International Journal of Plant & Soil Science

Volume 38, Issue 1, Page 296-307, 2026; Article no.IJPSS.151498
ISSN: 2320-7035

Evaluation of Clusterbean (*Cyamopsis tetragonoloba* L. Taub.) Genotypes for Saline Irrigation Water Stress Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i15949

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/151498

Original Research Article

Received: 22/11/2025
Accepted: 20/01/2026
Published: 23/01/2026

Abstract

The present study was conducted at the College of Agriculture, Mandor, Jodhpur, to assess genetic variability, heritability and genetic advance in 20 genotypes grown under three salinity levels: normal irrigation (S_0), moderate salinity at 5 dS/m (S_1) and high salinity at 10 dS/m (S_2). To assess

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Cite as: *Bhanwariya, Yudhishthir, Rupal Dhoot, Ramesh, Manish Kumar, L. Netajit Singh, Rahul Gurjar, Turfan Khan, Suman Yadav, and Anmol Jangir. 2026. "Evaluation of Clusterbean (Cyamopsis Tetragonoloba L. Taub.) Genotypes for Saline Irrigation Water Stress Conditions". International Journal of Plant & Soil Science 38 (1):296-307. https://doi.org/10.9734/ijpss/2026/v38i15949.*

genetic variability parameters in saline irrigation water. In this pot culture has been used and saline irrigation treatment has given to the 20 genotypes when irrigation required. Under non stress conditions (S_0) growth and biomass traits exhibited moderate phenotypic and genotypic variability indicating stable expression. High heritability and high genetic advance in traits such as total free amino acids (h^2 = 99.87 and GAM = 120.8) and proline content (h^2 = 99.56 and GAM = 90.68) along with moderate genetic advance for chlorophyll content (h^2 = 98.86 and GAM = 57.83) and root length (h^2 = 39.12 and GAM = 98.63) represent predominant additive gene action under normal irrigation. Under moderate salinity (S_1) increased GCV and PCV have been recorded particularly for biochemical and physiological parameters such as catalase, peroxidase, relative water content and membrane stability index. These increases indicated enhanced genetic divergence under stress. High heritability with substantial genetic advance in proline (h^2 = 99.94 and GAM = 181.93) and antioxidant enzymes. High salinity (S_2) produced the greatest variability across morphological, physiological and biochemical traits. Proline (GCV = 150.5 and PCV = 150.51), antioxidant enzymes and phenol (GCV = 94.77 and PCV = 101.89) showed maximum GCV and PCV confirming strong genotypic differentiation. Across all salinity levels, growth and chlorophyll content decreased while stress related traits increased. Genotypes HG-2-20, RGC-1031, RGr-18-1, HG-365, GG-2, RGC-589 and RGC-601 exhibited superior tolerance and are promising for cultivation and breeding under saline conditions.

Keywords: Salinity; tolerance; heritability; biochemical; physiological and morphological.

1. Introduction

Clusterbean [*Cyamopsis tetragonaloba* (L.) Taub.] is a legume crop. In different regions of country, it is also called as Khutti, Guar, Chavlikayi, Guari (Reddy *et al.* 2019). It is a drought tolerant leguminous crop mainly cultivated in semi-arid and arid regions. Clusterbean is a highly deep-rooted plant with 4-10 branches and it has highly drought and temperature tolerance capacity. Clusterbean is mainly grown in India in that also western India is leading and followed by Pakistan (Purohit *et al.*, 2011). Clusterbean is believed to have been originated from Africa, specifically from *C. senegalensis* and was domesticated in the northwestern regions of the Indo-Pakistan subcontinent (Kumar *et al.*, 2020, Reddy *et al.*, 2019). India is a major clusterbean-producing nation, followed by Pakistan, Sudan, United States, Italy, Morocco, Germany and Spain which together produce 15% to 20% of the world's output (Anonymous, 2022). India is the top worldwide producer of clusterbean with more than 80% of the total (Tripathy and Das, 2013). India produced 1.302 million tonnes of clusterbean across 2.707 million hectares in 2021–2022, yielding 481 kg ha^{-1} (Anonymous, 2022). Rajasthan accounts for most of India's clusterbean production (72% of the overall output); it also has the biggest acreage under clusterbean growing followed by Haryana, Gujarat, Uttar Pradesh, Punjab and Madhya Pradesh.

