

Salinity stress induced morphological, biochemical and genetic variations in soybean (*Glycine max* L.) genotypes

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Abstract

Soybean is a major source of protein and oil in Bangladesh. Despite its production remains limited due to the lack of high-yielding and stress-tolerant varieties. This study aimed to investigate the effect of three levels of salinity — 0, 6, and 10 dS m⁻¹ electrical conductivity (EC) — on shoot, root, pod, and biochemical traits of 13 soybean genotypes. At 10 dS m⁻¹ EC, plant height and shoot dry weight decreased by 18.7% and 47.9%, respectively, compared to control. The number of primary lateral roots significantly increased by 30.3%, although the dry weight of the roots and total number of seeds decreased by 34.3% and 80%, respectively, at 6 dS m⁻¹ EC compared to control. In response to stress, the contents of biochemical traits such as proline and ascorbate peroxidase showed dramatic upsurge by 271% and 159%, respectively, at 10 dS m⁻¹ EC compared to control. Principal component analysis (PCA) differentiated the genotypes under control and salt-treated conditions for their positive and negative PC1 scores, respectively. Plant height, total number of trifoliate, secondary lateral root diameter, total number of pods, total number of seeds, proline, hydrogen peroxide (H₂O₂), malondialdehyde (MDA), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) estimated high heritability coupled with a high genetic advance. The genotypes S-07 (MTD-176), S-31 (MTD-6), S-23 (Bragg), and BS-02 (Binasoybean-2) were salinity stress tolerant. These findings laid a foundation for developing salt-tolerant soybean varieties and identifying quantitative trait loci (QTL) associated with salinity stress tolerance.

Introduction

Soybean (*Glycine max* L., 2n = 40), the Golden Miracle bean of the Leguminosae family is a self-pollinated oilseed crop. It is one of the most important leguminous crops globally, valued for its high protein and oil content, making it a crucial component of food, feed, and industrial applications. In the crop year 2023–2024, soybeans were the most popular kind of oilseed globally with an annual production of around 394 million tons, which surpassed 427 million tons in the next year (Statista 2024, USDA 2024).

Bangladesh demands 2.5 to 3.0 million metric tons soybeans annually primarily for poultry feed, edible oil production and livestock feed (USDA 2024). Though Bangladesh has ideal meteorological and edaphic conditions for soybean production, the yield is barely 1.7 t ha⁻¹, compared to the global average of 3 t ha⁻¹, covering only about 5% of its total demand (USDA 2024). In 2023, Bangladesh imported \$962M in soybeans, becoming the 16th largest importer of soybeans in the world (OEC, 2024). Lack of high yielding varieties and poor agronomic practices in Bangladesh are the causes of the low production and acreage of soybeans. Moreover, soybean productivity is severely impacted by abiotic stresses such as salinity, which adversely affect growth, development, and yield of soybean plants. Out of 1.689 million hectares of coastal areas used for agriculture, around 1.056 million hectares are afflicted by salt (Shawkhatuzamman et al. 2023). Therefore, to sustain soybean production under such challenging conditions, one of the strategies would be to develop high-yielding and stress-tolerant soybean cultivars.

Plants have evolved various mechanisms to cope with environmental stresses like drought, heat, salinity, and disease. When exposed to such stresses, they undergo metabolic adjustments to help them survive and adapt (Amirijani 2010). Ion toxicity and osmotic stress are two principal mechanisms via which salinity alters the metabolism of soybean plants as well as affects the root system (Mittler 2002). The root is the primary organ responsible for water and nutrient uptake, and its performance under saline conditions is crucial for the overall health and productivity of the plant. Even the root system exhibits early signs of stress (Ao et al. 2010). Thu et al. (2014) reported that many soybean germplasms under drought stress condition had shorter roots and accumulated dry biomass.

Several studies have strongly demonstrated that roots with wider xylem diameters and/or more developed lateral root systems, along with a greater number of root hairs, contribute to improved drought tolerance (Tanaka et al. 2014, Vadez 2014). These root characteristics help the plant better absorb water and nutrients, thus enhancing its ability to withstand drought conditions by increasing root surface area and improving water uptake efficiency. A primary root (tap root) and lateral (basal) roots make up the allorhizic root system (Fig. 1) of soybean (Ao et al. 2010, Fenta et al. 2014). These roots typically have a greater surface area, which enhances their ability to efficiently absorb moisture and nutrients, thereby supporting photosynthesis (Blum 2011, Lopes et al. 2011, Comas et al. 2013). However, there is a lack of studies investigating these root characteristics under saline conditions.

Furthermore, salinity stress increases the generation of reactive oxygen species (ROS) such as superoxide radical, H₂O₂, hydroxyl radical and singlet oxygen, as well as other abiotic stresses like osmotic stress and oxidative stress in plant cells. The production of ROS disrupts a number of physiological and metabolic processes in plants, such as photosynthesis and the antioxidant defense system, which results in DNA strand cleavage, membrane disintegration, lipid peroxidation, chlorophyll degradation, biological macromolecule degradation, and ion leakage (Hossain et al. 2013).

The best way to study root systems is to investigate them directly, non-destructively, and continuously in the soil where they are growing. Unfortunately, these kinds of measurements are challenging due to the opacity of the soil. Using sodium chloride (NaCl) as a source of salt in hydroponic culture is one method for simulating stress conditions in order to examine the impact of salinity stress on root traits. This study was therefore planned to investigate the effect of salinity stress on morphological alteration with special emphasis on root morphology along with shoot, pod and biochemical traits of soybean genotypes.

Materials and Methods

Experimental site, plant materials, design and conditions for plant growth

This experiment was conducted in a glass-house. Thirteen soybean genotypes were chosen with three treatments i.e., untreated or control (no salt), 6 dS m⁻¹ EC, and 10 dS m⁻¹ EC (Table 1). About 10 g seeds of each genotype were germinated in petri-dishes. No nutrient solution was provided in the petri-dishes during germination of the seeds. Seedlings were transferred into the squared perforated styrofoam sheets at about 10–12 days age on a hydroponic solution (Robin et al. 2016). Thirty individual plastic containers were used for this experimentation, 10 randomized trays for each treatment. Each tray contained 13

randomized plants, one plant from each variety (Table 3). In this case, each plant was considered as a replicate. Thus, the experimental design was a completely randomized design. The first set of five trays were harvested for studying root and shoot traits and the second set of five trays were harvested to study pod traits. The Peter's Professional (Urea:TSP:MoP = 20:20:20 General Purpose Fertilizer, 1 g L⁻¹, The Scotts Company, Marysville, OH) was the source of nutrients, along with Ferrous Sulphate (FeSO₄) fertilizer (200 mg L⁻¹). The nutrient solution was changed after every week. The pH of the solution was checked at two-day interval using a pH meter (HI 9811-0; Hanna Instruments, Woonsocket, RI) and adjusted to around 5.7 and 5.8. All plants were raised in homogenous environmental settings until 20 days. After 20 days, NaCl solution was applied in order to induce salt stress. and the amount of salt solution was adjusted by using an EC meter (HI 9811-0; Hanna Instruments, Woonsocket, RI) at every two-days interval.

Table 1
List of genotypes used in the experiment

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Sl. No.	Genotypes	Code Given	Source	Sl. No.	Genotypes	Code Given	Source
1	Lokon	S-03	GPB, BAU	8	AGS-79	S-28	GPB, BAU
2	Shohag	S-05		9	MTD-6	S-31	
3	MTD-176	S-07		10	MINA-HAI	S-32	
4	K-16	S-09		11	Asset-93-19-1	S-35	
5	Asset-93-19-5	S-11	BINA	12	Binasoybean-2	BS-02	BINA
6	Bragg	S-23		13	Binasoybean-5	BS-05	
7	BS-13	S-25					
Here, GPB, BAU: Department of Genetics and Plant Breeding, Bangladesh Agricultural University; BINA: Bangladesh Institute of Nuclear Agriculture.							

Table 3
List of genotypes used for collecting data on morphological and biochemical traits at different growth stages.

Shoot and root traits	Pod and reproductive traits	Biochemical traits
S-03	S-03	S-03
S-05	S-05	S-23
S-23	S-23	BS-02
S-25	S-25	S-07
S-35	S-35	S-09
BS-02	BS-02	
S-31	S-31	
S-11	S-11	
S-07	S-07	
S-28	S-28	
S-09		
S-32		
BS-05		
Number of genotypes = 13	10	5
Number of replicates = 5	5	2
Number of treatments = 3	3	3
Here, S-03 = Lokon, S-05 = Shohag, S-23 = Bragg, S-25 = BS-13, S-35 = Asset-93-19-1, BS-02 = Binasoybean-2, S-31 = MTD-6, S-11 = Asset-93-19-5, S-07 = MTD-176, S-28 = AGS-79, S-09 = K-16, S-32 = MINA-HAI, BS-05 = Binasoybean-5.		

Data collection on morphological traits

The leaves of thirteen soybean genotypes from control and salt-treated plants were graded based on damage at 21 days after applying salt stress. Stress symptoms were scored optically on a scale from 1 to 9 (Table 2, Fig. 2) (modified from Ledesma et al. 2016).

Table 2
Scoring categories for the visible injury scoring of soybean leaves

Score	Observations
1	Healthy, dark green colored leaflets
3	Moderately healthy with slight chlorosis and curling at leaflet tip
5	Mostly pale green in color due to moderate chlorosis of leaflet
7	Leaflets become mostly dry and pale yellow in color
9	Dead or near to die leaflets

Chlorophyll content of soybean leaves was measured three weeks after salt stress application using a chlorophyll meter (SPAD-502, Minolta, Japan). 21 days after the application of salt stress, plants were harvested from 15 plastic trays (3 treatments x 5 trays) and data were recorded on different shoot and root traits. Total number of branches, trifoliates, and primary lateral roots of soybean genotypes were counted. Plant height, length of main axis root, length of primary lateral root and length of secondary lateral root were measured on a centimeter scale. All other root traits (main axis and lateral root diameter) were measured under a light microscope at 100X magnification using a micrometer scale. Roots and shoots were dried at 60°C in an air-dry oven for three days before recording their dry weights. Due to salinity stress, three genotypes died at the reproductive stage. Therefore, the remaining 10 genotypes were considered for data collection on number of pods plant⁻¹, seeds plant⁻¹, pod length, and thousand seed weight from the remaining 15 plastic trays (3 treatments x 5 trays) after harvest, at 60 days after salt stress application (Table 3).

Biochemical analyses

After 21 days of applying salt stress, five soybean genotypes were selected based on their high morphological performance (Table 3). Leaf samples were collected from the selected plants -during destructive harvest of morphological traits of both control and salt-treated conditions for biochemical analysis, along with two replications. Proline, H₂O₂, and MDA activity was assessed using the Bates (1973), the Alexieva et al. (2000), and the Heath and Packer (1968) techniques, respectively. For POD, CAT, and APX determination, Hemeda and Klein (1990), Beer and Sizer (1952), and Nakano and Asada (1981) techniques were followed, respectively.

