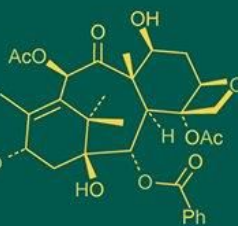
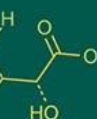
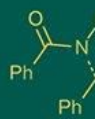
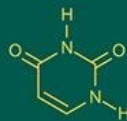
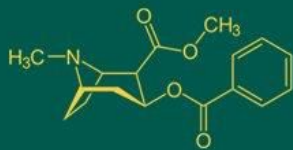


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(2): 598-602
www.biochemjournal.com
Received: 09-12-2024
Accepted: 13-01-2025

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Genotypic variability in salt tolerance of clusterbean genotypes during early growth stages

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DOI: <https://doi.org/10.33545/26174693.2025.v9.i2Sh.3856>

Abstract

Genetic variability is prerequisite for any crop improvement in a crop. Assessment of clusterbean genotypes for tolerance to salt stress has prime importance. The survey of genetic variability with the help of suitable genetic parameters like genotypic and phenotypic coefficients of variations, heritability estimates and genetic advance as percentage of mean are indispensable in breeding programmes aimed at improvement of seed yield. The current study was aimed to assess variability for germination and seedling traits in clusterbean genotypes during seedling growth and early growth stages. In this laboratory experiment, 10 genotypes of clusterbean were evaluated at different salinity levels (0.0%, 0.4% and 0.6% NaCl concentrations) in two sets i.e. set-I and set-II for analyzing the effect of salinity on germination (%) and seedling traits viz; shoot length, root length, seedling length, root to shoot length ratio, shoot fresh weight, root fresh weight, seedling fresh weight, shoot dry weight, root dry weight, seedling dry weight and seedling vigour index in clusterbean genotypes. Observations were recorded on 12th day and 17th day of sowing for set-I and set-II, respectively. The genotypes, salinity levels and their interaction exhibited significant differences in all the salinity levels for most of the traits in both the sets. The GCV and PCV values were observed in root dry weight followed by shoot fresh weight in set-I; and in set-II these were highest for root dry weight and root fresh weight. It indicated that variation was highest for these traits among the genotypes.

Keywords: Clusterbean, salinity, variation, GCV, PCV

Introduction

Clusterbean [*Cymopsis tetragonoloba* (L.) Taub] also known as guar, gavar, guwar or guvar bean is a self-pollinated leguminous crop which is grown during *kharif* as well as summer seasons in arid and semi-arid regions of India. Under saline conditions, germination of seeds under saline conditions differs from one crop to other and even a significant variation is recorded among the different cultivars of the same crop (Kumar and Bhardwaj, 1981) [13]. To some extent, it is salt tolerant crop which is used for human consumption and cattle forage as well as green manure for crops (Choy *et al.*, 2015) [8]. Its seeds contain a considerable amount of galactomannan gum, which is used in food processing, paper manufacturing, textile printing and in pharmaceutical and cosmetic industries (Alexander *et al.*, 1988) [2]. Salinity is one of the major constraints which exerts an adverse effect on germination, plant vigour and crop yield (Munns and Tester, 2008) [16] particularly in arid and semi-arid regions of the world (Ahmed, 2009) [1].

Seed germination is very sensitive to salt stress (Begum *et al.*, 2013) [6]. Salinity reduces the speed and percentage germination of the seeds; decreases shoot length, fresh and dry weights of shoots and roots of plants (Fateme *et al.*, 2010) [9]. Soil salinity may affect the germination of seeds either by creating a lower osmotic potential external to the seed preventing uptake of water or through the toxic effects of Na⁺ and Cl⁻ ions on germinating seeds (Khajeh-Hosseini *et al.*, 2003) [12].

Materials and Methods

The experiment was carried out at the laboratory of Department of Plant Breeding and Genetics, Sri Karan Narendra College of Agriculture, Jobner (Rajasthan) during the period from July, 2018 to October, 2018 at a temperature of 32±2° room temperature with 53% relative humidity.

For evaluation, seeds of 10 genotypes of clusterbean namely, RGC-1066, RGC-986, RGC-197, RGC-1033, RGC-1038, RGC-1055, RGC-1031, RGC-1003, RGC-936 and Mayur-31 were used. Before sowing, selected seeds were surface sterilized with 0.1% mercuric chloride for 1 minute and washed repeatedly for two to three times under running tap water followed by washing with distilled water. Seeds after presoaking coated with fungicide (Thiram @ 3 g/kg. seed) to protect from fungal infestation.

