Original Research Article

Screening blackgram (*Vigna mungo* (L.) Hepper) genotypes for salt tolerance at germination and seedling stages

ABSTRACT

Aim: Salt stress negatively affects the growth and development of plants. An effective and important strategy to develop salinity tolerant crops is to harness the genetic diversity within the crop germplasm by identification of salinity tolerant genotype. The present study was carried out with an objective to screen blackgram genotypes (A31, DPU 968, KU 7720, LBG 787, PLU 621, TMV 1, Tutiminimum, VBG 18028, VBG 18032, and VBN 2) for salt tolerance at seedlings stage and identification of saline tolerant blackgram genotype based on salt stress tolerance indices.

Study design: Factorial and arranged in a completely randomized design.

Place and Duration of Study: Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore; May-June 2022

Methodology: Using petriplate and roll towel techniques, blackgram seed germination indices and seedling growth indices such as germination percentage, germination index, mean germination time, seedling vigour index, shoot and root length, fresh and dry seedling weight, and stress tolerance indices were recorded. Two-way ANOVA, Principle Component Analysis (PCA) and Cluster Heat-map Analysis were used to analyze the data.

Results: PCA indicates that salinity stress accounted for 86.2 percent of the variation for salt tolerance, whereas the individual genotype contributes only 5.4 percent of the variation observed in germination and growth parameters that were studied in blackgram under control and 130mM NaCl salt stress conditions. Among the ten blackgram genotypes, stress tolerance index was highest for LBG 787 (0.831) followed by KU7720 (0.722). On the other side, the stress susceptible index was found to be lower for the blackgram genotype KU7720 (0.335) followed by LBG 787 (0.592), as compared to other genotypes screened. Cluster heat-map analysis for all the measured parameters revealed that blackgram genotype LBG 787 was clustered with PLU 621 and VBG 18028 and found to tolerate the salt stress level (130mM NaCl stress) compared to other blackgram genotypes.

Conclusion: Observations indicate that the blackgram genotypes KU 7720, LBG 787, PLU 621, and VBG 18028 exhibit greater tolerance towards salinity during the germination and seedling growth stages.

Keywords: Abiotic stress, Stress tolerance index, Stress susceptibility index and Cluster heatmap

1. INTRODUCTION

Salinity is an important abiotic stress factor which negatively affects the growth and development of crop plants across the globe [1]. World-wide about, 397 Mha of land area is affected by salinity and 434 Mha of land is affected by sodicity, which accounts for 3.4 percent of the world's land area [2]. According to FAO's soils portal (2022), nearly 19.5 percent of the World's irrigated land (45 million hectares) is salt-affected. This may increase due to erratic rainfall, inundation of sea water into coastal regions, poor-quality irrigation water due to groundwater depletion, and deterioration of high-salt rocks [3 and 4]. According to Patel *et al*., (2011), saline soil covers almost 7 Mha of land in India [5].

At whole plant level, salinity stress has negative impact on plant growth, development, and metabolism, since it affects, many physiological and biochemical processes including photosynthetic activity, protein synthesis, lipid metabolism, ion homeostasis and osmotic adjustment [1]. High ion concentrations (primarily Na^+ and Cl^-), results in a substantial reduction in water potential. Therefore, salinity stress has a dual effect on plant growth and development, functioning either as an osmotic barrier for plant water absorption or as an accumulator of Na^+ and Cl^- ions with harmful consequences [6].

The blackgram (*Vigna mungo* L. Hepper), is a nitrogen-fixing, short-duration tropical pulse crop cultivated across India. It has occupied an important place in human nutrition as rich source of protein and other nutrients. Blackgram is the most abundant source of phosphorus among the many pulses, being 5–10 times more abundant than other pulses. It also contains 55–60% carbohydrates, 22–25% protein, and 1-1.3% fat. Blackgram is sensitive to abiotic stresses such as high and low temperatures, freezing, drought, heavy metals, and hypoxia, and it is especially sensitive to salinity [7].

During seed germination, salinity inhibits seed water imbibition and absorption also induce harmful effects on germinating seeds by promoting the accumulation of Na^+ and Cl^- ions [8]. Such effects typically lead to reduction in the enzyme hydrolysis of stored carbohydrates into simple sugar, thus inhibiting seed germination and results in incomplete seedling emergence, and poor plant population establishment [9]. Salinity has significant impact on germination and early seedling growth, since germination occurs in the uppermost topsoil where most of the soluble salts are concentrated [10].