Statistical analyses

The statistical software program MINITAB 19 (Minitab Inc., State College, Pennsylvania, USA) was used to examine the data. A two-way analysis of variance (ANOVA) was conducted following a general linear model (GLM) for several morphological and biochemical traits to investigate genotype, treatment and genotype by treatment interaction (genotype × treatment) effects. ANOVA was conducted to evaluate the variation in root and shoot traits, pod-related traits, and biochemical traits across 13, 10, and 5 genotypes, respectively (Table 3). As a post-hoc analysis, Tukey's pairwise comparison was used to identify any significant differences between the genotypes, treatments and genotype by treatment interaction effects. To identify a pattern of genotype-trait associations and correlations between certain traits, principal component analysis (PCA) and Pearson correlation analyses were performed on the studied traits. ANOVA of the PC scores was performed using the GLM procedure to explore the statistical significance among genotype, treatment and genotype by treatment interaction.

Estimation of genetic parameters

Genetic parameters such as genotypic and phenotypic variances, heritability, genotypic co-efficient of variation (GCV), phenotypic co-efficient of variation (PCV) and genetic advance were estimated according to the formula outlined by Johnson et al. (1955), Singh and Chaudhary (1985), and Hanson et al. (1956).

Heritability in broad sense was calculated from the ANOVA table. The following formula was used-

$$h^2b = \frac{\sigma^2g}{\sigma^2p} \times 100$$

Here,

$$\sigma^2g = \text{Genetic variance} = \frac{\text{Mean square for genotypes} - \text{Mean square for error}}{r}$$

r = Replication number

$$\sigma^2p = \text{Phenotypic variance} = \sigma^2g + \sigma^2e$$

σ^2e = Environmental/error variance

Results

Treatment effect on morphological traits

Salt stress markedly affected the shoot and root traits of soybean genotypes (Table 4). At 10 dS m⁻¹ EC, plant height, chlorophyll content, and shoot dry weight decreased by 18.7%, 33.9%, and 47.9%, respectively, compared to control (Table 5). Regarding root traits, length of main root axis and total number of primary lateral roots significantly increased by 3.38% and 30.3%, respectively, at 6 dS m⁻¹ EC compared to control. Conversely, the diameters of the main, primary, and secondary axis roots declined by 25.1%, 42.4%, and 36.4%, respectively, at 10 dS m⁻¹ EC compared to control (Table 5).

Table 4
Analysis of variance for shoot and root traits of 13 soybean genotypes

Sources of variation	df	Mean sum of squares													
		LIS	PH (cm)	TB	TT	ChlC (%)	SDW (g)	MAL (cm)	MAD (mm)	TLR1	LRL1 (cm)	LRD1 (mm)	LRL2 (cm)	LRD2 (mm)	RDW (g)
Treatment (T)	2	487.2	4311.9	99.4	627.7	2079.8	265.0	6.8	38	655.3	58.7	7.5	26.8	0.03	16
		***	***	***	***	***	***	NS	***	***	**	***	**	***	***
Genotypes (G)	12	9.2	2932.1	3.9	100.4	84.4	19.2	32.9	3	196.8	40.1	0.6	6.8	0.4	0.6
		***	***	***	***	***	***	***	***	***	***	***	*	***	NS
G × T	24	4.9	374.2	2.5	25.0	37.2	17.7	9	1.6	51.1	15	0.5	3.6	0.013	0.6
		***	***	**	***	***	***	NS	**	*	*	***	NS	***	*
Error	153	0.4	128	1.1	9.7	0.17	2.2	8.2	0.7	26.5	7.9	0.09	3.7	0.0008	0.3
*, ** and *** indicate significant at 5%, 1% and 0.1% levels of probability, respectively and NS = non-significant. Here, G × T = Genotype by treatment interaction, df = degrees of freedom, LIS = Leaf injury scoring, PH = Plant height, TB = Total number of branches, TT = Total number of trifoliates, ChlC = Chlorophyll content, SDW = Shoot dry weight, MAL = Length of main axis root, MAD = Diameter of main axis root, TLR1 = Total number of primary lateral roots, LRL1 = Length of primary axis root, LRD1 = Diameter of primary axis root, LRL2 = Length of secondary axis root, LRD2 = Diameter of secondary axis root, RDW = Root dry weight.															

Table 5
Comparison of means between treatments for shoot, root, pod traits and biochemical parameters

Trait Type	Traits	Control	6 dS m ⁻¹ EC	% Change	10 dS m ⁻¹ EC	% Change	Mean
Shoot	PH (cm)	82.9	80.2	3.25	67.4	18.7	76.8
	TB	3.78	2.31	38.8	1.27	66.4	2.45
	TT	13.6	10.5	22.8	7.28	46.5	10.5
	ChIC (%)	34.2	28.3	17.2	22.6	33.9	28.4
	SDW (g)	7.69	4.31	43.9	4.00	47.9	5.33
Root	MAL (cm)	17.7	18.3	3.38	17.9	1.12	17.9
	MAD (mm)	6.22	5.55	10.7	4.66	25.1	5.47
	TLR1	19.1	24.9	30.3	19.8	3.66	21.3
	LRL1 (cm)	14.7	14.6	0.68	13.0	11.6	14.1
	LRD1 (mm)	1.58	1.12	29.1	0.91	42.4	1.20
	LRL2 (cm)	4.82	4.55	5.60	3.58	25.7	4.32
	LRD2 (mm)	0.11	0.09	18.2	0.07	36.4	0.09
	RDW (g)	2.86	1.88	34.3	2.18	23.7	2.31
Pod	TP	27.2	5.72	78.9	-	-	10.9
	PL (cm)	3.74	3.21	14.2	-	-	2.32
	TS	56.2	11.3	79.9	-	-	22.5
	TSW (g)	163	115	29.4	-	-	92.8
Biochemical	Proline (μg g ⁻¹)	7.14	27.9	291	26.5	271	20.5
	H ₂ O ₂ (μM g ⁻¹)	3.19	3.97	24.5	4.95	55.2	4.04
	MDA (μM g ⁻¹)	0.25	0.35	40.0	0.37	48.0	0.32
	POD (μM min ⁻¹ g ⁻¹)	2.61	3.86	47.9	4.76	82.4	3.74
	CAT (μM min ⁻¹ g ⁻¹)	0.48	0.94	95.8	1.04	116	0.82
	APX (μM min ⁻¹ g ⁻¹)	41.2	81.6	98.1	107	159	76.6

Here, LIS = Leaf injury scoring, PH = Plant height, TB = Total number of branches, TT = Total number of trifoliate, ChIC = Chlorophyll content, SDW = Shoot dry weight, MAL = Length of main axis root, MAD = Diameter of main axis root, TLR1 = Total number of primary lateral roots, LRL1 = Length of primary axis root, LRD1 = Diameter of primary axis root, LRL2 = Length of secondary axis root, LRD2 = Diameter of secondary axis root, RDW = Root dry weight, TP = Total number of pods plant⁻¹, PL = Length of pod, TS = Total number of seeds plant⁻¹, TSW = Thousand (1000) seed weight, Proline = Proline content, H₂O₂ = Hydrogen peroxide content, MDA = Malondialdehyde content, POD = Peroxidase content, CAT = Catalase content, APX = Ascorbate peroxidase content.

Salinity stress significantly influenced the timing of flowering and pod formation. Plants exposed to 6 dS m⁻¹ EC initiated flowering at 40–45 days after sowing (DAS), whereas control plants commenced flowering at 50–55 DAS (Table S1). In contrast, plants subjected to an EC level of 10 dS m⁻¹ did not survive at the flowering stage. Under 6 dS m⁻¹ EC, notable reductions were observed in key yield attributes compared to the control. The total number of pods plant⁻¹, pod length, total number of seeds plant⁻¹, and 1000-seed weight decreased by 78.9%, 14.2%, 79.9%, and 29.4%, respectively (Table 5).

Genotypic differences for morphological traits

The genotypes S-23 and S-11 recorded the highest plant height (105.4 cm) and shoot dry weight (8.1 g), respectively (Table 6). On the other hand, genotype BS-02 had the lowest plant height (55.2 cm) and shoot dry weight (3.6 g) (Table 5). In terms of root traits, the highest diameter of the main root axis (6.3 mm), number of primary lateral roots (26.9) were found in genotype S-28 and root dry weight (2.7 g) were found in genotype BS-02 (Table 6). In addition, genotype S-09 recorded the highest primary (1.48 mm) and secondary (0.65 mm) lateral root diameter. In contrast, genotype BS-05 recorded the lowest number of primary lateral roots (15.6), and genotype S-31 recorded the minimum root dry weight (1.98 g) (Table 6). Moreover, genotypes S-23 and S-35 had the highest number of pods plant⁻¹ (17.8) and the highest thousand seed weight (103.8 g), respectively. Contrarily, the minimum number of pods plant⁻¹ and thousand seed weight were recorded in S-03 and S-11, respectively (Table 6).

Table 6
Comparison of means among soybean genotypes for shoot, root, pod traits and biochemical parameters

Traits	S-03	S-05	S-23	S-25	S-35	BS-02	S-31	S-11	S-07	S-28	S-09	S-32	BS-05	Mean
LIS	3.3	3.1	4.3	3.9	2.2	1.7	3.5	3.4	3.7	3.7	4.1	4.5	3.6	3.5
PH	64.9	70.7	105.4	78.2	78.5	55.2	89.4	79.8	79.4	78.5	68.6	94.3	56.1	76.8
TB	1.7	2.3	2.5	1.9	2.3	1.9	2.9	3.1	2.4	3.5	2.1	2.6	2.5	2.5
TT	7.5	8.6	11.1	8.7	10.5	6.3	13.7	13.9	11.9	14.8	8.9	11.1	9.3	10.5
ChIC	28.9	30	26.4	27.9	27.1	31.9	27.2	26.7	29.7	26.9	33.2	28.6	24	28.3
SDW	5.8	5.1	5.3	5.3	4.1	3.6	5.9	8.1	4.2	5.1	6.5	5.2	4.9	5.3
MAL	15.6	15.9	17.9	17.7	17.5	19.8	20.4	17.8	19.2	19.5	16.3	18.4	17.2	17.9
MAD	4.8	5.4	5.1	5.7	5	5.6	5.8	6.3	5.3	6.3	5.5	5.2	5.3	5.5
TLR1	18.7	20.1	21.5	18.5	23.5	16.5	25.9	20.7	26.7	26.9	22.4	19.7	15.6	21.3
LRL1	12.9	11.5	14.8	14.7	14.6	17.1	15.8	13.4	13.8	13.9	13.4	16.6	11.6	14.2
LRD1	0.9	0.91	1.1	1.3	1.2	1.05	1.5	1.41	1.09	1.22	1.48	1.19	1.4	1.2
LRL2	3.9	3.6	4.4	3.5	3.4	4.3	4.8	5.61	5.27	4.9	3.94	3.99	4.3	4.3
LRD2	0.04	0.044	0.04	0.04	0.07	0.05	0.05	0.038	0.03	0.04	0.65	0.035	0.04	0.09
RDW	2.2	2.2	2.3	2.5	2.2	2.7	1.98	2.38	2.32	2.68	2.22	2.33	2.2	2.3
TP	4.9	12.9	17.8	11.5	12.4	7.1	12.7	10.8	10.1	9.3	Mean		10.9	
PL	2.2	2.1	2.4	1.8	2.35	2.6	2.3	2.4	2.6	2.5	2.32			
TS	10.9	23.1	31.9	24.8	24.3	13.3	32.8	20.3	23.3	20.5	22.5			
TSW	92.8	90.7	91.3	86.6	103.8	91.5	96.9	81	94.8	98.7	92.8			
	S-3		S-23		BS-2		S-7		S-9		20.53			
Proline	22.839		14.769		23.633		18.399		23.032					
H ₂ O ₂	4.798		2.988		4.354		4.071		3.978		4.0378			
MDA	0.273		0.378		0.381		0.297		0.291		0.324			
POD	6.004		2.095		4.296		1.573		4.745		3.7426			
CAT	0.663		0.834		0.898		1.026		0.673		0.8188			
APX	64.161		84.483		69.739		114.122		50.739		76.6488			

Here, LIS = Leaf injury scoring, PH = Plant height, TB = Total no. of branch, TT = Total no. of trifoliate, ChIC = Chlorophyll content, SDW = Shoot dry weight, MAL = length of main axis root, MAD = diameter of main axis root, LRL1 = length of primary axis root, LRD1 = diameter of primary axis root, LRL2 = length of secondary axis root, LRD2 = diameter of secondary axis root, RDW = root dry weight, TP = Total no. of pods per plant, PL = length of pod, TS = Total number of seeds per plant, TSW = Thousand (1000) seed weight, Proline = Proline content, H₂O₂ = Hydrogen peroxide content, MDA = Malondialdehyde content, POD = Peroxidase content, CAT = Catalase content, APX = Ascorbate peroxidase content and S-03 = Lokon, S-05 = Shohag, S-23 = Bragg, S-25 = BS-13, S-35 = Asset-93-19-1, BS-02 = Binasoybean-2, S-31 = MTD-6, S-11 = Asset-93-19-5, S-07 = MTD-176, S-28 = AGS-79, S-09 = K-16, S-32 = MINA-HAI, BS-05 = Binasoybean-5.