Salinity in the soil is one of the main abiotic stresses that limits crop productivity and dispersion. Salinity especially in arid areas, poses a major constraint as it disturbs cellular functions through osmotic stress, ion toxicity and oxidative injury, primarily resulting from the excessive buildup of sodium (Na^+) and chloride (Cl^-) ions (Munns, 2009). Understanding genetic variability in the available germplasm is essential for the successful selection of superior genotypes. Hence, it is important to identify or develop clusterbean genotypes suitable for vegetable, fodder and gum production in saline condition. Information on the extent and nature of genetic variability, along with heritability and genetic advance over mean for seedling parameters, growth parameters, physiological parameters and biochemical related traits, plays a vital role in an effective selection program.

Salt tolerance is polygenic trait makes even more difficult the process of creating a crop genotype that is tolerant to salinity. To advance in this area, it is imperative to integrate research regarding the physiological, biochemical and genetic traits of salt tolerance (Ashraf and Foolad, 2007). Thus, there has been a strong push to use genetic engineering to make our traditional crops more salt-tolerant so that they can produce higher yields in regions that are impacted by salt (Flowers, 2004). In view of the significance of variability and trait association in crop improvement, the present investigation was undertaken.

2. Materials and Methods

2.1 Plant Materials and Location

In the present experiment, 20 Clusterbean genotypes were grown in FCRD (Factorial completely randomized design) with two replications during *Kharif*-2024 under different saline irrigation water having salinity levels (control, 5 dSm^{-1} and 10 dSm^{-1}) in pots at an Instructional farm of College of Agriculture, Jodhpur. Plastic pots have been used. Three plants were sampled from each pot.

2.2 Observations

2.2.1 Seedling Parameters

2.2.1.1 Germination Percentage

Seeds were grown in petri plate on germination paper.

$$\%seed\ germination = \frac{Number\ of\ seeds\ germinate}{Total\ number\ of\ seeds\ sown} x100$$

2.2.1.2 Root and Shoot Length

Root and shoot length were calculate using cm scale.

2.2.1.3 Total Length

Total length was the sum of shoot length and root length.

2.2.1.4 Shoot and Root Length Ratio

Shoot and root length ratio were calculate using shoot length value divisible by root length.

2.2.1.5 Vigour Index (VI) – [after Perry, 1981]

This is used to evaluate the seedling vigour. This was calculated by following formula:

VI = Per cent germination x seedling length

2.2.2 Growth Attributing Parameters

2.2.2.1 Plant Height (cm)

Plant height was measured in centimeter from ground level to the tip of the main axis at the time of 30th day and 60th day from three randomly selected plants and was averaged.

2.2.2.2 Number of Branches

Number of branches per plant was recorded by counting all the branches per plant from three randomly selected plants and was averaged.

2.2.3 Bio-chemical Parameters

Biochemical composition was determined by McCreddy *et al*. (1958) using the Anthrone reagent.

2.2.3.1 Proline Content

Free proline was measured by use of spectrophotometrically according to method of Bates *et al.* (1973).

2.2.3.2 Total Soluble Sugar

Soluble carbohydrate content (mgg^{-1}) was determined by method Yemm and Willis (1954).

2.2.3.3 Starch

Starch content (mgg^{-1}) was determined by method Yemm and Willis (1954).

2.2.3.4 Total Free Amino Acid

The total free amino acid (TFAA) contents were estimated using the ninhydrin assay described by Hamilton and Van Slyke (1943).

2.2.3.5 Antioxidant Enzymes Assay

This has estimated using the method of Chance and Maehly (1955).

Table 1. List of clusterbean genotypes

Genotype	Name of Genotype	Genotype	Name of Genotype
G1	RGC-1033	**G11**	RGC-570
G2	RGC-1031	**G12**	RGr-20-15
G3	RGC-1002	**G13**	RGr-20-7
G4	RGC-936	**G14**	RGr-18-1
G5	RGC-1038	**G15**	GG-1
G6	RGC-589	**G16**	GG-2
G7	RGC-565	**G17**	HG-884
G8	RGC-601	**G18**	HG-365
G9	RGC-582	**G19**	HG-563
G10	RGC-567	**G20**	HG-2-20

2.2.4 Physiological Parameters

2.2.4.1 Fresh Weight (FW)

Weight has been taken of the third fully fresh expanded leaf (grams).

2.2.4.2 Turgid Weight (TW)

Weight of the third fully expanded leaf which was submerged into water for 24 hours by which it gets fully turgid.

2.2.4.3 Dry weight (DW)

Weight of the third fully expanded leaf which has been turgid used for drying in oven for 12 h at 65°C use for dry weight.