Three NaCl solutions viz. control (0.0%), 0.4% and 0.6% were used and depicted as SL₁, SL₂ and SL₃, respectively. Tap water was used as control and 0.4% and 0.6% NaCl solutions were prepared by dissolving 4 g and 6 g NaCl in double distilled water, accordingly. The experiment with ten genotypes and three NaCl concentrations was carried out in completely randomized design (CRD) with three replications in two sets. 180 plastic pots (90 for set-I and 90 for set-II) with the dimensions of 8.5 cm length and 8.2 cm diameter were used for sowing seeds. There were 10 pots per replication for each salinity level, in this way there were 30 plastic pots for each salinity level and all genotypes were replicated 3 times with each treatment in one plot. Six disinfected seeds of each clusterbean genotype were placed at a depth of 1.5 cm at equal distances in each plastic pot filled with 458 g sandy soil.

In set-I and set-II, five seedlings were randomly selected and the observations on various seedling characteristics except germination (%) were recorded on 12th and 17th day, respectively in each replication from each treatment.

Methods used for recording following observations are described below:

Germination (%)

A seed was considered to have germinated at the emergence of both radical and plumule up to 2 mm length (Chartzoulakis and Klapaki, 2000) [7]. The numbers of germinated seeds were recorded on 8 days after sowing and the final germination (%) was determined by using the following formula (Aniat *et al.*, 2012) [4]:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Root length, Shoot length and Seedling length (cm)

The root length (the distance from crown to root tip) and shoot length (the distance from crown to leaf tip) were measured by using a measuring scale in centimeter and average for each pot was recorded. The hypocotyl length was also included in shoot length. For seedling length, shoot length and root length of five seedlings which had already been selected from each replication and in each treatment were added and all five seedling lengths were averaged.

Root fresh weight, Shoot fresh weight and Seedling fresh weight (mg)

The fresh weight of roots and shoots of the concerned five seedlings was measured in milligrams by using a sensitive electronic balance and averaged. Shoot fresh weight and root fresh weight were added to obtain seedling fresh weight.

Root dry weight, Shoot dry weight and Seedling dry weight (mg)

The roots and shoots which were taken for fresh weight

were kept into labeled paper bags and dried in hot air oven at 65 °C for 48 hours. After drying, the dried roots and shoots were weighted by sensitive electronic balance in milligrams and average was recorded. Seedling dry weight was obtained by adding root dry weight and shoot dry weight.

Root to shoot length ratio

The root to shoot length ratio of the concerned seedlings were calculated by the following formula:

$$\text{Root to Shoot Length Ratio} = \frac{\text{Root Length}}{\text{Shoot Length}}$$

Seedling vigour index

The seedling vigour index was determined by multiplying the sum total of mean length of shoot and root of concerned seedlings with concerned germination (%) by the following formula (Iqbal and Rahmati, 1992) [11]:

$$\text{Seedling Vigour Index (SVI)} = (\text{Mean Root Length} + \text{Mean Shoot length}) \times (\text{Germination \%})$$

The data obtained were subjected to analysis of variance following standard statistical methods (Panse and Sukhatme, 1985) [17] and significant differences among the mean values were compared by least significant difference (LSD) test ($p < 0.05$). Variability parameters were calculated using various formulae given by Burton 1952 and Johnson *et al.* 1955.

Results and Discussion

Analysis of variance in each salt (NaCl) concentration namely 0.0%, 0.4% and 0.6% (SL₁, SL₂ and SL₃, respectively) showed significant differences among the genotypes for most of the traits under study, except few in both the sets. Significant difference indicated that there was sufficient genetic variability in the genotypes used for investigation (Table 1 and Table 2). Variability parameters and other attributes of different traits are presented in Table 3 and Table 4 for set-I and set-II, respectively.

A wide range of variability was found for all the characters studied. The mean values of all the characters varied along the salinity gradient in both the sets. The values were found to be maximum in the control (0.0%) and were minimum at the highest salinity level (0.06%) for almost all the characters.

In general, the PCV values were higher than GCV values for all the characters indicating a positive effect of environment on the character expression. The differences were however low for all the traits while screening of salinity tolerance. The highest GCV and PCV values were observed in root dry weight followed by shoot fresh weight in set-I and In set-II highest GCV and PCV values were observed in root dry weight followed by root fresh weight.