To understand the genotypic performances of shoot, root, and pod traits at control, 6 dS m⁻¹ EC and 10 dS m⁻¹ EC, a ranking table was created depending on the number of letters found during post-hoc analysis. Genotypes having better performance received higher grades. The overall score of a genotype was then calculated from the trait-based scores of that genotype, and genotypes were ranked accordingly. The genotype S-32, followed by S-07, S-23, and S-31 were ranked as toppers for various shoot traits under both control and treated conditions (Table S2). For root traits, the genotype S-09 outperformed all other genotypes, followed by S-28, S-31 and S-11 (Table S3). The genotype S-31 showed superior performance for various pod traits under both control and treated conditions (Table S4).

Genotype × Treatment interactions on morphological traits

This study evaluated the responses of 13 soybean genotypes to salinity stress, focusing on shoot, root, and pod morphology. Analysis of variance revealed that the majority of shoot, root, and pod traits—except for main axis root length and secondary lateral root length—exhibited significant genotype-by-treatment interactions (Table 4, 7). At 10 dS m⁻¹ EC, plant height was reduced by 31.3% in S-07, 17.4% in S-31, and 12.7% in BS-02 compared to control. Conversely, at 6 dS m⁻¹ EC, genotype S-23 exhibited a 28% increase in plant height (Table S5, Fig. 3). Furthermore, at 10 dS m⁻¹ EC, shoot dry weight declined significantly in S-07 (51%), S-31 (45.6%), S-23 (46%), and BS-02 (29%) compared to control (Table S5, Fig. 3).

Table 7
Analysis of variance for pod traits of 10 soybean genotypes

Sources of variation	df	Mean sum of squares			
		TP	PL (cm)	TS	TSW (g)
Treatment (T)	2	10101.9***	200.2***	43382.4***	348257***
Genotypes (G)	9	171***	0.73***	676.9***	524***
G × T	18	264.3***	0.32***	757.7***	1052***
Error	113	6.1	0.048	47.3	9
*** indicates significant at 0.1% level of probability. Here, G × T = Genotype by treatment interaction, df = Degrees of freedom, TP = Total number of pods plant ⁻¹ , PL = Length of pod, TS = Total number of seeds plant ⁻¹ , TSW = Thousand (1000) seed weight.					

At 10 dS m⁻¹ EC, primary lateral root length increased in S-31 (7.9%), S-5 (14%), and S-9 (3.6%), while a decline was observed in the remaining genotypes compared to control (Table S6, Fig. 4). For root dry weight, genotypes S-5 and S-35 exhibited an increase of 23.94% and 5.9%, respectively, whereas all other genotypes recorded a reduction in root biomass (Table S6). In contrast, primary lateral root diameter and main axis root diameter decreased under salinity stress compared to the control (Table S6, Fig. 4), suggesting that salinity stress adversely affects the structural integrity of roots despite variations in genotype-specific responses.

Among pod-related traits, total number of seeds plant⁻¹ decreased significantly at 6 dS m⁻¹ EC, with reductions of 68.8% in S-07, 70% in S-31, 90.6% in S-23, and 63% in BS-02 compared to control (Table S7, Fig. 3). Similarly, thousand seed weight declined by 13.4% in S-07, 14.7% in S-31, 31.8% in S-23, and 40% in BS-02 at 6 dS m⁻¹ EC compared to control (Table S7, Fig. 3). These findings indicate that salinity stress severely impacts seed yield and quality, with genotype-specific variations in tolerance.

Biochemical responses under salinity stress

All the biochemical traits in this study were significantly increased under salinity stress compared to the control in the five soybean genotypes (Table 8). At 6 dS m⁻¹ EC, proline underwent a significant rise of 291% in response to stress. Increase of salinity from 6 to 10 dS m⁻¹ EC, resulted in an increase of CAT by 21% and APX by 62%, respectively (Table 5).

Table 8
Analysis of variance for biochemical traits of 5 soybean genotypes

Sources of variation	df	Mean sum of squares					
		Proline	H ₂ O ₂	MDA	POD	CAT	APX
		(µg g ⁻¹)	(µM g ⁻¹)	(µM g ⁻¹)	(µM min ⁻¹ g ⁻¹)	(µM min ⁻¹ g ⁻¹)	(µM min ⁻¹ g ⁻¹)
Treatment (T)	2	1351.9***	7.9***	0.04***	11.7***	0.88***	11025.5****
Genotypes (G)	4	88.4***	2.7***	0.02***	20.8***	0.14***	3510.9***
G × T	8	133.7***	1.6***	0.009***	2.3***	0.2***	905.9***
Error	15	0.96	0.09	0.00009	0.07	0.003	54.2
*** indicates significant at 0.1% level of probability. Here, G × T = Genotype by treatment interaction, df = Degrees of freedom, Proline = Proline content, H ₂ O ₂ = Hydrogen peroxide content, MDA = Malondialdehyde content, POD = Peroxidase content, CAT = Catalase content, APX = Ascorbate peroxidase content.							

At 6 dS m⁻¹ EC, proline accumulation significantly increased in genotypes S-03 (941%), S-23 (486%), and S-09 (55.5%) compared to control (Table S8, Fig. 5). Similarly, at 10 dS m⁻¹ EC, genotypes BS-02 and S-07 exhibited a 765% and 718% increase in proline content, respectively, compared to control (Table S8, Fig. 5). Furthermore, ascorbate peroxidase (APX) activity was significantly upregulated under 10 dS m⁻¹ EC, with an increase of 623% in S-23, 135.5% in BS-02, 94.4% in S-07, and 509% in S-09 compared to control (Table S8, Fig. 5). In addition to APX, other oxidative stress-related biochemical parameters, including hydrogen peroxide (H₂O₂), malondialdehyde (MDA), peroxidase (POD), and catalase (CAT), were significantly elevated under salt stress conditions (Table S8, Fig. 5).

Trait association

PCA for morphological traits

The first five principal components (PCs) explained 76.2% of the total data variation revealing the effect of salinity stress on thirteen genotypes on 18 shoot, root and pod traits (Table 9). PC1, PC2, PC3, PC4 and PC5 explained 44.2%, 11.5%, 9%, 6.4%, and 5.2% data variation, respectively (Table 9). The first principal component (PC1) accounted for positive coefficients for all the shoot, root and pod traits except leaf injury score (Table 9). PC1 clearly divided all the genotypes in controlled condition from 10 dS m⁻¹ EC condition due to their positive and negative PC scores respectively (Fig. 6). PC2 was dominated by the negative coefficients of majority of the traits excluding chlorophyll content, diameter of secondary lateral roots, root dry weight, total number of pods, total

number of seeds, pod length, and thousand seed weight. PC2 scores evidently separated the genotypes S-03 (Lokon), S-05 (Shohag), S-25 (BS-13) and BS-02 (Binasoybean-2) from other genotypes in controlled and treated condition by their contrasting setting in PCA-biplot (Fig. 6). Both PC1 and PC2 were highly significant for treatment, genotype and genotype $\times$ treatment interaction (Table 9).

Table 9
Coefficients of principal components for shoot, root and pod traits

Variables		PC1	PC2	PC3	PC4	PC5
Shoot traits	Leaf Injury Score	-0.286	-0.185	-0.000	0.202	0.003
	Plant Height (cm)	0.122	-0.227	0.288	0.230	0.534
	Total Number of Branches	0.250	-0.215	-0.023	0.106	-0.248
	Total Number of Trifoliates	0.216	-0.322	-0.161	0.229	-0.143
	Chlorophyll Content (%)	0.267	0.254	0.114	-0.158	-0.110
	Shoot Dry Weight (g)	0.195	-0.041	0.225	0.441	0.129
Root traits	Main Axis Root Length (cm)	0.009	-0.454	0.175	-0.384	0.225
	Main Axis Root Diameter (mm)	0.228	-0.262	0.068	0.014	-0.230
	Total number of Primary Lateral Roots	0.016	-0.158	-0.535	0.065	0.269
	Primary Lateral Root Length (cm)	0.082	-0.319	0.305	-0.482	0.236
	Primary Lateral Root Diameter (mm)	0.232	-0.234	0.179	0.186	-0.025
	Secondary Lateral Root Length (cm)	0.127	-0.361	0.029	0.055	-0.304
	Secondary Lateral Root Diameter (mm)	0.137	0.136	0.103	-0.048	0.471
	Root Dry Weight (g)	0.109	0.065	0.451	0.201	0.131
Pod traits	Total Number of Pods	0.271	0.171	0.121	0.154	0.133
	Pod Length (cm)	0.306	0.069	-0.152	-0.211	-0.042
	Total Number of Seeds	0.279	0.144	0.130	0.151	0.125
	Thousand Seed Weight (g)	0.313	0.136	-0.088	-0.138	-0.017
Eigen value		8.832	2.302	1.791	1.272	1.043
Variation explained (%)		44.2	11.5	9.0	6.4	5.2
Total variation explained (%)		44.2	55.7	64.6	71.0	76.2
P (treatment)		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
P (genotype)		< 0.01	< 0.01	< 0.01	< 0.01	0.87
P (genotype $\times$ treatment)		< 0.01	0.076	< 0.05	< 0.01	0.19
Here, p denotes the significance level						

PCA for biochemical traits

The first two principal components (PCs) accounted for 74.2% of the total data variation revealing the effect of salinity stress on five genotypes and six important biochemical traits (Table 10). PC1 and PC2 explained 50.5% and 23.7% data variation, respectively (Table 10). The first principal component (PC1) explained highest variation (50.5%) of the data with strong positive coefficients for all biochemical traits (Table 10). PC1 clearly separated all the genotypes in controlled condition from treated conditions for both 6 dS m^{-1} EC and 10 dS m^{-1} EC (Fig. 7). PC2 was dominated by the positive coefficients of proline, H_2O_2 , and POD, and negative coefficients of MDA, CAT, and APX. It is evident from the biplot that, PC2 separated the genotypes S-03 (Lokon), BS-02 (Binasoybean-2) and S-09 (K-16) from other genotypes in controlled and treated conditions (Fig. 7). Both PC1 and PC2 were highly significant for treatment, genotype and genotype $\times$ treatment interaction (Table 10).