2.2.4.4 Dry Weight and Fresh Weight Ratio (DW/FW)

Dry weight and fresh weight ratio have been calculated by division of dry weight value to fresh weight value.

2.2.4.5 Fresh Weight and Turgid Weight Ratio (FW/TW)

Fresh weight and turgid weight ratio have been calculated by division of fresh weight value to turgid weight value.

2.2.4.6 Relative Water Content (RWC)

This has estimated through Weatherley's (1950) formula:

RWC = [(Fresh weight – dry weight/Turgid weight – dry weight)] × 100

2.2.4.7 Membrane Stability Index (MSI)

MSI was assessed by following the equation that is [1-(C_1/C_2)]x100.

Here, C_1 is incubation of sample at 40°C for 30 minutes and C_2 is incubation of sample at 100°C for 15 minutes and take electrical conductivity.

2.2.4.8 Total Chlorophyll Content

Total chlorophyll has measured from method of Arnon's (1949) equation.

Total Chl. (mg/g FW) = [(20.2 x OD645) + (8.02 x OD663)] x V /1000 x W

2.2.4.9 Chlorophyll – a and b Content

Chlorophyll - a, Chlorophyll - b has measured from method of Arnon's (1949) equation.

Chl. a (mgg^{-1} FW) = [(12.7 x OD663) – (2.69 x OD645)] x V /1000 x W

Chl. b (mgg^{-1} FW) = [(22.9 x OD645) – (4.68 x OD663)] × V / 1000 × W

2.2.4.10 Carotenoid Content (mgg^{-1} FW)

Carotenoid has measured from method of Arnon's(1949) equation.

Carotenoids = {OD480 + (0.114 × OD663) − (0.638 × OD645)} x V/(1000 x W)

2.3 Statistical Analysis

Analysis of variance was computed based on factorial completely randomized design (FCRD) for each of the characters like seedling parameters, growth parameters, physiological parameters and biochemical parameters by use of R studio software version 4.5.1.

3. Results and Discussion

The analysis of genetic variability parameters across the three salinity levels revealed significant differences in the expression of morphological, physiological, biochemical, and yield-related traits. Salinity stress had a pronounced influence on the extent of phenotypic and genotypic variance, heritability (h^2) estimates, and expected genetic advance, indicating that stress intensity alters the genetic architecture and selection efficiency for most traits.

3.1 Variability Under Normal Irrigation (S0)

Under non stress conditions (S_0), most traits displayed moderate phenotypic variance (PCV) and genotypic variance (GCV), implying stable trait expression in the absence of salt stress. Higher means were recorded for growth traits such as plant height at 30 DAS (19.7 cm to 35.26 cm), plant height at 60 DAS (30.79 cm to 60.80 cm), number of leaves (6.5 to 9.3) and biomass components like fresh weight (207.1mg to 436.79 mg), dry weight (7.2 mg to 22.5 mg), turgid weight (235.85 mg to 496.6 mg), indicating

optimum physiological performance under normal irrigation. The GCV and PCV values were relatively low to moderate, signifying that the genetic component of variation was comparatively limited and that environmental influence played a smaller role. High heritability with moderate genetic advance for traits like chlorophyll content a (h^2 = 98.12 and GA = 0.87), chlorophyll content b (h^2 = 98.66 and GA = 0.62), total chlorophyll (h^2 = 98.86 and GA = 1.48) and root length (h^2 = 98.63 and GA = 2.79) suggests additive gene action and suitability for selection under normal growing conditions.

3.2 Variability Under Moderate Salinity Stress (S_1 Represents 5 dS/m)

Exposure to moderate salinity (S_1) increased variability for several morphological and physiological traits. GCV and PCV increased for traits such as antioxidant enzymes like catalase (GCV = 48.63 and PCV = 51.56), peroxidase (GCV = 83.88 and PCV = 84.10), membrane stability index (GCV = 19.87 and PCV = 19.93) and relative water content (GCV = 9.44 and PCV = 9.96) reflecting enhanced genetic divergence in stress responsive mechanisms. Moderate to high heritability along with high genetic advance was observed for biochemical traits like proline (h^2 = 99.94 and GA = 6.64), antioxidant enzymes like catalase (h^2 = 88.95 and GA = 0.41) and peroxidase (h^2 = 99.48 and GA = 0.48) indicating the predominance of additive gene effects under moderate stress. Growth related traits showed reduced means, as expected with salinity effects, but the increase in variability suggests that genotypes responded differently to the stress level.