Heritability in broad sense was generally high for seedling dry weight and shoot dry weight in salinity level SL₁ and SL₂ and for root dry weight and root fresh weight in salinity level SL₃ in set-I. In set-II it was high for root dry weight at SL₁, SL₃ and for seedling dry weight at SL₂ salinity level. Hence, it is suggested that during screening for salinity tolerance major emphasis should be given on these characters.

Genetic advance as percent of mean was highest for root dry weight followed by shoot fresh weight in set-I while for root dry weight and root fresh weight in set-II, across all the salinity levels. It was highest for root dry weight in both the sets.

In the present investigation the pooled analysis of variance showed that the mean sum of squares due to salinity levels and genotypes were significant for almost all characters. It revealed that there was significant difference among genotypes and the effect of salinity on the genotypes. Sprague and Federer (1951) ^[18] suggested that the biasness caused by genotype x environment interaction in the estimates of genetic parameters is of unknown magnitude and direction and it may not be same for each parameter. The genotype x salinity (G x S) interaction was also found significant for most of the characters examined which indicated differential response of genotypes to different salinity levels. Allard and Bradshaw (1964) ^[3] reported that genotype x environment is a common phenomenon present

in crop plant species. Similar results have also been reported in cowpea by Gogile *et al.* (2013) ^[10]. Since the genotype x salinity interaction was significant, it would be worthwhile to compare the genotypes over different salinity levels for different characters rather than the pooled mean alone. In view of this, the analysis of variance was carried out for each salinity level separately.

Analysis of variance in the each salt (NaCl) concentration namely 0.0%, 0.4% and 0.6% (SL₁, SL₂ and SL₃, respectively) showed significant differences among the genotypes for most of the traits under study, except few in both the sets. Mshelmbula *et al.* (2015) ^[15] also reported that the germination of cowpea seeds, growth and yield were highly affected by salinity at all levels of treatments as compared to control. Similar findings were also reported in clusterbean by Lahiri *et al.* (1987) ^[14] and in lentil by Tesfaye *et al.* (2015) ^[19]. Significant difference indicated that there was sufficient genetic variability in the material used for this investigation.

Table 1: Pooled ANOVA showing mean sum of squares of various characters in clusterbean for set-I

Source	df	G (%)	SL (cm)	RL (cm)	SLL (cm)	RFW (mg)	SFW (mg)	SLFW (mg)	RDW (mg)	SDW (mg)	SLDW (mg)	RL/SL	SVI
Genotypes (G)	9	233.531**	3.127**	0.865**	3.702**	68.977**	149859.076**	152557.71**	11.096**	346.798**	414.607**	0.011**	28308.281*
Salinity levels (S)	2	2873.295**	67.303**	11.572**	134.687**	527.037**	737441.166**	777057.28**	45.927**	1738.164**	2348.769**	0.002	3225115.295**
Genotypes x Salinity (G X S)	18	44.235	0.552*	0.113	0.573*	12.987**	14795.067**	15230.523**	1.056**	15.610**	15.734**	0.002*	17758.075
Pooled Error	60	46.296	0.248	0.073	0.263	2.069	3533.428	3490.009	0.214	5.636	5.718	0.001	15557.533

Table 2: Pooled ANOVA showing mean sum of squares of various characters in clusterbean for set-II

Source	df	G (%)	SL (cm)	RL (cm)	SLL (cm)	RFW (mg)	SFW (mg)	SLFW (mg)	RDW (mg)	SDW (mg)	SLDW (mg)	RL/SL	SVI
Genotypes (G)	9	432.384**	2.707**	1.072**	4.120**	276.082**	162044.513**	166613.58*	52.638**	187.122**	368.275**	0.008**	117526.943**
Salinity levels (S)	2	2678.658**	77.612**	15.572**	162.570**	713.570**	1440602.716**	1504120.7*	81.907**	366.930**	795.520**	0.002*	4165295.483 **
Genotypes x Salinity (G X S)	18	100.101**	0.369*	0.136**	0.260	5.070	4551.022	4430.412	0.911**	0.931	2.130	0.002**	37658.880**
Pooled Error	60	30.862	0.179	0.049	0.194	3.271	6569.587	6517.708	0.242	1.261	1.443	0.000	12333.920

*, ** =Significant at 5% and 1% levels, respectively

G-Germination, SL-Shoot length, RL-Root length, SLL-Seedling length, RFW-Root fresh weight, SFW-Shoot fresh weight, SLFW-Seedling fresh weight, RDW-Root dry weight, SDW-Shoot dry weight, SLDW-Seedling dry weight, RL/SL-Root length/ Shoot length, SVI-Seedling Vigour Index