Table 10
Coefficients of principal components for biochemical parameters

Variables	PC1	PC2
Proline ($\mu\text{g g}^{-1}$ FW)	0.517	0.149
Hydroden peroxide ($\mu\text{M g}^{-1}$ FW)	0.473	0.278
Malondialdehyde ($\mu\text{M g}^{-1}$ FW)	0.386	-0.283
Peroxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ FW)	0.362	0.607
Catalase ($\mu\text{M min}^{-1} \text{g}^{-1}$ FW)	0.325	-0.384
Ascorbate peroxidase ($\mu\text{M min}^{-1} \text{g}^{-1}$ FW)	0.351	-0.552
Eigen value	3.029	1.425
Variation explained (%)	50.5	23.7
Total variation explained (%)	50.5	74.2
P (treatment)	< 0.001	< 0.001
P (genotype)	< 0.001	< 0.001
P (genotype $\times$ treatment)	< 0.001	< 0.001
Here, p denotes the significance level		

Correlation among morphological traits

Correlation coefficient analysis was conducted for measuring the mutual relationship among the various shoot, root and pod traits. Most of the traits showed highly significant ($p < 0.001$) relationship among each other. Correlation study revealed that out of 153 associations, 82 associations were highly significant and 37 associations were non-significant. Positive correlation was found in 102 associations and the rest were negatively correlated (Table 11). Plant height had significant positive correlation with all other morphological traits, except chlorophyll content and root dry weight (Table 11). Total number of primary lateral roots had no significant relationship with any other traits. Total number of pods and total number of seeds had significant positive relation with total number of branches, total number of trifoliate, chlorophyll content, shoot dry weight and root (both main axis and lateral) diameter (Table 11).

Table 11
Correlation coefficients among morphological traits of 10 soybean genotypes

Traits	LIS	PH	TB	TT	ChIC	SDW	MAL	MAD	TLR1	LRL1	LRD1	LRL2	LRD2	RDW	TP	PL
PH	-0.228 **															
TB	-0.575 ***	0.301 ***														
TT	-0.458 ***	0.449 ***	0.855 ***													
ChIC	-0.767 ***	0.107 NS	0.465 ***	0.315 ***												
SDW	-0.43 ***	0.333 ***	0.407 ***	0.4 ***	0.45 ***											
MAL	-0.003 NS	0.199 **	0.154 *	0.217 **	-0.082 NS	-0.001 NS										
MAD	-0.49 ***	0.245 **	0.653 ***	0.596 ***	0.387 ***	0.417 ***	0.214 **									
TLR1	-0.024 NS	0.326 ***	-0.018 NS	0.2 **	-0.043 NS	-0.08 NS	0.072 NS	-0.035 NS								
LRL1	-0.168 *	0.251 ***	0.236 **	0.223 **	0.207 **	0.037 NS	0.569 ***	0.261 ***	-0.104 NS							
LRD1	-0.406 ***	0.218 **	0.583 ***	0.531 ***	0.438 ***	0.578 ***	0.149 *	0.538 ***	-0.14 NS	0.246 **						
LRL2	-0.223 **	0.17 *	0.428 ***	0.449 ***	0.21 **	0.249 **	0.288 ***	0.38 ***	0.012 NS	0.302 ***	0.379 ***					
LRD2	-0.048 NS	-0.071 NS	0.016 NS	-0.031 NS	0.326 ***	0.204 **	-0.1 NS	0.058 NS	0.064 NS	-0.038 NS	0.244 **	-0.028 NS				
RDW	-0.312 ***	0.077 NS	0.245 **	0.218 **	0.376 ***	0.511 ***	0.105 NS	0.279 ***	-0.145 *	0.197 **	0.294 ***	0.04 NS	-0.029 NS			
TP	-0.668 ***	0.232 **	0.524 ***	0.391 ***	0.728 ***	0.48 ***	-0.132 NS	0.393 ***	-0.075 NS	0.09 NS	0.532 ***	0.18 *	0.441 ***	0.318 ***		
PL	-0.846 ***	0.319 ***	0.587 ***	0.482 ***	0.752 ***	0.394 ***	0.004 NS	0.565 ***	0.117 NS	0.204 *	0.484 ***	0.274 **	0.289 ***	0.18 *	0.644 ***	
TS	-0.667 ***	0.251 **	0.567 ***	0.443 ***	0.727 ***	0.479 ***	-0.101 NS	0.431 ***	-0.088 NS	0.12 NS	0.581 ***	0.173 *	0.433 ***	0.343 ***	0.976 ***	0.6 ***
TSW	-0.851 ***	0.295 ***	0.581 ***	0.466 ***	0.833 ***	0.448 ***	-0.076 NS	0.526 ***	0.042 NS	0.165 *	0.511 ***	0.217 **	0.343 ***	0.248 **	0.723 ***	0.9 ***

*, ** and *** indicate significant at 5%, 1% and 0.1% levels of probability, respectively and NS = non-significant. Here, LIS = Leaf injury scoring, PH = Plant height, TB = Total number of branches, TT = Total number of trifoliate, ChIC = Chlorophyll content, SDW = Shoot dry weight, MAL = Length of main axis root, MAD = Diameter of main axis root, TLR1 = Total number of primary lateral roots, LRL1 = Length of primary axis root, LRD1 = Diameter of primary axis root, LRL2 = Length of secondary axis root, LRD2 = Diameter of secondary axis root, RDW = Root dry weight, TP = Total number of pods plant⁻¹, PL = Length of pod, TS = Total number of seeds plant⁻¹, TSW = Thousand (1000) seed weight.

Correlation among biochemical traits

Furthermore, correlation coefficient analysis among the various biochemical traits, revealed that out of fifteen associations, eight associations were significant and the rest were non-significant (Table 12). All the associations were positively correlated. Correlation coefficients among biochemical traits

showed positive and significant relationship between proline with all other parameters (Table 12). Hydrogen peroxide showed highly significant positive relationship with peroxidase. Ascorbate peroxidase showed significant positive association with malondialdehyde and catalase (Table 12).

Table 12
Correlation coefficients among biochemical parameters of five soybean genotypes

Biochemical parameters	Proline	H ₂ O ₂	MDA	POD	CAT
H ₂ O ₂	0.7***				
MDA	0.504**	0.319 ^{NS}			
POD	0.658***	0.688***	0.229 ^{NS}		
CAT	0.484**	0.315 ^{NS}	0.245 ^{NS}	0.007 ^{NS}	
APX	0.366*	0.349 ^{NS}	0.595**	-0.065 ^{NS}	0.466**
*, ** and *** indicate significant at 5%, 1% and 0.1% levels of probability, respectively and NS = non-significant. Here, Proline = Proline content, H ₂ O ₂ = Hydrogen peroxide content, MDA = Malondialdehyde content, POD = Peroxidase content, CAT = Catalase content, APX = Ascorbate peroxidase content.					

Estimation of genetic parameters

The true strength of variability can be determined by comparing the relative amounts of PCV and GCV. Total number of trifoliate (40.65%) showed the highest GCV and chlorophyll content (14.46%) showed the lowest GCV for shoot traits (Table 13). Secondary lateral root diameter (322.09%) showed relatively higher GCV than other root traits. Total number of pods (52.36%) had higher GCV for pod traits. Proline showed the highest GCV value (129.18%), while malondialdehyde showed the lowest GCV (44.14%) among biochemical traits (Table 13).

Table 13
Estimation of genetic parameters for shoot, root, pod traits and biochemical parameters

Traits	Traits	GV	PV	GCV	PCV	$\frac{2}{\sqrt{\text{varvec}h_{\text{varvec}b}}}$	GA	GAM
				(%)	(%)	(%)		(%)
Shoot traits	LIS	1.76	2.13	38.41	42.28	82.52	2.48	71.88
	PH	560.8	688.8	30.82	34.15	81.42	44.02	57.28
	TB	0.55	1.67	30.24	52.72	32.91	0.88	35.74
	TT	18.14	27.88	40.65	50.39	65.06	7.08	67.54
	ChIC	16.84	17.01	14.46	14.53	99	8.41	29.64
	SDW	3.39	5.63	34.56	44.46	60.43	2.95	55.34
Root traits	MAL	4.96	13.14	12.4	20.19	37.73	2.82	15.69
	MAD	0.46	1.18	12.39	19.86	38.9	0.87	15.92
	TLR1	34.05	60.59	27.42	36.58	56.19	9.01	42.35
	LRL1	6.43	14.38	17.91	26.79	44.68	3.49	24.66
	LRD1	0.099	0.197	26.19	36.92	50.30	0.46	38.26
	LRL2	0.629	4.32	18.36	48.13	14.56	0.62	14.44
	LRD2	0.084	0.085	322.09	323.72	98.99	0.59	660.1
	RDW	0.057	0.41	10.29	27.79	13.72	0.18	7.86
Pod traits	TP	32.98	39.08	52.36	56.99	84.39	10.87	99.09
	PL	0.137	0.185	15.97	18.55	74.05	0.66	28.3
	TS	125.9	173.2	49.85	58.47	72.69	19.71	87.56
	TSW	103	112	10.94	11.4	91.96	20.05	21.61
Biochemical parameters	Proline	703.7	704.6	129.18	129.27	99.86	54.61	265.93
	H ₂ O ₂	3.89	3.98	48.82	49.43	97.56	4.01	99.34
	MDA	0.0205	0.0205	44.14	44.24	99.59	0.29	90.75
	POD	5.83	5.9	64.51	64.9	98.79	4.94	132.1
	CAT	0.44	0.44	80.87	81.15	99.32	1.36	166.03
	APX	5486	5540	96.63	97.11	99.02	151.8	198.1

Here, LIS = Leaf injury scoring, PH = Plant height, TB = Total number of branches, TT = Total number of trifoliates, ChIC = Chlorophyll content, SDW = Shoot dry weight, MAL = Length of main axis root, MAD = Diameter of main axis root, TLR1 = Total number of primary lateral roots, LRL1 = Length of primary axis root, LRD1 = Diameter of primary axis root, LRL2 = Length of secondary axis root, LRD2 = Diameter of secondary axis root, RDW = Root dry weight, TP = Total number of pods plant⁻¹, PL = Length of pod, TS = Total number of seeds plant⁻¹, TSW = Thousand (1000) seed weight, Proline = Proline content, H₂O₂ = Hydrogen peroxide content, MDA = Malondialdehyde content, POD = Peroxidase content, CAT = Catalase content, APX = Ascorbate peroxidase content. GV = Genotypic variation, PV = Phenotypic variation, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, h^2_b = Heritability, GA = Genetic advance, GAM = Genetic advance as percentage of mean.