Therefore, seedlings capacity to tolerate salt stress during seed germination and seedling emergence determines the plants establishment and its survival under saline soils [11]. According to Alvarado *et al*. 1987 increased salinity generally reduces the plants' establishment, its growth, development and biomass accumulation [12]. In agriculture to lessen the effect of salt stress on the crop growth and development appropriate viable solutions must be identified. One of the effective and promising techniques is to screen and identify genotypes that perform better than others under saline stress situations [13].

Therefore, the current study objective is to assess how the 10 blackgram genotypes respond to 13 mM NaCl salt stress throughout the germination and seedling stages and to determine whether or not they have the potential to tolerate salt stress at germination and seedling stage. we hypothesized the blackgram genotypes differing their salt tolerance at germination and seedling stage.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

The ten blackgram genotypes including A31, DPU 968, KU 7720, LBG 787, PLU 621, TMV 1, Tutiminimum, VBG 18028, VBG 18032 and VBN 2 were used in this study. Germination assay were carried out using Petri dish and roll towel method. Blackgram seeds were sterilized using 4% sodium hypochlorite solution for three minutes followed by washing thrice with sterile distilled water. After draining the excess water, 25 blackgram seeds were placed in the petriplates and roll towel paper moistened with water (control) and 13 mM NaCl solution (salt stress). Three replications were maintained for each treatment. The petriplates and roll towel containing blackgram seeds were placed in the growth chamber maintained at 25 °C, 16 hrs light cycle and 70 % relative humidity. At regular intervals, the petriplates and roll towels were moistened with water and 13 mM salt (NaCl) water. The rate of germination of recorded continuously for eight days.

2.1 ASSESSING SEED GERMINATION

We analyzed the following factors:

2.1.1 Germination percentage (GP): The germination rate was calculated as follows,

$$\textbf{GP (\%)} = \frac{\text{No. of seeds germinated}}{\text{No. of total seeds taken}} \text{ X } 100$$

2.1.2 Germination Index (GI):

$$\textbf{GI} = \sum \frac{Gt}{Tt} \text{ X } 100$$

Where, Gt represents the number of seeds germinated on day t and Tt represents the number of days [11] [(14)].

2.1.3 Mean Germination Time (MGT):

$$\textbf{MGT} = \sum \frac{Ti \times Ni}{Tt} \text{ X } 100$$

Where, Ni is the number of seeds that have just germinated at time Ti [15].

2.1.4 Seedling vigour index (SVI):

SVI = Mean germination percentage × (Mean shoot length + Mean root length) [15].

2.2 ASSESMENT OF SEEDLING GOWTH

At the end of eighth day, ten blackgram seedlings were randomly selected from all the replications of the treatment and its shoot length (SL) and root length (RL) was measured. Similarly, seedlings were selected randomly and its fresh weight (FW) was recorded using weighing balance. The seedlings were oven dried for 72 hours at 80°C and its dry weight (DW) was recorded.

2.3 STRESS TOLERANCE INDICES

Stress Susceptibility Index (SSI) and Stress Tolerance Index (STI) were calculated each genotype as follows [16,17],

$$\textbf{Stress Susceptibility Index (SSI)} = \frac{1-\frac{Ys}{Yp}}{\Sigma\, 1-\frac{\overline{Ys}}{\overline{Yp}}}$$

$$\textbf{Stress Tolerance Index (STI)} = \frac{Ys \times Yp}{\overline{Yp^2}}$$

In the formulas above, Yp and Ys stand for the mean seedling dry weight of a certain genotype under control and 130 mM NaCl-stress conditions, respectively. $(\overline{Yp})$ and $(\overline{Ys})$, respectively, represent the average seedling dry weights of all genotypes under control and 130Mm NaCl stress conditions.

2.4 STATISTICAL ANALYSIS

Data was arranged in a completely randomized design model with three replications as factorial experiment was used to statistically analyze each parameter. Specific pairwise differences between means were evaluated at the 0.05 significance level using the Fisher's least significant difference (LSD) test. The interrelationships between the recorded parameters were evaluated using Principle Component Analysis (PCA) and a cluster heatmap. Statistical tool R-Studio was used to conduct all tests and analysis