Table 3: The general mean, range, genotypic and phenotypic coefficient of variation (GCV & PCV), heritability (in broad sense) and genetic advance (GA) over different salinity levels for different characters for set-I

Salinity Levels	Germination (%)						Salinity Levels	Shoot Length (cm)					
	Mean	Range	GCV	PCV	h ² (%)	GA (%)		Mean	Range	GCV	PCV	h ² (%)	GA (%)
SL ₁	95.56	88.89-100.00	3.41	7.90	18.68	3.04	SL ₁	12.81	11.52-14.07	5.49	7.63	51.83	8.15
SL ₂	90.55	83.33-100.00	5.64	9.40	36.02	6.97	SL ₂	11.14	10.31-12.10	6.18	7.29	71.68	10.77
SL ₃	76.67	66.67-83.33	6.43	10.96	34.47	7.78	SL ₃	9.82	8.93-10.46	4.48	5.48	66.74	7.54
Root Length (cm)							Seedling Length (cm)						
SL ₁	4.71	4.30-5.32	7.11	9.79	52.83	10.65	SL ₁	17.52	16.29-19.11	4.61	6.03	58.41	7.26
SL ₂	4.03	3.66-4.77	8.27	9.20	80.69	15.30	SL ₂	15.17	14.08-16.76	5.07	5.87	74.74	9.04
SL ₃	3.47	3.02-4.16	7.49	11.51	42.28	10.03	SL ₃	13.30	12.47-13.90	2.47	3.62	46.59	3.47
Root Fresh Weight (mg)							Shoot Fresh Weight (mg)						
SL ₁	25.83	19.77-35.73	17.64	18.95	86.69	33.84	SL ₁	904.90	592.40-1301.87	19.57	20.87	88.00	37.83
SL ₂	20.54	18.20-26.07	11.47	13.40	73.27	20.22	SL ₂	740.59	472.37-916.43	17.06	18.25	87.38	32.85
SL ₃	17.56	15.03-20.13	10.29	11.75	76.67	18.55	SL ₃	591.45	439.60-734.50	15.99	19.25	69.02	27.37
Seedling Fresh Weight (mg)							Root Dry Weight (mg)						
SL ₁	930.73	618.90-1337.60	19.32	20.54	88.46	37.43	SL ₁	6.34	4.40-8.83	22.96	24.55	87.50	44.25
SL ₂	761.14	491.03-936.03	16.70	17.85	87.62	32.21	SL ₂	5.18	3.73-7.68	24.53	26.01	88.95	47.66
SL ₃	609.01	456.27-754.63	15.61	18.73	69.46	26.79	SL ₃	3.87	2.73-4.80	17.45	19.93	76.64	31.47
Shoot Dry Weight (mg)							Seedling Dry Weight (mg)						
SL ₁	66.76	52.80-74.50	10.33	10.84	90.82	20.27	SL ₁	73.10	59.37-83.33	10.15	10.62	91.38	19.99
SL ₂	60.08	45.60-69.40	12.01	12.52	92.15	23.76	SL ₂	65.26	50.33-77.08	12.13	12.52	93.90	24.21
SL ₃	51.57	42.87-59.23	8.83	10.33	73.01	15.54	SL ₃	55.44	46.67-63.63	9.06	10.38	76.20	16.30
Root Length / Shoot Length							Seedling Vigour Index						
SL ₁	0.37	0.31-0.44	8.70	12.82	46.04	12.16	SL ₁	1673.02	1558.13-1823.00	1.52	7.94	3.68	0.60
SL ₂	0.36	0.30-0.41	9.92	12.00	73.22	17.49	SL ₂	1372.79	1223.01-1535.33	4.70	10.13	21.55	4.49
SL ₃	0.36	0.29-0.44	10.87	14.82	53.83	16.44	SL ₃	1018.02	882.31-1096.90	5.45	10.34	27.79	5.92

Table 4: The general mean, range, genotypic and phenotypic coefficient of variation (GCV & PCV), heritability (in broad sense) and genetic advance (GA) over different salinity levels for different characters for set-II