Heritability in broad sense (h^2_b) along with genetic advance (GA) are more significant those help predict how selection will eventually affect phenotypic expression. High heritability ($h^2_b > 60\%$) with high genetic advance as percentage of mean (GAM > 20%) showed in plant height ($h^2_b = 81.42$, GAM = 57.28), total number of trifoliates ($h^2_b = 65.06$, GAM = 67.54), secondary lateral root diameter ($h^2_b = 98.99$, GAM = 660.1), total number of pods ($h^2_b = 184.39$, GAM = 99.09), total number of seeds ($h^2_b = 172.69$, GAM = 87.56), proline ($h^2_b = 199.86$, GAM = 265.92), peroxidase ($h^2_b = 98.79$, GAM = 132.1), ascorbate peroxidase ($h^2_b = 99.02$, GAM = 198.1), malondialdehyde ($h^2_b = 99.59$, GAM = 90.75), catalase ($h^2_b = 99.32$, GAM = 166.03), and hydrogen peroxide ($h^2_b = 97.56$, GAM = 99.34) (Table 13).

Discussion

Morphological changes due to salinity stress

Salt stress prevents proper cell growth and development and hinders the uptake and transfer of water and nutrients, growth is obviously inhibited as a consequence (Hasanuzzaman et al. 2022). In this study, soybean plants were exposed to three different salt stress levels, 0 dS m⁻¹, 6 dS m⁻¹ and 10 dS m⁻¹

EC, to evaluate their impact on morphological traits. The results revealed that salt stress significantly reduced key morphological traits including plant height, total number of branches, total number of trifoliates, and chlorophyll content, indicating impaired growth and photosynthetic efficiency under saline conditions (Table 5) (Anjum et al. 2011, Din et al. 2011). Additionally, shoot dry weight, a critical measure of biomass accumulation, decreased significantly, reflecting the adverse effects of salinity on overall plant development (Table 5). This decline indicates that high salinity levels hinder biomass accumulation by disrupting essential physiological processes, such as nutrient uptake, water absorption, and photosynthesis. As a result, overall plant development is adversely affected, leading to stunted growth and reduced productivity. Root system architecture was also negatively affected, with reductions observed in the length and diameter of the main axis root, primary axis root, and secondary axis root, as well as a decline in root dry weight, highlighting the detrimental impact of salt stress on root growth and function (Table 5) (Otie et al. 2021, Amirijani 2010). However, some other crops, including rice (Hazman and Brown 2018), rapeseed (Dai et al. 2020, Arif et al. 2019), and wheat (Robin et al. 2016) exhibited greater lateral root elongation under stress environment. Furthermore, reproductive traits such as total number of pods plant⁻¹, pod length, total number of seeds plant⁻¹, and thousand seed weight were significantly reduced, underscoring the negative influence of salinity on yield-related parameters (Table 5) (Ghassemi-Golezani et al. 2010)

Biochemical changes due to salinity stress

One of the most damaging abiotic stressors, salt stress, causes oxidative stress through a variety of mechanisms such as, changed enzyme activity, interrupted stomatal conductance and intrusions into photosynthesis. But the best part is that plants can use a variety of mitigation techniques or adaptive mechanisms to help them recover from or be protected from the cellular damages brought on by salt stress (Hasanuzzaman et al. 2013).

In this study, soybean plants exposed to saline conditions demonstrated significant biochemical adaptations as part of their natural defense mechanisms against stress. When subjected to salt stress, these plants exhibited substantial increases in proline, a key osmoprotectant and antioxidant that helps stabilize cellular structures and scavenge reactive oxygen species (ROS) to mitigate oxidative damage (Table 5, Fig. 5) (Mittler 2002). Elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation, indicated oxidative stress and membrane damage resulting from the accumulation of ROS (Table 5, Fig. 5) (Esfandiari et al. 2007). Additionally, hydrogen peroxide (H₂O₂), a reactive oxygen species, accumulated under saline conditions, serving both as a damaging agent and as a signaling molecule to activate stress responses (Table 5, Fig. 5) (Abid et al. 2018, Mittler 2004). To counteract the oxidative stress, soybean plants significantly enhanced the activity of antioxidant enzymes such as ascorbate peroxidase (APX) and catalase (CAT), which play crucial roles in detoxifying H₂O₂ and other ROS (Table 5, Fig. 5) (Abid et al. 2018). These coordinated responses - proline accumulation, MDA production, H₂O₂ signaling, and increased activity of ascorbate peroxidase and catalase - collectively represent the plant's adaptive strategies to manage the detrimental effects of saline stress, maintain cellular homeostasis, and improve survival under adverse conditions (Islam et al. 2015).

Trait association

A useful tool for choosing desirable features in a breeding program is still the degree of association between characters, as shown by the correlation coefficients. It gives an insight into the genetic variability present in populations. In this study, all the shoot traits were positively and significantly correlated with each other except leaf injury score, total number of trifoliates and chlorophyll content (Table 11). Total number of seeds plant⁻¹, a critical determinant of soybean yield, was positively and significantly correlated with other key traits - such as plant height, total number of branches, total number of trifoliates, chlorophyll content, shoot dry weight, total number of pods plant⁻¹, pod length, and 1000 seed weight - suggesting that these traits are interrelated and can collectively contribute to higher seed yield (Table 11) (Guleria et al. 2019, Ghodrati 2013). Similarly, majority of the root traits showed significant and positive association except secondary lateral root diameter (Table 11). The biochemical traits also showed positive and significant relationship among them (Table 12) (Mehra et al. 2020).

Genetic parameter analysis

The study of the phenotypic coefficient of variance (PCV) and the genotypic coefficient of variance (GCV) is not only useful for comparing the relative amounts of phenotypic and genotypic variations among different traits, but it is also pivotal in estimating the extent to which selection might enhance a trait. This is due to the fact that most characters exhibit complex inheritance and are significantly influenced by several genes interacting with various environmental conditions. In this study, PCV surpassed GCV for all the characters; however, the smaller difference observed between PCV and GCV in certain cases suggested that these characters were less influenced by the environment (Table 13) (Reni and Rao 2013). Total number of trifoliates, secondary lateral root diameter, total number of pods, and proline content showed the highest values for both GCV and PCV among the analyzed shoot, root, pod, and biochemical traits. (Table 13) (Mahbub et al. 2016). This suggested that these traits exhibited significant genetic and phenotypic variability, making them suitable candidates for selection in breeding programs aimed at developing saline-tolerant soybean genotypes. High GCV indicated substantial genetic variation for these traits, which means that they were more likely to respond to selection and can be improved through breeding. Additionally, high PCV indicated that both genetic factors and environmental influences contributed to the expression of these traits, but the larger genetic contribution allows for better control and selection under different environmental conditions. Conversely, chlorophyll content, root dry weight, 1000 seed weight, and malondialdehyde showed the lowest GCV and PCV values, indicating a narrow genetic base for these traits and limited potential for improvement through traditional breeding methods (Table 13) (Gohil et al. 2007). These low values suggested that genetic variation for these traits was minimal, meaning that the observed differences in these traits were more likely to be influenced by environmental factors rather than genetic differences.

Determining the heritability of the variables is indeed necessary for the selection process, as heritability estimates assist plant breeders in selecting elite genotypes from various genetic populations (Singh et al. 2011, Chandrawat 2017). To predict the effectiveness of selecting the best candidates, heritability estimates (above 60%) combined with genetic advance (above 20%) are more beneficial than heritability alone (Johnson et al. 1955, Islam et al. 2018). In this experiment, high heritability with high genetic advance was observed in plant height, total number of trifoliates, secondary lateral root diameter, total number of pods, total number of seeds, proline, peroxidase, ascorbate peroxidase, malondialdehyde, catalase, and hydrogen peroxide (Table 13) (Karnwal and Singh

2009, Baraskar et al. 2014). These findings suggest that additive gene action predominates in the expression of these traits, which can be improved in future generations. Furthermore, it implies that these factors can be adjusted as needed and that significant improvement is possible through careful selection.

Conclusion

Abiotic stressors such as salinity stress is one the key causes of low soybean production in Bangladesh. Understanding the specific responses of different soybean genotypes to salinity stress is crucial for developing effective stress coping strategies. This study provides valuable insights into the impact of salinity on various aspects of soybean growth, including morphological traits, biochemical properties, and genetic parameters. Based on the findings, genotypes such as S-07 (MTD-176), S-31 (MTD-6), S-23 (Bragg), and BS-02 (Binasoybean-2) exhibit promising tolerance to salinity stress, making them suitable candidates for further research on stress resilience. The study also highlights traits with high heritability and significant genetic improvement, such as plant height, secondary lateral root diameter, proline content, and peroxidase levels. These traits are crucial for salinity tolerance and should be prioritized in future breeding programs. However, salinity stress involves complex physiological mechanisms, and addressing it effectively, requires a comprehensive approach. Focusing on a combination of traits, rather than just one or two parameters, will provide a more holistic understanding and better strategy for improving salinity tolerance in soybean. Thus, a cumulative association of traits is essential for developing stress-resistant cultivars in the face of increasing salinity challenges.

Declarations

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Author contributions and author agreement

Arif Hasan Khan Robin and Tridiba Das conceived and designed the study. Tridiba Das conducted the experiment and collected data. Sifate Rabbana Khanom assisted in biochemical analysis. Tridiba Das and Abu Musa Md Main Uddin Tareque wrote the experiment. Sifate Rabbana Khanom, Shamsun Nahar Begum, and Arif Hasan Khan Robin supervised the experiment. Sifate Rabbana Khanom and Arif Hasan Khan Robin critically revised the manuscript. All authors approved the final version of the manuscript.

Ethics approval and consent to participate

This study follows the institutional and international guidelines. All experiments were conducted following the ethical standards. The self-pollinated soybean genotypes were collected and conserved by the Department of Genetics and Plant Breeding of Bangladesh Agricultural University, Mymensingh. The collection of the plants/plant parts used in this study/NAME OF PLANT complied with local or national guidelines. The plant materials are cultivated and no licences are required for cultivations. As the study did not involve human participants or animals, ethical approval was not required.

Data availability statement

Data will be available upon reasonable request from the authors.

Consent to publish

All authors have contributed significantly to the work and approved of the final version being submitted.

Ethics & guidelines statement

Not applicable. The authors declare that they do not have any conflict of interest (financial or non-financial).

Plant reproducibility

Not applicable. The source of genotypes has been described in the materials and methods section of this manuscript.

References

1. Abid M, Ali S, Qi LK, Zahoor R, Tian Z, Jiang D, Dai T. Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L). *Sci Rep.* 2018;8(1):4615.
2. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* 2001;24:1337–44.
3. Amirijani MR. Effects of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. *Am J Plant Physiol.* 2010;5(6):350–60.
4. Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W. Morphological, physiological and biochemical responses of plants to drought stress. *Afr J Agric Res.* 2011;6(9):2026–32.
5. Ao J, Fu J, Tian J, Yan X, Liao H. Genetic variability for root morph-architecture traits and root growth dynamics as related to phosphorus efficiency in soybean. *Funct Plant Biol.* 2010;37:302–12.

