SOD exposed to 7.0 dS m^{-1} of salt strain, in contrast to ambasidar (22.03%) and green arrow (23.38%) (Figure. 2 c). When compared to ambasidar (55.54%) and green arrow (58%) salt treatments, the salts stressed at 7.0 dS m^{-1} POD showed the highest performance, followed by samrena zard (70.58%) and climax (62.55%) (Figure. 2 a). Samrena zard (44%) and climax (40.9%) performed the best among all the genotypes examined in relation to CAT exposed to 7.0 dS m^{-1} salt stress, in contrast to ambasidar (26.31%) and green arrow (35%), as shown in (Figure. 2b).





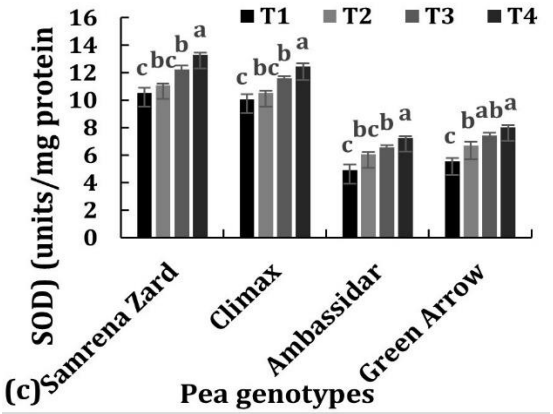
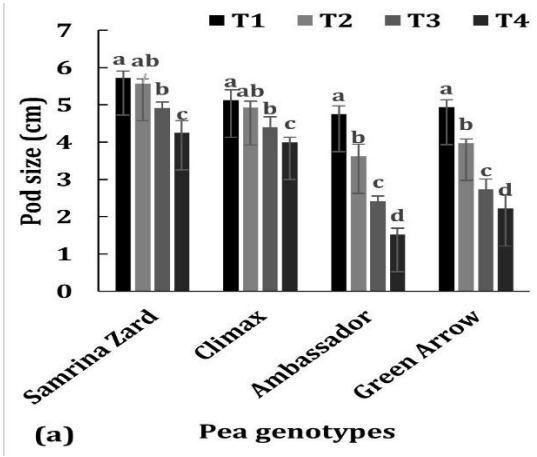
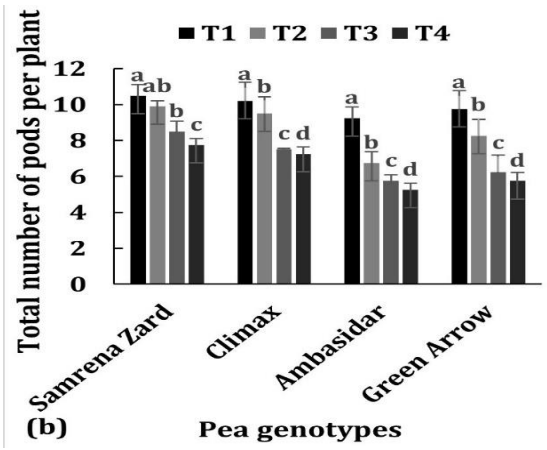


Figure.2 a, b, c & d: Effect of salts on CAT, POD & SOD activities of salt-tolerant (Samrena Zard and Meteor) and salt-sensitive (Ambasidar and Green arrow) pea genotypes under salts stress. Each value in the figure is the mean of four replicates and the vertical bars give the standard error (SE) of the mean. Least significance difference (LSD) test was used to evaluate the significance of differences between the treatments (T_1) control, (T_2) 2.5, (T_3) 5.0 and (T_4) 7.0.; dS m^{-1}.

Impact of salinity on yield attributes

A remarkable reduction in pod size, seeds per pod, and pods per plant was noted under salt stress. Comparison of the means showed that a substantial fall in the total pods number per plant was attained in ambasidar (43.24%), and green arrow (41.21%) genotypes under 7.0 dS m^{-1} of salt strain. The samrena zard (26.19%) and climax (29.41%) genotypes performed better with the lowest percentage of decline in the number of seeds per pod under 7.0 dS m^{-1} of stress (Figure. 3c). The maximum seeds per pod were recorded in control, while the highest fall in the seeds per pod was observed in climax (51.82%) and samrena zard (54.53%) genotypes under 7.0 dS m^{-1}. The pod weight (g) decreased by the least percentage at 7.0 dS m^{-1} climax (31.6%) and samrena zard (30.4%) (Figure. 3d). Pod weight (g) reduced in number of seeds per pod at 7.0 dSm^{-1} ambasidar (41.98%) and green arrow (42.55%) (Figure. 3d). At 7.0 dS m^{-1} of stress, the samrena zard (28%) and climax (21.87%) genotypes showed least percentage drop in pod size (Figure. 3a). Green arrow (55.17%) and ambasidar (68%) reduced the pod size (cm) at 7.0 dSm^{-1} (Figure. 3a). In comparison to ambasidar and green arrow, the aforementioned results demonstrated that all examined genotypes exhibited the best performance in terms of yield attributes when exposed to 7.0 dS m^{-1} salt stress.