3. RESULTS AND DISCUSSION

3.1 Overall genotype and salinity effects

In this study, observations indicate that seed germination and seedling growth were found to be affected because of salt stress. The individual effects of genotype (ten genotypes) and treatments (control and 130mM NaCl), as well as the interaction effects between them were significant (Table 1). Salt stress (130mM NaCl) reduces all the germination parameters (Germination Percentage (GP) and Germination Index (GI)) and seedling growth parameters (Shoot length (SL), Root Length (RL), Seedling Vigour Index (SVI), Fresh Weight (FW), and seedling Dry Weight (DW). However, Mean Germination Time (MGT) between blackgram genotypes increased at the same time (Figure 1). However, the response blackgram towards salinity stress was found to be genotype-dependent, as shown on the PCA diagram (Figure 1), where the first ordination PCA axis shows salinity gradient. genotypes are arranged from the left (treatments with 130 mM NaCl) to the right side of the diagram (0 mM NaCl). The measured parameters' variance is explained by this axis in 86.2 percent of cases. 5.4 percent of the overall variance is accounted by the second PCA axis, which represents variations in 10 genotype responses and genotypes arranged from lower to upper.

Table 1. Analysis of variance (mean squares) for ten blackgram genotypes in control and 130mM NaCl salt treatment

Traits	Source of variation			
	G	T	G x T	Error
df	**9**	**1**	**9**	**40**
GP	125.07**	2509.07**	66.99*	30.400
MGT	0.7948**	16.3386**	0.182**	0.043
GI	19.43**	914.39**	14.080**	1.360
SVI	78360**	9155797**	61187**	6893
SL	1.22**	347.62**	2.120**	0.230
RL	1.481**	63.613**	1.334**	0.182
FW	0.00232**	0.390**	0.002**	0.0004
DW	0.00001515**	0.001**	0.000**	0.0000018

GP = Germination Percentage; GI = Germination Index; MGT = Mean Germination Time; SL = Shoot length of seedling; RL = Root length of seedling; SVI = Seedling vigour index; FW = Fresh weight of seedling; DW = Dry weight of seedling; G = Blackgram genotypes; T= 130Mm NaCl salinity; df = degrees of freedom; Error = within group variance; * = $p < 0.05$; ** = $p < 0.01$

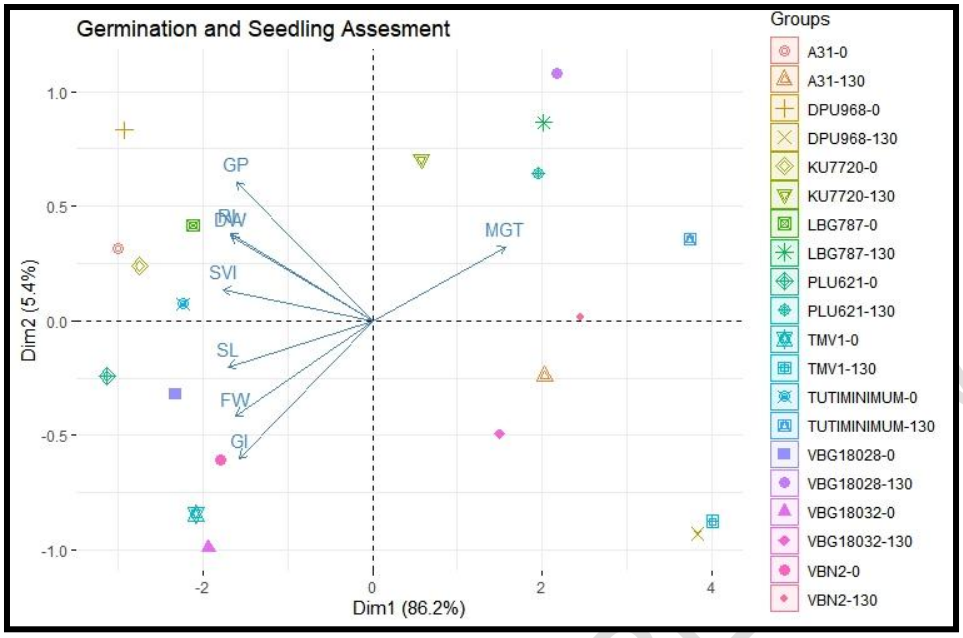

Figure 1: Principle Component Analysis Biplot (Standardized data) for ten blackgram genotypes in control and 130mM NaCl salt treatment.
GP = Germination Percentage; GI = Germination Index; MGT = Mean Germination Time; SL = Shoot length of seedling; RL = Root length of seedling; SVI = Seedling vigour index; FW = Fresh weight of seedling; DW = Dry weight of seedling; A31-0 and A31-130 - A31 denotes genotype, 0 denotes - Salinity level (0 mM NaCl) while, 130 denotes - Salinity level (130 mM NaCl).