3.3 Variability Under High Salinity Stress (S_2 Represents 10 dS/m)

High salinity induced the greatest genetic variability across most traits and showed strong differentiation among genotypes. GCV and PCV values were highest in S_2 for almost all biochemical traits, including proline content (GCV = 150.59 and PCV = 150.51), catalase (GCV = 48.15 and PCV = 49.40), peroxidase (GCV = 0 and PCV = 79.77) and membrane stability index (GCV = 43.48 and PCV = 43.53) signifying that maximum genetic divergence emerged under severe stress. Heritability values were generally high and many traits also expressed high genetic advance as percent of mean (GAM). This combination of high heritability and high GAM indicates predominantly additive gene action, allowing effective improvement through direct selection even under severe stress. Growth and yield related traits like fresh weight (mean = 157.86 mg and range = 98 mg to 260.88 mg) and dry weight (mean = 20.71 mg and range = 11.50 mg to 36.98 mg) showed large reductions in mean performance but higher variability representing wide genetic differences in stress tolerance capability. Traits directly associated with salinity tolerance like proline, antioxidant enzyme activity, membrane stability index, Na^+/K^+ maintenance were most responsive and discriminating at S_2 which is 10 dS/m.

3.4 Comparative Variability Trends Across S_0, S_1, and S_2

3.4.1 Trait Mean Performance

Traits such as plant height, biomass (fresh weight, dry weight and turgid weight), chlorophyll content and RWC decreased progressively from S_0 to S_1 to S_2. Stress responsive traits such as proline, antioxidant enzymes and membrane stability index increased with salinity level.

3.4.2 Variance Components (GCV and PCV)

GCV and PCV were lowest in S_0, moderate in S_1 and highest in S_2 for most biochemical and physiological parameters. This indicates that salinity stress, especially severe stress, enhances genetic expression differences among genotypes.

3.4.3 Heritability (h^2)

High heritability was common under S_1 and S_2 for biochemical traits, reflecting stable genetic control under stress. Under S_0, heritability was high for some morphological and physiological traits but generally lower for biochemical traits.

3.4.4 Genetic Advance and Genetic Advance Mean (GA and GAM)

Greatest GAM values occurred under S_2, particularly for traits strongly linked to stress physiology (proline and antioxidant enzymes).

4. Discussion

The present study has evaluated the genetic variability and response of multiple morpho-physiological and biochemical traits under three distinct salinity levels: S_0 (irrigation water), S_1 (5 dS/m) and S_2 (10 dS/m). The variability

parameters like means, GCV, PCV, heritability, GA, GAM revealed that salinity stress altered both the magnitude and expression of genetic variation in the studied genotypes.

4.1 Effect of Salinity on Trait Expression

With increasing salinity from S_0 to S_1 (5 dS/m) and S_2 (10 dS/m), there is a progressive reduction in growth traits such as plant height, number of branches, germination percentage, root and shoot length, relative water content, chlorophyll content and biomass due to the well-known inhibitory effects of salt stress on cell division, photosynthesis, nutrient uptake, water relations and tissue integrity, whereas under S_0 the genotypes express their full growth potential, and the declines at S_1 and S_2 reflect intensifying osmotic stress and ion toxicity that reduce turgor and metabolic efficiency; however, stress-responsive biochemical traits including proline, total soluble sugars, starch, antioxidant enzymes (catalase, peroxidase) and membrane stability index increase substantially at S_1 and S_2, indicating activation of osmotic adjustment and membrane protection mechanisms that mitigate salinity damage, in agreement with earlier reports by Abbas *et al*. (2024), Riaz *et al*. (2021), Akram *et al*. (2020), Deepika & Dhingra (2014) and Garg *et al*. (1986). Vishnyakova *et al*. (2023) seen similar results like increase in antioxidant enzymes and decrease in morphological observations.