6. Arif MR, Islam MT, Robin AHK. Salinity stress alters root morphology and root hair traits in *Brassica napus*. *Plants*. 2019;8(7):192.
7. Baraskar VV, Kachhadia VH, Vachhanl JH, Barad HR, Patel MB, Darwankar MS. Genetic variability, heritability and genetic advance in soybean [*Glycine max* (L.) Merrill]. *Electron J Plant Breed*. 2014;5(4):802–6.
8. Bates LS. Rapid determination of free proline for water-stress studies. *Plant Soil*. 1973;39:205–7.
9. Beer JR, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem*. 1952;195:133–40.
10. Blum A. Drought resistance—is it really a complex trait? *Funct Plant Biol*. 2011;38:753–7.
11. Chandrawat KS, Baig KS, Hashmi S, Sarang DH, Kumar A, Dumai PK. Study on genetic variability, heritability and genetic advance in soybean. *Int J Pure Ap Biosci*. 2017;5(1):57–63.
12. Comas LH, Becker SR, Cruz VM, Byrne PF, Dierig DA. (2013) Root traits contributing to plant productivity under drought. *Front Plant Sci* 4(442).
13. Dai L, Li J, Harmens H, Zheng X, Zhang C. Melatonin enhances drought resistance by regulating leaf stomatal behaviour, root growth and catalase activity in two contrasting rapeseed (*Brassica napus* L.) genotypes. *Plant Physiol Biochem*. 2020;149:86–95.
14. Din J, Khan SU, Ali I, Gurmani AR. Physiological and agronomic response of canola varieties to drought stress. *J Anim Plant Sci*. 2011;21(1):78–82.
15. Esfandiari E, Shakiba MR, Mahboob SA, Alyari H, Toorchi M. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. *J Food Agri Environ*. 2007;5(1):149.
16. Fenta BA, Beebe SE, Kunert KJ, Burrridge JD, Barlow KM, Lynch PJ. (2014) Field phenotyping of soybean roots for drought stress tolerance. *Agronomy* 4 418 – 35.
17. Ghassemi-Golezani K, Taifeh-Noori M, Oustan S, Moghaddam M, Seyyed-Rahmani S. Oil and protein accumulation in soybean grains under salinity stress. *Not Sci Biol*. 2010;13:64–7.
18. Ghodrati GR, Sekhavat R, Mahmoodinezhadedezfully SH, Gholami A. Evaluation of correlations and path analysis of components seed yield in soybean. *Int J Agric*. 2013;3(4):795.
19. Gohil VN, Mehta DR, Pandya HM. Genetic divergence in soybean (*Glycine max* (L.) MERR). *Legume Res*. 2007;30(3):224–6.
20. Guleria H, Kumar P, Jyoti B, Kumar A, Paliwal A, Paliwal A. Genetic variability and correlation analysis in soybean (*Glycine max* (L.) Merrill) genotypes. *Int J Chem Stud*. 2019;7(1):1928–32.
21. Hanson CH, Robinson HF, Comstock RE. Biometrical studies of yield in segregating populations of Korean lespedeza. *Agron J*. 1956;48(6):268–72.
22. Hasanuzzaman M, Nahar K, Fujita M. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In: Ahmad P, Azooz MM, Prasad MN, editors. *Ecophysiology and Responses of Plants under Salt Stress*. New York: Springer; 2013. pp. 25–87.
23. Hasanuzzaman M, Parvin K, Anee TI, Masud AAC, Nowroz F. Salt stress responses and tolerance in soybean. *Plant Stress Physiology-Perspectives in Agriculture*. IntechOpen: London; 2022. pp. 47–82.
24. Hazman M, Brown KM. Progressive drought alters architectural and anatomical traits of rice roots. *Rice*. 2018;11(1):1–16.
25. Heath RL, Packer L. Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 1968;125(1):189–98.
26. Hemeda HM, Klein BP. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *J Food Sci*. 1990;55:184–85.
27. Hossain MA, Mostofa MG, Fujita M. Cross protection by cold-shock to salinity and drought stress-induced oxidative stress in mustard (*Brassica campestris* L.) seedlings. *Mol Plant Breed*. 2013;4:50–70.
28. Islam MZ, Chakrabarty T, Akter N, Rashid ES, Khalequzzaman M, Chowdhury MA. Genetic variability, character association and path analysis in boro rice (*Oryza sativa* L.) germplasm from Bangladesh. *Bangladesh Rice J*. 2018;22(1):35–43.
29. Islam M, Begum MC, Kabir AH, Alam MF. Molecular and biochemical mechanisms associated with differential responses to drought tolerance in wheat (*Triticum aestivum* L.). *J Plant Interact*. 2015;10(1):195–201.
30. Johnson HW, Robinson HF, Comstock RE. Estimates of genetic and environmental variability in soybeans. *Agron J*. 1955;47(7):314–18.
31. Karnwal MK, Singh K. Studies on genetic variability, character association and path coefficient for seed yield and its contributing traits in soybean [*Glycine max* (L.) Merrill]. *Legume Res*. 2009;32(1):70–3.
32. Ledesma F, Lopez C, Ortiz D, Chen P, Korth KL, Ishibashi T, Zeng A, Orazaly M, Florez-Palacios L. A simple greenhouse method for screening salt tolerance in soybean. *Crop Sci*. 2016;56:1–10.
33. Lopes MS, Araus JL, Van Heerden PD, Foyer CH. Enhancing drought tolerance in C4 crops. *J Exp Bot*. 2011;62:3135–53.
34. Mahbub MM, Rahman MM, Mahmud F, Kabir MM. (2016) Genetic Variability Analysis in Different Genotypes of Soybean (*Glycine max* (L.) Merrill).
35. Mehra S, Shrivastava MK, Amrate PK, Yadav RB. Studies on variability, correlation coefficient and path analysis for yield associated traits in soybean [*Glycine max* (L.) Merrill]. *J Oilseeds Res*. 2020;37(1):56–9.
36. Mittler R. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. 2002;7:405–10.
37. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. *Trends Plant Sci*. 2004;9(10):490–98.
38. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 1981;22:867–80.
39. OEC. (2024) *Soybeans in Bangladesh*. Retrieved December 10, 2024, from <https://oec.world/en/profile/bilateral-product/soybeans/reporter/bgd?utm>
40. Otie V, Udo I, Shao Y, Itam MO, Okamoto H, An P. Salinity effects on morpho-physiological and yield traits of soybean (*Glycine max* L.) as mediated by foliar spray with brassinolide. *Plants*. 2021;10:541.
41. Reni YP, Rao YK. Genetic variability in soybean [*Glycine max* (L) Merrill]. *Int J Plant Anim Environ Sci*. 2013;3(4):35–8.

42. Robin AHK, Matthew C, Uddin MJ, Bayazid KN. Salinity-induced reduction in root surface area and changes in major root and shoot traits at the phytomer level in wheat. *J Exp Bot.* 2016;67(12):3719–29.
43. Singh RK, Chaudhary BD. Biometrical methods in quantitative genetic analysis. Ludhiana, New Delhi: Kalyani Publication; 1985.
44. Singh SK, Singh CM, Lal GM. Assessment of genetic variability for yield and its component characters in rice (*Oryza sativa* L). *Res Plant Biol.* 2011;1(4):73–6.
45. Shawkhatuzamman M, Roy SR, Alam MZ, Majumder P, Anka NJ, Hasan AK. Soil salinity management practices in coastal area of Bangladesh: a review. *Res Agric Livest Fish.* 2023;10(1):1–7.
46. Statista. (2024) *Global oilseed production 2023/24, by type.* <https://www.statista.com/statistics/267271/worldwide-oilseed-production-since-2008>. Accessed on January 18, 2025.
47. Tanaka N, Kato M, Tomioka R, Kurata R, Fukao Y, Aoyama T. Characteristics of a root hair-less line of *Arabidopsis thaliana* under physiological stresses. *J Exp Bot.* 2014;65:1497–512.
48. Thu NB, Nguyen QT, Hoang XL, Thao NP, Tran LS. (2014) Evaluation of drought tolerance of the Vietnamese soybean cultivars provides potential resources for soybean production and genetic engineering. *Biomed Res Int* 2014(9).
49. USDA. (2024) <https://ipad.fas.usda.gov/countrysummary/Default.aspx?id=BG&crop=Soybean>. Accessed on December 10, 2024.
50. Vadez V. Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Res.* 2014;165:15–24.

Figures

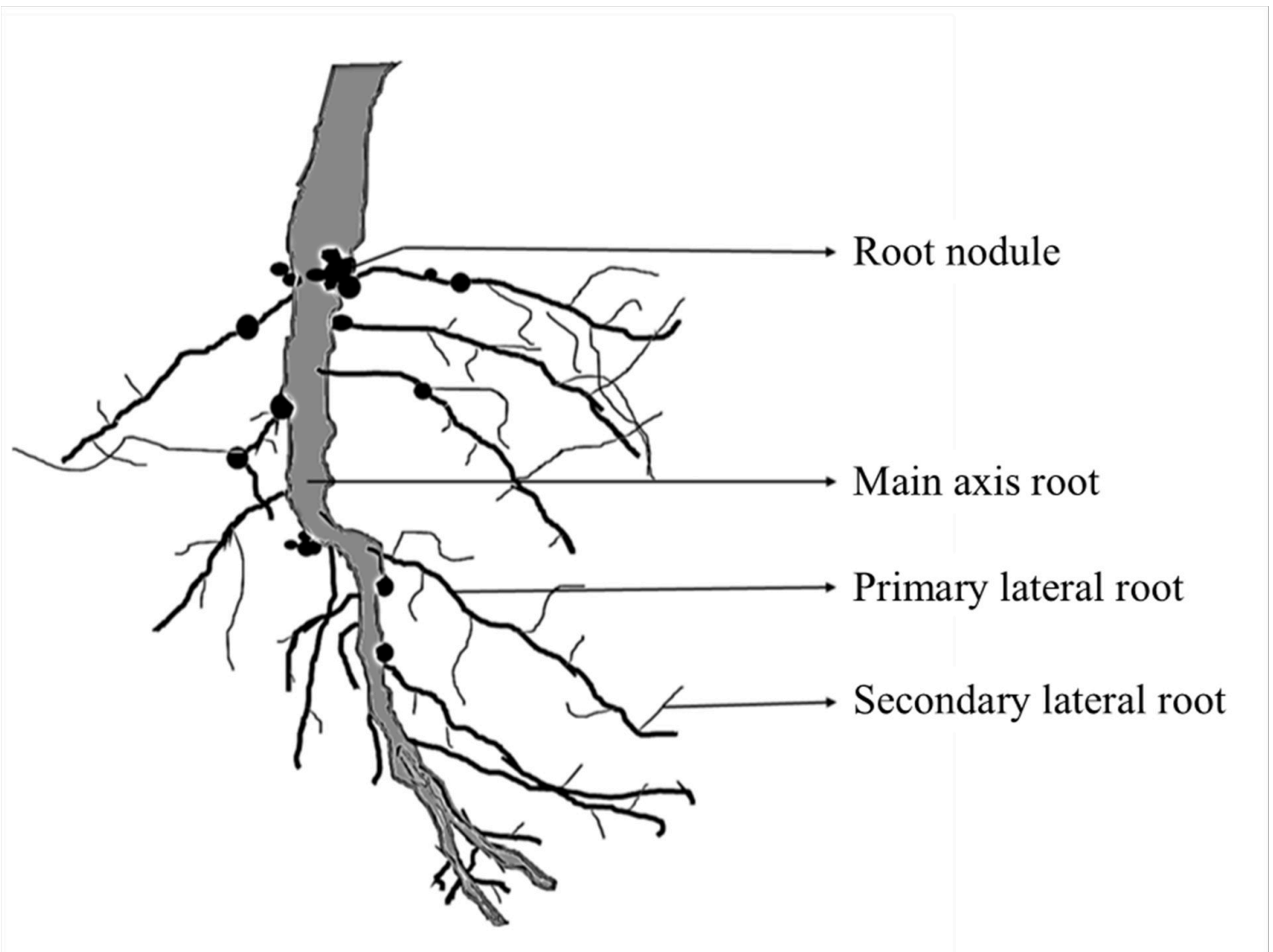


Figure 1

A hypothetical drawing of allorhizic root system of soybean. (A) main axis root; (B) primary lateral root; (C) secondary lateral root; (D) root nodule.