3.2 GERMINATION ASSESSMENT:

3.2.1 Germination Percentage (GP):

The germination process is a crucial and significant stage in plant's life cycle and in different crop species this phase is known to be most vulnerable to stresses especially salinity [18]. The present study's findings revealed that the mean genotype germination percentage was 94.67 percent in the control and it was 81.73 percent when the blackgram genotypes were evaluated in presence of 130 mM NaCl. This results in 14.22 percent reduction in salinity stress compared to the control (130 mM NaCl) (Table 2). Low hormones level, an imbalance in ionic and water absorption, interferes with the effective use of food reserves and regular metabolism and these perturbations reduce the germination percentage under saline conditions [11]. Several reports indicates that genotypes that maintain a greater germination percentage or a lower GP reduction level are considered as salt tolerant and these genotypes produce more biomass and yield when grown in saline environments [7 and 19]. Several reports indicate that genotype maintain a greater germination percentage or a lower GP reduction level are considered as salt tolerant and these genotypes produce more biomass and yield when grown in saline environments (Figure 2). These observations are in agreement with the findings of Mensah and Ihenyen., 2009 in black gram [20], Naher and Alam., 2010 in greengram [21], El Sabagh *et al*., 2015 in soybean [22] and Hasan *et al.,* 2017 in blackgram [19].

3.2.3 Mean Germination Time (MGT):

Results clearly indicate that the Mean Germination Time (MGT) was increased by 39.01 percent compared to the control, with the mean genotype - MGT being 2.75 in the control and 3.79 in presence of 130 mM NaCl stress condition (Table 2 and Figure 2). Decreased osmotic potential of germination media, slows down the rate of water absorption by the germinating blackgram seedling and thus delays and reduces the germination process thereby increasing the germination time under high salinity stress conditions [23 and 24]. Contrarily, high concentration of Na^+ ions in the medium induces ion toxicity and this situation negatively affects the enzymes activity [25]. These conditions, during seed germination induces alterations in the metabolism of nucleic acids and proteins [26], hormonal imbalance [27] and reduction in the seed reserves [28 and 29]. Salinity stress has increased MGT in all blackgram genotypes (Table 2). At 130 mM NaCl salt stress, compared to the control situation, highest difference in MGT was observed in the blackgram genotype PLU621 (78.57%) followed by Tutiminimum (77.78%), and the lowest rate of increase was seen in genotype A31 (40.63%) followed by DPU968 (55.69 %) (Table 2). These results are similar to that of Hasan *et al* 2018 and Priyadharshini *et al*., 2019 in blackgram [7 and 30].

Table 2. Germination Percentage and Mean Germination Time of ten blackgram genotypes in control and 130mM NaCl salt treatments (means).

S. No	Blackgram Genotypes	Germination Percentage			Mean Germination Time		
		Control	Salt stress (130mM NaCl)	Percent Change	Control	Salt stress (130mM NaCl)	Percent Change
1	A31	96.00	86.67	-9.72	2.56	3.24	26.56
2	DPU968	98.67	72.00	-27.03	3.28	4.392	33.90
3	KU7720	98.67	90.67	-8.10	2.48	3.672	48.06
4	LBG787	97.33	84.00	-13.70	3.12	3.716	19.10
5	PLU621	97.33	89.33	-8.22	2.24	3.5	56.25
6	TMV1	88.00	70.67	-19.70	2.72	4.176	53.53
7	TUTIMINIMUM	97.33	76.00	-21.92	2.88	4.508	56.53
8	VBG18028	93.33	85.33	-8.57	3.08	3.78	22.73
9	VBG18032	90.67	81.33	-10.30	2.24	3.24	44.64
10	VBN2	89.33	81.33	-14.93	2.88	3.708	28.75
	Mean	**94.67**	**81.73**	**-14.22**	**2.75**	**3.79**	**39.01**
		G	**T**	**G x T**	**G**	**T**	**G x T**
	SEd	3.183	1.424	4.502	0.119	0.053	0.168
	CD ($p<0.05$)	7.200	18.094	10.184	0.269	0.673	0.380

G = Blackgram genotypes; T = 130mM NaCl salt treatment; G x T = Interaction between Genotypes and 130mM NaCl salt treatment; SEd = Standard Error Difference; CD = Critical Difference.