Table 2. Anova table of seedling parameters

		SGP	RL	SL	VI	TL	SL/RL
Source of variation	**df**	**MSS**					
Treatment	59	298.70	5.47	3.21	149326.92	11.49	0.09
Genotype	19	490.57	8.18	2.94	267797.84	17.74	0.06
Salinity	2	2969.73	15.79	34.46	880436.30	20.62	1.34
G*S	38	62.19	3.57	1.70	51612.02	7.88	0.04
Error	60	7.17	0.04	0.04	2050.38	0.07	0.00

Table 3. Anova of growth related and biomass related traits

		PH30	PH60	NOB	DW	FW	DW/FW	FW/TW
Source of variation	**df**	**MSS**						
Treatment	59	41.08	153.42	2.26	92.15	10368.34	0.004	0.02
Genotype	19	71.36	161.85	2.89	69.04	5045.70	0.002	0.02
Salinity	2	396.45	1367.64	22.38	633.57	176133.99	0.074	0.20
G*S	38	7.24	85.30	0.89	75.21	4305.16	0.002	0.01
Error	60	1.06	3.36	0.13	3.76	333.88	0.000	0.00

Table 4. Anova of biochemical related traits

		PRO	TSS	CAT	STA	TFAA	PER	PHE
Source of variation	**df**	**MSS**						
Treatment	59	27.63	142.12	0.1183	13.06	60.64	0.0818	128.13
Genotype	19	46.37	276.34	0.1346	17.75	62.06	0.0673	158.50
Salinity	2	9.93	38.24	1.3139	6.98	35.00	0.0449	0.48
G*S	38	19.19	80.47	0.0472	11.04	61.28	0.0909	119.67
Error	60	0.01	0.69	0.0004	0.03	0.07	0.0002	0.35

Table 5. Anova of physiological parameters

		Chl a	Chl b	Chl T	Caro	MSI	RWC
Source of variation	**df**	**MSS**					
Treatment	59	0.954	0.22	2.04	0.001513	263.80	239.18
Genotype	19	0.253	0.14	0.74	0.000487	282.01	234.42
Salinity	2	21.005	3.52	41.53	0.033819	1498.71	2712.46
G*S	38	0.249	0.09	0.61	0.000325	189.71	111.38
Error	60	0.0028	0.0012	0.0046	0.000018	1.30	26.25

Table 6. In this general mean, range, genotypic variance (σ^2 g), phenotypic variance (σ^2 p), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), genetic advance (GA), genetic advance as percent of mean (GAM) and heritability (h^2) data shown of normal irrigation water treatment

Trait	Mean	Range	σ g^2	σ p^2	GCV	PCV	GA	GAM	H^2%
SGP	71.2	54-88	63.66	66.06	11.21	11.42	16.14	22.66	96.37
RL	7.13	4.9-10.1	1.86	1.89	19.12	19.25	2.79	39.12	98.63
SL	7.8	6-10.3	1.22	1.24	14.19	14.27	2.27	29.05	98.82
TL	14.93	11.86-19.7	4.63	4.66	14.42	14.45	4.42	29.62	99.48
VI	1064.16	702-1694.2	43163.87	43895.51	19.52	19.69	424.4	39.88	98.33
SL/RL	1.12	0.8-1.66	0.04	0.04	17.81	18.27	0.4	35.78	95.05
FW	287.19	207.1-436.79	3251.2	3445.24	19.85	20.44	114.1	39.73	94.37
DW	13.48	7.2-22.5	9.17	9.95	22.46	23.4	5.99	44.43	92.17
TW	344.92	235.85-496.6	4024.84	4026.57	18.39	18.4	130.66	37.88	99.96
DW/FW	0.05	0.03-0.07	0	0	22.43	23.78	0.02	43.58	88.95
FW/TW	0.83	0.65-0.98	0.01	0.01	8.6	9.73	0.13	15.66	78.13
RWC	82.71	63.88-98.29	55.75	71.1	9.03	10.19	13.62	16.47	78.41
PH30	24.17	19.7-35.26	17.94	18.39	17.53	17.75	8.62	35.66	97.54
CHLa	1.73	1.06-2.51	0.18	0.19	24.76	24.99	0.87	50.52	98.12
Chl.b	0.86	0.42-1.58	0.09	0.09	35.5	35.74	0.62	72.63	98.66
Chlt	2.58	1.47-3.96	0.52	0.53	28.02	28.18	1.48	57.38	98.86
Caro	0.07	0.04-0.11	0	0	25.2	26.19	0.03	49.96	92.6
PH60	47.19	30.79-60.8	63.73	65.28	16.92	17.12	16.25	34.43	97.61
PRO	2.8	0.77-5.71	1.52	1.53	44.12	44.21	2.53	90.68	99.56
TSS	28.42	6.98-43.95	110.94	111.42	37.07	37.15	21.65	76.19	99.57
CAT	0.14	0.04-0.42	0.01	0.01	72.13	74.14	0.2	144.56	94.65
STA	6.5	1.56-12.38	9.09	9.11	46.37	46.43	6.2	95.42	99.77
TFAA	8.84	2.43-22.81	26.88	26.92	58.68	58.71	10.67	120.8	99.87
PER	0.31	0.04-0.77	0.05	0.05	70.12	70.25	0.45	144.18	99.63
PHE	11.16	2.75-31.25	24.54	25.63	44.4	45.38	9.98	89.47	95.72
MSI	41.3	11.57-58.09	109.65	110.85	25.35	25.49	21.45	51.95	98.92
NOB	8.25	6.5-9.3	0.49	0.56	8.47	9.07	1.34	16.31	87.34