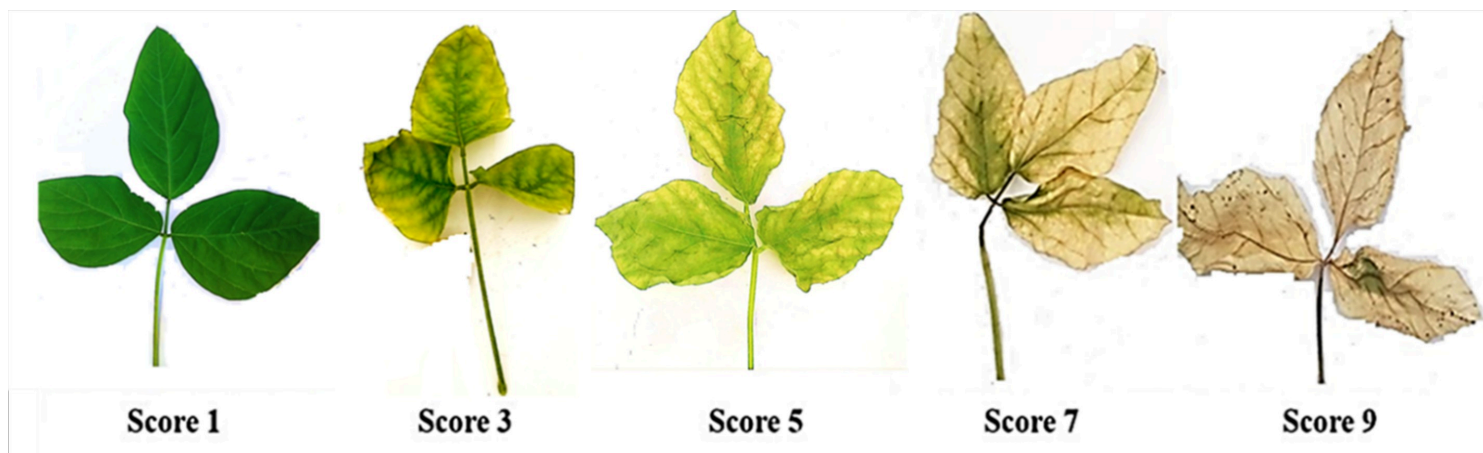


Figure 2

Injury scoring of soybean leaves under control and various salt stress conditions.

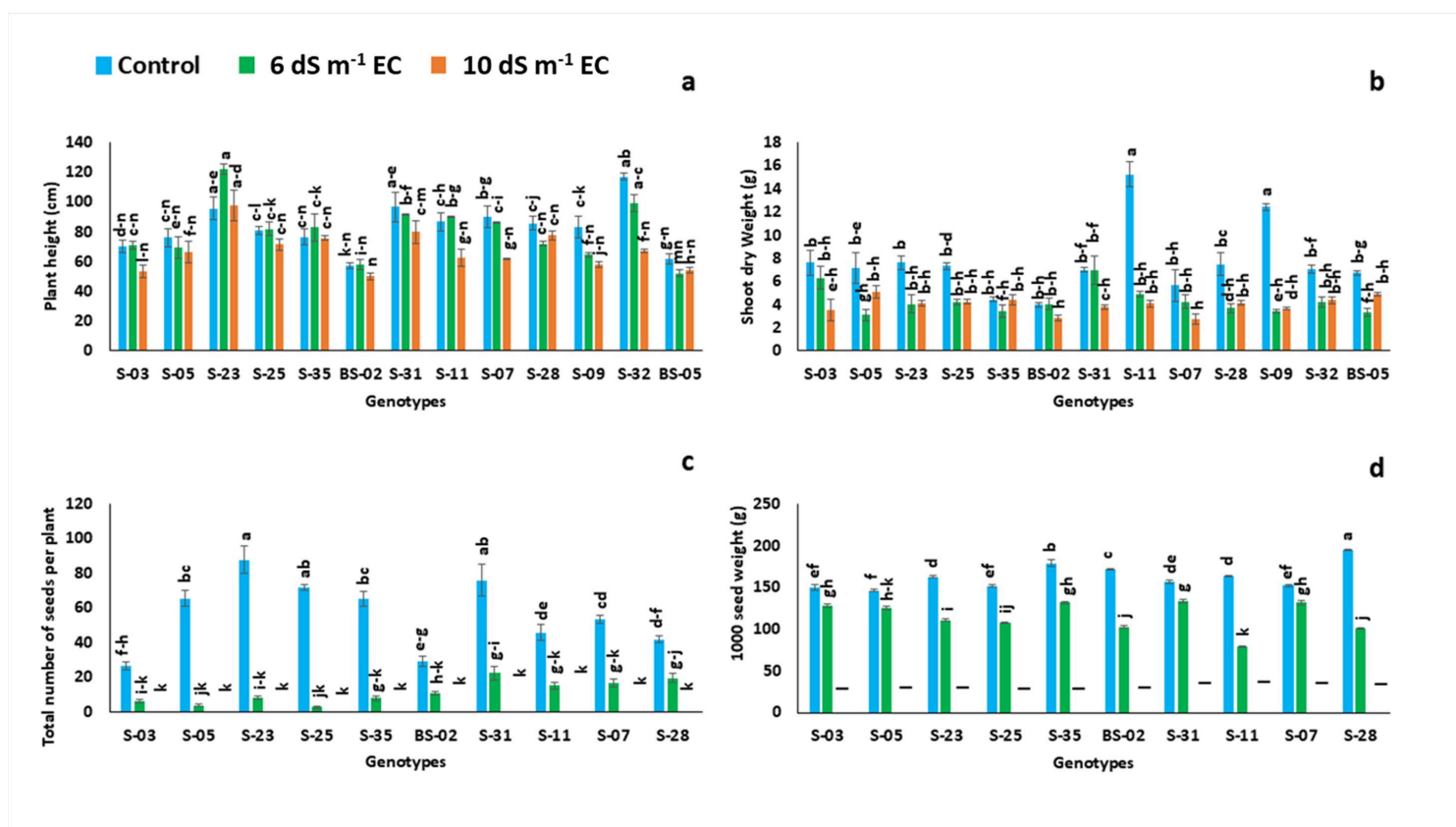


Figure 3

Treatment effect, genotypic variation and genotype $\times$ treatment interaction for: (a) Plant height; (b) Shoot dry weight; (c) Total number of seeds plant⁻¹ and (d) 1000 seed weight of soybean genotypes under control and two saline treatments. Here, S-03 = Lokon, S-05 = Shohag, S-23 = Bragg, S-25 = BS-13, S-35 = Asset-93-19-1, BS-02 = Binasoybean-2, S-31 = MTD-6, S-11 = Asset-93-19-5, S-07 = MTD-176, S-28 = AGS-79, S-09 = K-16, S-32 = MINA-HAI and BS-05 = Binasoybean-5. Vertical bars indicate standard error of means and different letters denote significant differences at 0.1% level of probability.

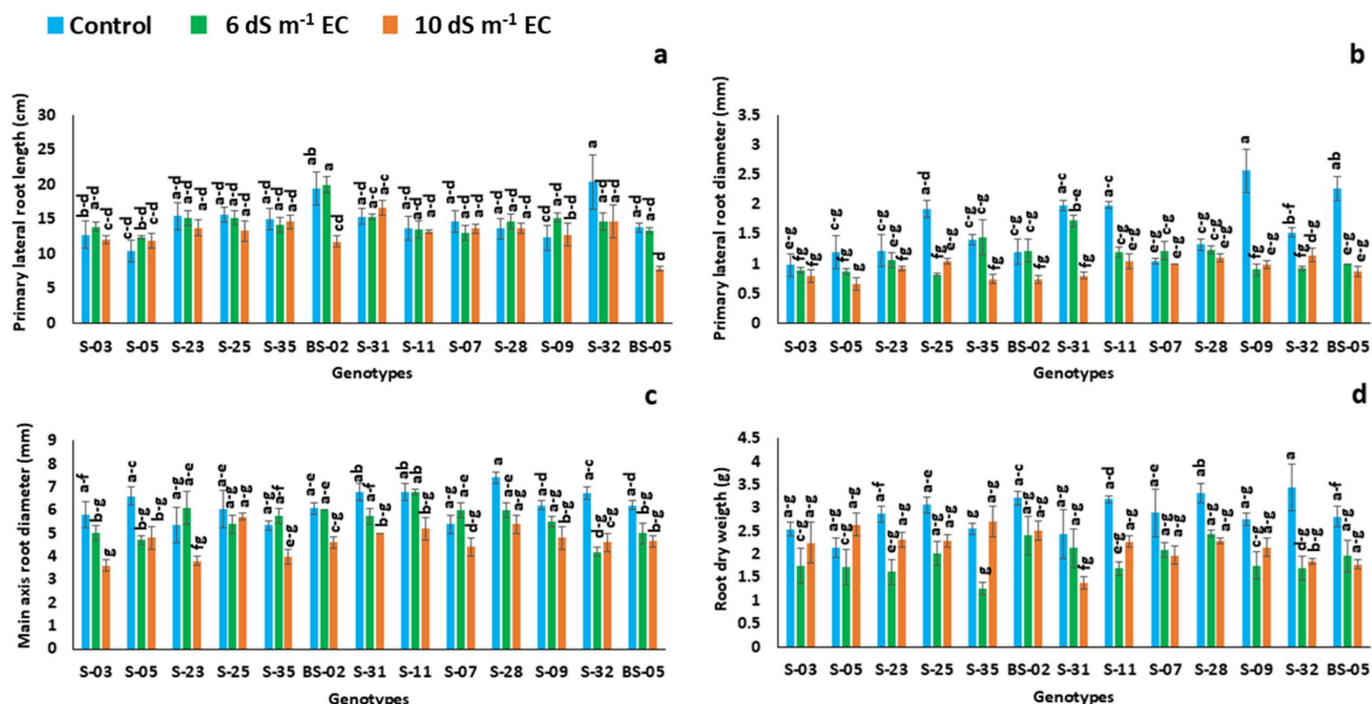


Figure 4

Treatment effect, genotypic variation and treatment $\times$ genotype interaction for: (a) Primary lateral root length; (b) Primary lateral root diameter; (c) Main axis root diameter and (d) Root dry weight of soybean genotypes under control and two saline treatments. Here, S-03 = Lokon, S-05 = Shohag, S-23 = Bragg, S-25 = BS-13, S-35 = Asset-93-19-1, BS-02 = Binasoybean-2, S-31 = MTD-6, S-11 = Asset-93-19-5, S-07 = MTD-176, S-28 = AGS-79, S-09 = K-16, S-32 = MINA-HAI and BS-05 = Binasoybean-5. Vertical bars indicate standard error of means and different letters denote significant differences at 1% and 5% levels of probability.