3.2.3 Germination Index (GI):

According to Dash and Panda (2001) [31] and Soltani *et al*., 2006 [32] salinity decreases germination index in blackgram and mung bean. The current study's findings also indicated that the salinity stress has reduced the GI by 30.86 percent compared to the control, with the mean genotype-averaged germination index being 21.66 percent in the control and 13.85 percent under salt stress conditions (Table 3). In our experiment, the lowering of germination index under salinity stress is consistent with these findings of Hasan *et al* 2018 and Priyadharshini *et al*., 2019 in blackgram [7 and 30]. According to Kandil *et al.,* 2012 [33], salinity stress increases the dormancy in agricultural seeds, and this may be a mechanism used by plants to survive in highly salinized soils. In our investigation, under 130 mM NaCl treatment, blackgram genotype Tutiminumum (60.24%) has showed the greater decline in GI followed by VBG 18028 (51.31%), while the genotypes A31 (20.29 %). and DPU 968 (23.86 %) has showed lesser reduction in GI (Table 3 and Figure 2).

3.2.4 Seedling Vigour Index (SVI):

Salinity significantly decreased the seedling vigour index (SVI) of all blackgram genotypes, which measures the health of young plants (Table 3). The results of the current investigation indicated in all the genotypes studied, there was 47.35 percent reduction in SVI due to salinity stress as compared to the control. (Table 3 and Figure 2). Reduced SVI caused by the reduction in transfer of endosperm materials such as carbohydrates and proteins to seedlings. Reduced specific ion action with lower potentiality in ambient H_2O is another explanation for this phenomenon [34]. Under salt treatment, blackgram genotype DPU 968 (63.3%) showed the greatest reduction in SVI, followed by TMV 1 (59.79%) and genotype KU7720 showed the least decrease (30.5%) followed by genotype VBG18032 (32.23%) (Table 3).

Table 3. Germination Index and Seedling Vigour Index of ten blackgram genotypes in control and 130mM NaCl salt treatments (means).

S. No	Blackgram Genotypes	Germination Index			Seedling Vigour index		
		Control	Salt stress (130mM NaCl)	Percent Change	Control	Salt stress (130mM NaCl)	Percent Change
1	A31	21.833	17.417	-20.23	1727.56	1559.61	-53.41
2	DPU968	22.000	16.750	-23.86	1802.72	1315.50	-63.30
3	KU7720	22.333	17.310	-28.98	1768.90	1625.47	-30.50
4	LBG787	20.500	11.495	-43.93	1678.30	1448.40	-43.02
5	PLU621	23.500	13.833	-41.13	1687.76	1549.04	-42.72
6	TMV1	20.500	13.283	-35.20	1547.33	1242.56	-59.79
7	TUTIMINIMUM	21.167	8.417	-60.24	1594.32	1244.88	-54.67
8	VBG18028	24.500	11.929	-51.31	1663.03	1520.48	-42.37
9	VBG18032	23.500	17.000	-27.66	1460.97	1310.58	-32.23
10	VBN2	21.750	16.086	-26.04	1552.17	1413.17	-51.50
	Mean	**21.66**	**13.85**	**-35.86**	**1648.31**	**1422.97**	**-47.35**
		G	**T**	**G x T**	**G**	**T**	**G x T**
	SEd	0.673	0.301	0.952	47.935	21.437	67.790
	CD ($p<0.05$)	1.522	3.825	2.154	108.437	272.383	153.352

G = Blackgram genotypes; T = 130mM NaCl salt treatment; G x T = Interaction between Genotypes and 130mM NaCl salt treatment; SEd = Standard Error Difference; CD = Critical Difference