SGP- Seed germination percentage, RL- root length, SL- shoot length, TL- total length, VI- vigour index, SL/RL- shoot length per root length, FW- fresh weight, DW- dry weight, TW- turgid weight, DW/FW- dry weight per fresh weight, FW/TW- fresh weight per turgid weight, RWC- relative water content, PH30- plant height at 30 days after sowing, CHLa- chlorophyll a, Chlb- chlorophyll b, Chlt- total chlorophyll, Caro- carotenoid, PH60- plant height at 60 days after sowing, PRO- Proline, TSS- total soluble sugar, CAT- catalase, STA- starch, TFAA- total free amino acids, PER- peroxidase, PHE- phenol, MSI- membrane stability index, NOB- number of branches

Table 7. In this general mean, range, genotypic variance (σ^2 g), phenotypic variance (σ^2 p), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), genetic advance (GA), genetic advance as percent of mean (GAM) and heritability (h^2) data shown of moderate saline (5dS/m) water treatment

Traits	Mean	Range	σ g^2	σ p^2	GCV	PCV	GA	GAM	H^2%
SGP	64.5	46-84	78.89	82.89	13.77	14.12	17.85	27.68	95.17
RL	8.33	5.78-12.5	2.37	2.39	18.49	18.57	3.16	37.92	99.14
SL	7.13	5.4-9.7	1.17	1.19	15.19	15.32	2.21	31.04	98.37
TL	15.46	11.22-22.1	5.88	5.93	15.69	15.75	4.97	32.18	99.16
VI	1003.2	592.48-1543.92	54509.72	55707.29	23.27	23.53	475.76	47.42	97.85
SL/RL	0.87	0.66-1.14	0.01	0.01	13.15	13.35	0.23	26.7	97.1
FW	248.33	175.3-339.13	1874.86	2087.42	17.44	18.4	84.53	34.04	89.82
DW	19.99	12.8-51.1	56.23	58.08	37.52	38.13	15.2	76.05	96.82
TW	307.96	215.3-407.6	2616.11	2748.14	16.61	17.02	102.8	33.38	95.2
DW/FW	0.08	0.05-0.22	0	0	37.99	38.97	0.06	76.28	95.01
FW/TW	0.81	0.65-0.97	0	0.01	8.47	8.99	0.13	16.45	88.77
RWC	79.35	62.36-96.39	56.09	62.49	9.44	9.96	14.62	18.42	89.75
PH30	21.44	15.87-31.65	11.67	12.2	15.94	16.29	6.88	32.1	95.64
CHLa	0.84	0.31-1.84	0.16	0.17	48.43	48.5	0.83	99.62	99.71
Chl.b	0.44	0.11-1.07	0.05	0.05	51.22	51.33	0.46	105.3	99.59
Chlt	1.27	0.45-2.9	0.39	0.39	49	49.03	1.28	100.86	99.86
Caro	0.04	0.03-0.09	0	0	30.76	30.91	0.03	63.04	99.01
PH60	43.39	28-54.74	50.69	52.94	16.41	16.77	14.35	33.08	95.75
PRO	3.65	1.61-16.38	10.41	10.41	88.35	88.37	6.64	181.93	99.94
TSS	30.31	12.24-42.9	52.96	53.37	24.01	24.1	14.93	49.26	99.22
CAT	0.43	0.11-0.94	0.04	0.05	48.63	51.56	0.41	94.47	88.95
STA	6.35	2.28-10.15	5.22	5.23	35.99	36.03	4.7	74.05	99.78
TFAA	10.07	2.08-22.23	18.5	18.55	42.69	42.75	8.85	87.81	99.7
PER	0.28	0.02-0.82	0.05	0.05	83.88	84.1	0.48	172.34	99.48
PHE	11.22	0.87-32.5	27.21	39.07	46.48	55.7	8.97	79.92	69.66
MSI	37.7	18.8-56.45	56.13	56.44	19.87	19.93	15.39	40.82	99.45
NOB	7.72	6.3-9.3	0.44	0.52	8.6	9.34	1.26	16.31	84.8