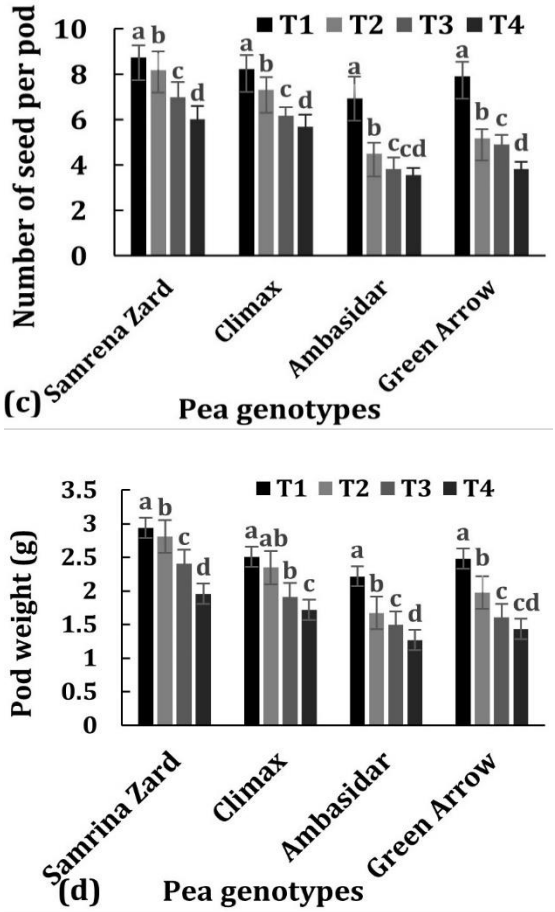


Figure.3 a, b, c & d: Effect of salts on pod size, total number of pods, number of seed per pod & pod weight of salt-tolerant (Samrena Zard and Meteor) and salt-sensitive (Ambasidar and Green arrow) pea genotypes under salts stress. Each value in the figure is the mean of four replicates and the vertical bars give the standard error (SE) of the mean. Least significance difference (LSD) test was used to evaluate the significance of differences between the treatments (T_1) control, (T_2) 2.5, (T_3) 5.0 and (T_4) 7.0.; dS m^{-1}.

Discussion

Salt tolerance is a key target trait in pea breeding programs. Developing an effective and reliable identification method is essential for the successful evaluation of salt tolerance in pea germplasms. In this experiment, a pot culture technique was used to illustrate saline tolerance among four distinct pea germplasms. This method is both fast and cost-effective, and it can readily satisfy the necessary experimental conditions. It offers several key benefits: (i) salinity levels are standardized within each pot or across multiple pots, (ii) the amount of irrigation can be precisely controlled, and (iii) issues related to salt depletion have been effectively resolved. The efficacy of this approach has been further validated by prior studies on various crops, including rice [24], cotton [25], and garlic [26]. Compared to ambasidar and green arrow, resistant genotypes (samarina zard and climax) had higher levels of proline and glycine betaine. In directive to defend antioxidant system enzymes from the destructive impact of salt stress, proline is essential (Figure. 1a, b, c & d). High proline as well as glycine betaine levels are accumulated in plant chloroplasts under abiotic stressors like salt and drought. This indicates the plant's capacity for osmotic adjustment and promotes better plant growth. In order to mitigate the severe consequences of abiotic stressors, synthetic pure glycine betaine has been widely utilized [27]. The effects of adding glycine betaine to roots and leaves are equally important [28]. *Salicornia europaea* and *Suaeda maritima* have been shown to accumulate more suitable solutes under stressful situations [29], *Phragmites australis* [30], *Brassica nupus* [31] and *Zea mays* [32]. With a notable rise in POD activity, particularly at EC of 5.0 and 7.0 dS m^{-1}, all of the salt treatments markedly enhanced total SOD activity. The actions of APX and CAT usually diminished in seedlings in salt strain. According to our research (Figure.2 a, b &c) many acquired salt tolerance as a result of enhanced resistance to oxidative stress through elevated peroxidase and superoxide dismutase/ ascorbate-glutathione cycle [33]. To minimize oxidative harm, plants produce a range of antioxidants [34]. Our results are consistent [35] finds that plants combat ROS by a combination of enzymatic as well as non-enzymatic

progressions. Enzymes that scavenge ROS embrace glutathione reductase (GR), CAT, APX, SOD, POD, and glutathione-synthesizing enzymes [36, 37]. The findings similarly support those of [38] found that the genotype exposed to salt strain had the greatest level of antioxidant activities plant root and leaf. [39] confirmed that under salinity, the antioxidant enzymes were more vigorous in mung bean plants. Si application lowered sodium ion absorption and raised K^+ in mung bean plants. Our finding showed that the area of peas leaves, dry mass, pod number, and yield had decreased in comparison to the result mentioned by [40]. The reaction to salinity was a marked reduction in flower and pod production. The plants reproductive phase salt sensitivity was more noticeable at high salinity, as evidenced by an increase in flower shedding that resulted in fewer pods. Furthermore, the number and weight of seeds per plant exhibited a linear decline under salt stress. Similarly, effects on reproductive growth were observed in mung bean, where a 0.6% saline solution resulted in a 60 % reduction in pod production and a 12 % decrease in seed yield, as reported by [41,42]. Our findings (Figure.3 a, b & c) confirm the results of [34] a comparable decrease in pod in pod length was noted at all salinity levels rising at 75nm and minimum at 25 salt stress. On the other hand, 50 mM and the control group have the largest pod lengths.

Conclusion

The study emphasized the substantial interspecific variability in the accumulation of osmolytes, such as proline as well as glycine betaine, and the alternation of enzyme activity. Additionally, a recent study shows that in the root and shoot of samrena zard the higher activities of antioxidant and more gathering of osmolytes were observed, which indicate that it is more salt resistant than ambasidar. This variability can be utilized for early-stage screening to differentiate salt-resistant and susceptible genotypes. Samrena zard is more resistant to salt stress than ambasidar because it has more antioxidants and higher levels of proline and glycine betaine in below and above plant parts.

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