Salinity Levels	Mean	Range	GCV	PCV	h ² (%)	GA (%)	Salinity Levels	Mean	Range	GCV	PCV	h ² (%)	GA (%)
	Germination (%)							Shoot Length (cm)					
SL ₁	93.89	83.33-100.00	4.99	8.80	32.16	5.83	SL ₁	15.01	14.41-15.66	2.60	3.76	47.59	3.69
SL ₂	90.56	77.78-100.00	4.80	13.46	12.71	3.52	SL ₂	13.07	12.21-14.04	5.37	6.09	77.77	9.75
SL ₃	75.00	66.67-83.33	10.09	11.60	75.57	18.06	SL ₃	11.82	10.80-12.60	4.82	6.30	58.71	7.61
Root Length (cm)							Seedling Length (cm)						
SL ₁	5.93	5.34-6.37	5.088	6.706	57.572	7.953	SL ₁	20.95	20.43-21.86	2.103	2.774	57.463	3.284
SL ₂	5.14	4.69-5.82	8.051	8.667	86.294	15.406	SL ₂	18.21	17.07-19.63	4.474	5.004	79.952	8.241
SL ₃	4.50	4.05-5.36	8.226	9.648	72.699	14.449	SL ₃	16.32	15.13-17.49	4.313	5.364	64.664	7.145
Root Fresh Weight (mg)							Shoot Fresh Weight (mg)						
SL ₁	43.51	34.33-51.17	12.39	13.76	81.11	22.98	SL ₁	1578.89	1166.60-1769.57	9.12	12.96	49.51	13.22
SL ₂	38.53	29.70-47.83	15.97	16.18	97.46	32.49	SL ₂	1283.55	987.73-1404.50	9.03	10.86	69.08	15.46
SL ₃	33.76	28.60-41.07	14.87	15.47	92.46	29.46	SL ₃	1150.79	870.23-1266.17	9.93	10.80	84.46	18.80
Seedling Fresh Weight (mg)							Root Dry Weight (mg)						
SL ₁	1622.40	1209.17-1820.70	8.978	12.650	50.369	13.126	SL ₁	13.61	9.93-19.60	21.99	22.48	95.64	44.30
SL ₂	1322.07	1021.43-1442.93	8.927	10.673	69.954	15.380	SL ₂	11.39	8.70-15.80	19.18	19.70	94.80	38.47
SL ₃	1184.54	899.50-1300.77	9.841	10.669	85.073	18.698	SL ₃	10.38	8.17-14.87	19.73	19.86	98.65	40.36
Shoot Dry Weight (mg)							Seedling Dry Weight (mg)						
SL ₁	78.34	67.27-83.47	6.16	6.51	89.51	12.01	SL ₁	91.94	78.37-103.07	7.76	7.98	94.46	15.53
SL ₂	73.56	62.80-78.73	6.08	6.18	96.60	12.30	SL ₂	84.95	72.30-94.53	7.25	7.33	97.87	14.78
SL ₃	71.52	61.40-75.67	6.01	6.06	98.16	12.26	SL ₃	81.90	70.07-90.53	7.11	7.17	98.50	14.54
Root Length / Shoot Length							Seedling Vigour Index						
SL ₁	0.40	0.35-0.43	6.17	8.65	50.82	9.05	SL ₁	1964.50	1821.32-2130.67	3.67	7.84	21.85	3.53
SL ₂	0.40	0.34-0.47	9.57	10.50	83.06	17.96	SL ₂	1646.22	1436.34-1868.67	3.76	13.03	8.34	2.24
SL ₃	0.38	0.33-0.44	9.00	11.07	66.12	15.08	SL ₃	1221.01	1096.13-1374.95	7.94	10.55	56.67	12.32

Conclusion

The genotypes exhibited significant differences for all the characters in all the salinity levels except germination (%) in salinity level SL₁ and seedling vigour index in all the salinity levels in set-I and except seedling vigour index in SL₁ salinity level indicating inherent differences among the genotypes. The interactions between genotype x salinity levels also exhibited significant difference for all characters except germination (%), root length and seedling vigour index in set-I and except for seedling length, root fresh

weight, shoot fresh weight, seedling fresh weight, shoot dry weight and seedling dry weight in set-II, indicating differential response of genotypes to salinity for all the traits. The mean values were maximum in the control (0.0%) and were minimum at the highest salinity level (0.06%) for almost all the characters. The highest GCV and PCV values were observed in root dry weight followed by shoot fresh weight in set-I and In set-II highest GCV and PCV values were observed in root dry weight followed by root fresh weight. Heritability in broad sense was generally

high for seedling dry weight and shoot dry weight in salinity level SL₁ and SL₂ and for root dry weight and root fresh weight in salinity level SL₃ in set-I. In set-II it was high for root dry weight at SL₁, SL₃ and for seedling dry weight at SL₂ salinity level. Hence, it is suggested that during screening for salinity tolerance major emphasis should be given on these characters.

Acknowledgement

The authors are highly thankful to Sri Karan Narendra Agriculture University, Jobner for funding the research project.

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