SGP- Seed germination percentage, RL- root length, SL- shoot length, TL- total length, VI- vigour index, SL/RL- shoot length per root length, FW- fresh weight, DW- dry weight, TW- turgid weight, DW/FW- dry weight per fresh weight, FW/TW- fresh weight per turgid weight, RWC- relative water content, PH30- plant height at 30 days after sowing, CHLa- chlorophyll a, Chlb- chlorophyll b, Chlt- total chlorophyll, Caro- carotenoid, PH60- plant height at 60 days after sowing, PRO- Proline, TSS- total soluble sugar, CAT- catalase, STA- starch, TFAA- total free amino acids, PER- peroxidase, PHE- phenol, MSI- membrane stability index, NOB- number of branches

Table 8. In this general mean, range, genotypic variance (σ^2 g), phenotypic variance (σ^2 p), genotypic coefficient of variance (GCV), phenotypic coefficient of variance (PCV), genetic advance (GA), genetic advance as percent of mean (GAM) and heritability (h^2) data shown of severe saline (10 dS/m) water treatment

Traits	Mean	Range	σ^2 g	σ^2 p	GCV	PCV	GA	GAM	H^2%
SGP	54.1	32-82	154.17	158.52	22.95	23.27	25.22	46.63	97.26
RL	8.07	4.7-11.05	3.37	3.38	22.73	22.79	3.77	46.71	99.5
SL	5.96	4.06-7.88	0.71	0.73	14.15	14.36	1.71	28.72	97.07
TL	14.04	8.76-17.79	6.14	6.17	17.65	17.69	5.09	36.25	99.46
VI	782.2	302.4-1384.98	84761.78	85908.14	37.22	37.47	595.73	76.16	98.67
SL/RL	0.76	0.53-1.06	0.02	0.02	17.49	17.84	0.27	35.32	96.08
FW	157.86	98-260.88	1201.08	1295.32	21.95	22.8	68.75	43.55	92.72
DW	20.71	11.5-36.98	38.69	41.7	30.03	31.18	12.34	59.59	92.78
TW	227.79	148.4-363.5	2708.5	2711.25	22.85	22.86	107.15	47.04	99.9
DW/FW	0.13	0.07-0.23	0	0	28.36	29.41	0.08	56.35	93.02
FW/TW	0.7	0.47-0.88	0.01	0.01	12.1	13.28	0.16	22.7	82.97
RWC	67.07	44.5-86.25	77.38	95	13.12	14.53	16.35	24.38	81.45
PH30	17.89	13.89-30.1	11.72	12.32	19.13	19.62	6.88	38.44	95.1
Chl a	0.29	0.09-0.67	0.02	0.02	53.52	53.73	0.32	109.81	99.2
Chl b	0.29	0.1-0.6	0.02	0.02	43.52	43.88	0.26	88.91	98.35
Chl T	0.58	0.33-1.18	0.06	0.06	42.13	42.22	0.5	86.61	99.59
Caro	0.01	0-0.03	0	0	57.95	58.23	0.01	118.82	99.07
PH 60	35.71	21.71-48.31	46.77	47.99	19.15	19.4	13.91	38.94	97.45
PRO	3.67	1.11-26.49	30.43	30.43	150.5	150.51	11.36	310	99.98
TSS	28.96	14.29-39.59	53.71	53.85	25.31	25.34	15.08	52.06	99.73
CAT	0.47	0.06-0.98	0.05	0.05	48.15	49.4	0.46	96.69	95.02
STA	5.71	2-11.6	5.55	5.57	41.23	41.29	4.85	84.83	99.74
TFAA	8.24	0.4-21.21	46.82	46.84	83.03	83.05	14.09	171.01	99.96
PER	0.24	0.01-0.89	0	0.04	0	79.77	0	0	0
PHE	11.37	-1.88-52.63	116.12	134.24	94.77	101.89	20.65	181.56	86.5
MSI	29.37	3.5-58.18	162.99	163.43	43.48	43.53	26.26	89.44	99.73
NOB	6.77	45905	1.23	1.27	16.38	16.63	2.25	33.22	96.94