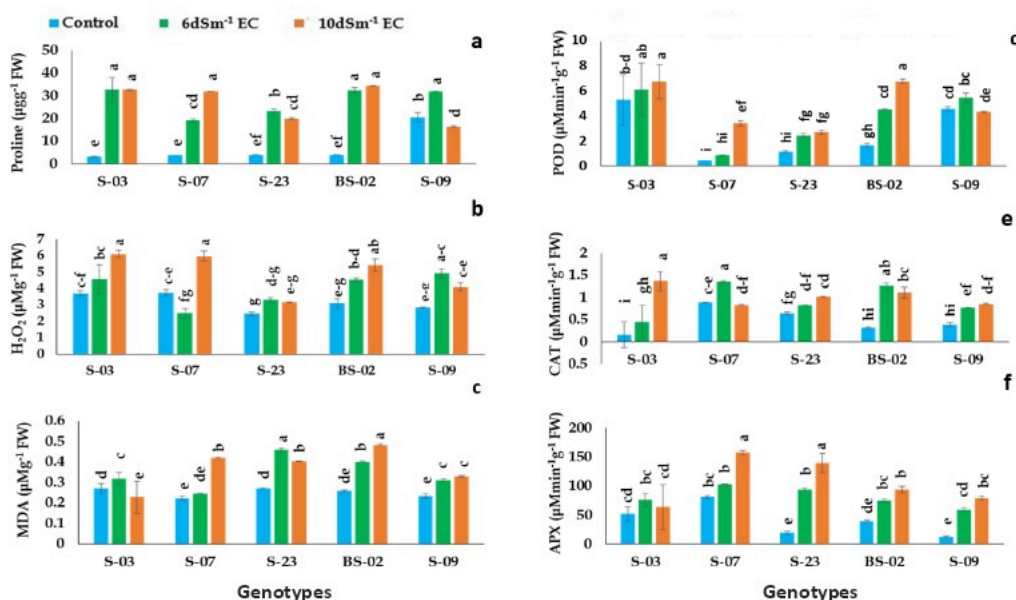


Figure 5

Treatment effect, genotypic variation and treatment $\times$ genotype interaction for Biochemical traits. (a) Proline, (b) H_2O_2 , (c) MDA, (d) POD, (e) CAT, and (f) APX of five soybean genotypes under control and salinity stress conditions. Here, S-03 = Lokon, S-07 = MTD-176, S-23 = Bragg, BS-02 = Binasoybean-2 and S-09 =

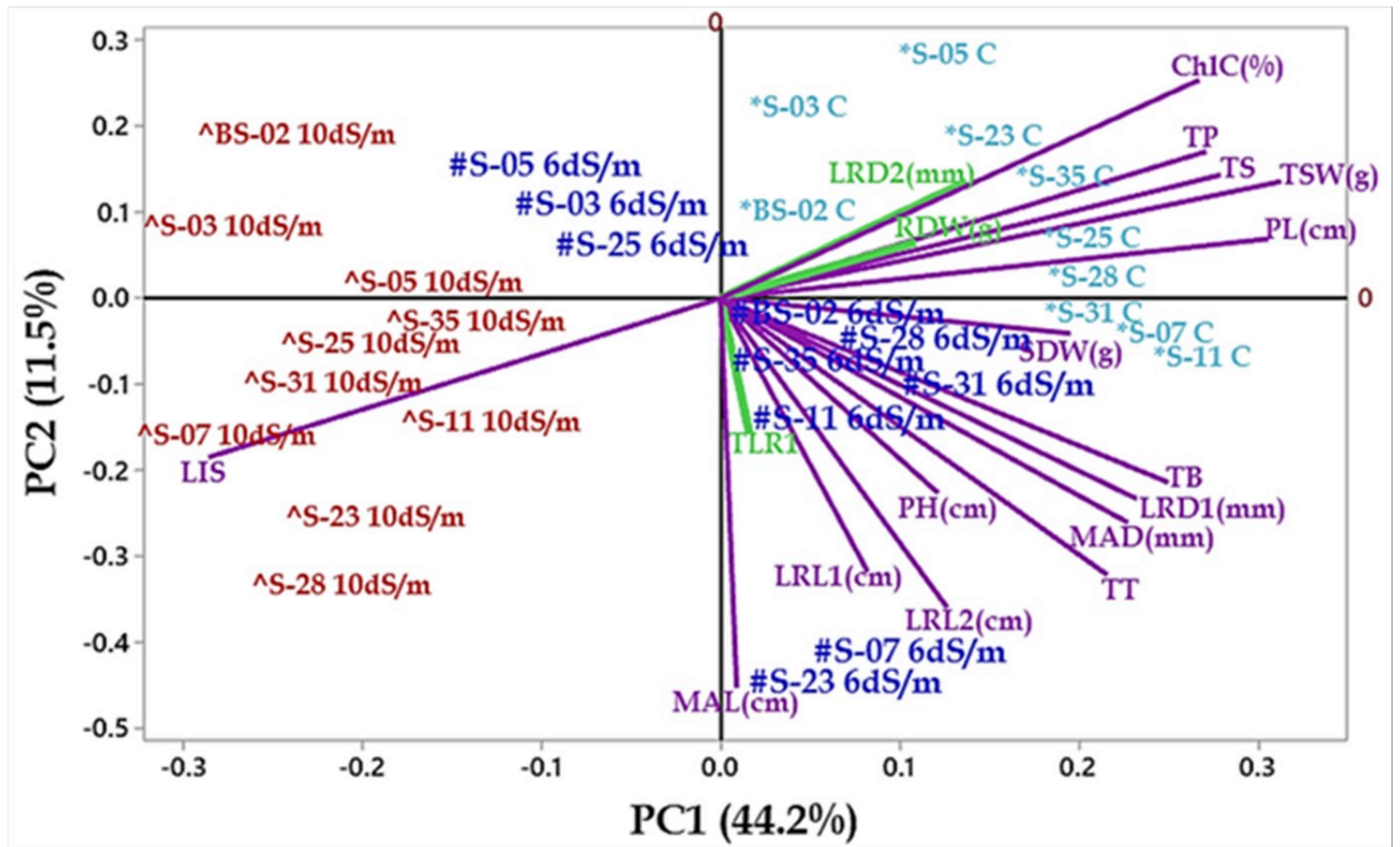


Figure 6

Biplot for shoot, root and pod traits of ten soybean genotypes under control and three saline treatments. Here, LIS = Leaf injury score, PH = Plant height, TB = Total number of branches, TT = Total number of trifoliates, ChIC = Chlorophyll content, SDW = Shoot dry weight, MAL & MAD = Main axis root length & diameter, TLR1 = Total number of primary lateral roots, LRL1 & LRD1 = Primary lateral root length & diameter, LRL2 & LRD2 = Secondary lateral root length & diameter, RDW = root dry weight, TP = Total number of pods, PL = Pod length, TS = Total number of seeds, TSW = 1000 seed weight, C = Control, S-03 = Lokon, S-05 = Shohag, S-23 = Bragg, S-25 = BS-13, S-35 = Asset-93-19-1, BS-02 = Binasoybean-2, S-31 = MTD-6, S-11 = Asset-93-19-5, S-07 = MTD-176 and S-28 = AGS-79.

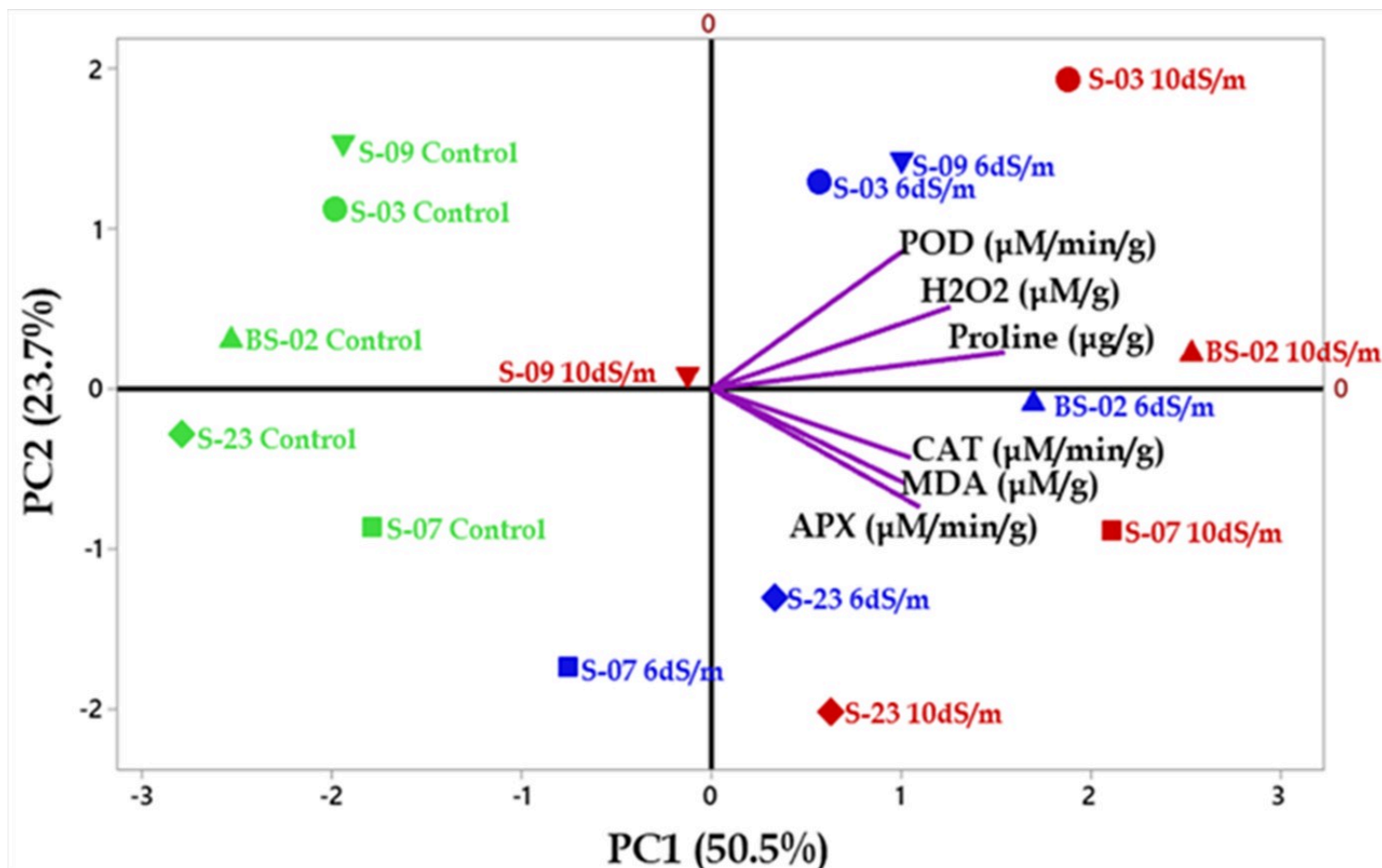


Figure 7

Biplot for biochemical parameters of five soybean genotypes under control and three saline treatments. Here, Proline = Proline content, H₂O₂ = Hydrogen peroxide, MDA = Malondialdehyde, POD = Peroxidase, CAT = Catalase, APX = Ascorbate peroxidase, S-03 = Lokon, S-23 = Bragg, BS-02 = Binasoybean-2, S-07 = MTD-176 and S-09 = K-16.

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