SGP- Seed germination percentage, RL- root length, SL- shoot length, TL- total length, VI- vigour index, SL/RL- shoot length per root length, FW- fresh weight, DW- dry weight, TW- turgid weight, DW/FW- dry weight per fresh weight, FW/TW- fresh weight per turgid weight, RWC- relative water content, PH30- plant height at 30 days after sowing, CHLa- chlorophyll a, Chlb- chlorophyll b, Chlt- total chlorophyll, Caro- carotenoid, PH60- plant height at 60 days after sowing, PRO- Proline, TSS- total soluble sugar, CAT- catalase, STA- starch, TFAA- total free amino acids, PER- peroxidase, PHE- phenol, MSI- membrane stability index, NOB- number of branches

4.2 Genetic Variability Across Salinity Levels (GCV and PCV)

A clear trend of increasing genotypic (GCV) and phenotypic (PCV) coefficients of variation emerged from S0 to S2 for most traits, with lower GCV/PCV under S0 indicating stable genetic expression for growth traits in no-stress conditions, moderate amplification under S1 revealing differential genotypic responses in biochemical/physiological traits to mild salinity, and highest values under S2 for stress-responsive traits due to severe salinity exposing broader genetic variability; this aligns with prior studies on cluster bean by Nayak *et al*. (2025), Kumar *et al*. (2024), Reddy *et al*. (2019), Jukanti *et al*. (2015), Kumar and Ram (2015), Jitender *et al*. (2014), Rai *et al*. (2014), Saini *et al*. (2010) and Dass *et al*. (1973), confirming salinity as a catalyst for unveiling hidden genetic differences, especially in osmotic and oxidative stress responses.

4.3 Heritability and Genetic Advance

Heritability estimates increased under salinity stress, particularly S2, for biochemical and physiological traits like proline content, antioxidant enzymes (catalase, peroxidase), membrane stability index, starch, total soluble sugars, total free amino acids, chlorophyll content and Na^+/K^+ parameters, where high heritability combined with high GAM indicates additive gene action ideal for selection; under S2, severe stress amplified genetic control over tolerance mechanisms over environmental effects, while morphological traits under S0 showed moderate heritability with low-moderate GAM reflecting partial non-additive control or greater environmental influence, consistent with Nayak *et al*. (2025), Reddy *et al*. (2019), Patil *et al*. (2014) and Rai *et al*. (2014).

4.4 Influence of Stress Intensity on Selection Efficiency

Differential genetic behaviour across S_0, S_1 (5 dS/m) and S_2 (10 dS/m) implies targeted breeding strategies: S_0 suits evaluating growth/yield potential under optimal conditions but misses tolerance mechanisms; S_1 reveals moderate variability in physiological/biochemical traits for identifying partial tolerance genotypes; S_2 proves most effective for selecting true salt-tolerant genotypes by amplifying genetic differences and additive variance in key tolerance traits, supporting screening under higher stress levels, as confirmed by Soni *et al.* (2025), Riaz *et al*. (2021) and Deepika & Dhingra (2014) in cluster bean.

4.5 Trait Categories Important for Breeding Salt Tolerance

The study identifies three key trait groups with high genetic significance under salinity stress: (a) osmotic adjustment traits like proline and osmolytes, showing high variability and GAM under S_2 for maintaining turgor and hydration; (b) antioxidant defense mechanisms including catalase, peroxidase and total free amino acids, with high heritability and variability as reliable markers for oxidative stress tolerance; and (c) membrane stability index, starch content and total soluble sugars, exhibiting strong genetic control as indicators of cell integrity, making them ideal for selecting promising salt-tolerant genotypes.

5. Conclusion

Salinity stress significantly influenced genetic variability with maximum divergence observed at S_2 (10 dS/m). Traits related to osmotic adjustment (like total soluble sugar and starch), antioxidant enzymes and membrane stability index exhibited the highest genetic advance and heritability making them reliable selection indicators for breeding salt tolerant varieties. S_1 (5dS/m) has allowed identification of moderately tolerant genotypes, while S_0 (available irrigation water) served as a baseline for evaluating potential under optimal conditions. Thus, the study concludes that progressive salinity stress strengthens the expression of genetic differences and selection for salt tolerance is most effective under high stress conditions using biochemical and physiological traits as primary indicators. This reflects strong additive genetic variance and suggests that these traits can serve as reliable selection criteria in breeding programs aimed at salinity tolerance.

Disclaimer (Artificial Intelligence)

Author's hereby declare that they have not used AI for this AI is showing on general words of departments and words which can not be change like GCV, PCV, growth, genetic advance etc. Author's hereby declare that no image have been generated through AI and generative AI tech, AI

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Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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