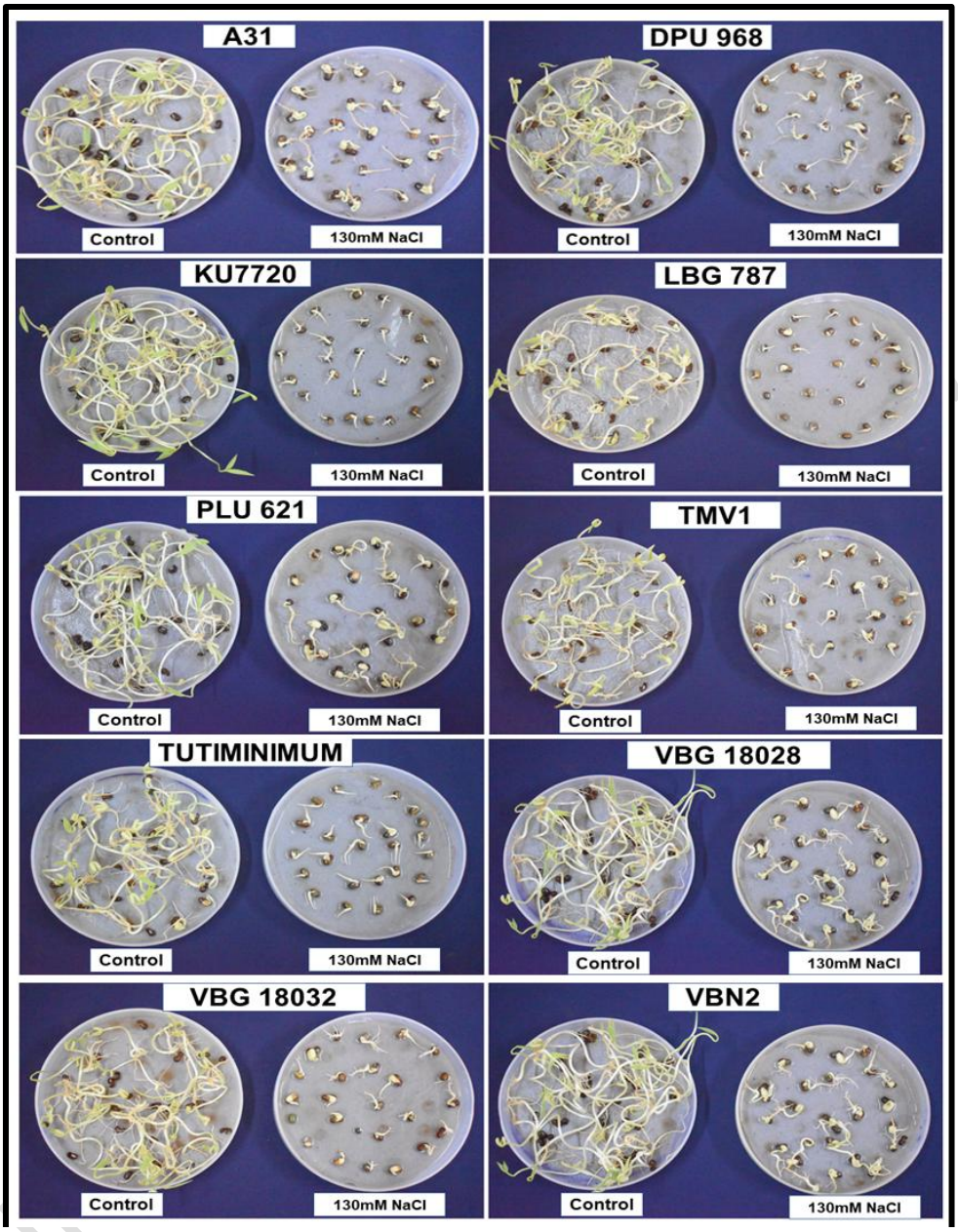

Figure 2: Germination assessment of blackgram genotypes in control and 130mM NaCl salt stress

3.3 SEEDLING GROWTH ASSESSMENT:

3.3.1 Shoot Length (SL):

According to Karim *et al*., 1992) [35], the seedling stage is more susceptible to salt stress, than its germination, and the shoot length of the seedling is more severely affected than root length. Salinity has significantly reduced SL of all the blackgram genotypes studied (Table 4 and Figure 3). The current findings also indicated that, under control conditions, the

genotype's mean shoot length was 9.76 cm (Table 3), while it was 4.96 cm under saline stress conditions. This means that, in comparison to non-stress conditions, there was a 48.87 percent reduction in shoot growth under salt stress (130mM NaCl) conditions. Osmotic stress was the key factor causing reduction in shoot length under salt stress conditions. As a result, the plant had to spend additional energy to exhibit diverse measures in order to reduce the effect of osmotic pressure. As a result, energy produced by plants was diverted to stabilize the osmotic pressure, which in turn will compromise the other growth activities [36]. Under non stress conditions, highest reduction was seen in A31 (58.65%), followed by VBG 18028 (55.96%), while lower percent reduction was observed in the genotypes KU7720 (11.78%) and VBG18032 (33.96%) respectively (Table 4).

3.3.1 Root Length (RL):

Roots are crucial for salt stress tolerance because they are in direct contact with saline medium and shoots are involved in ascent of sap [23]. Salinity considerably decreased the RL of the blackgram genotypes tested (Table 4 and Figure 3). Results indicate that the blackgram genotype's mean root length in the control group was 7.75 cm (Table 4), whereas it was only 5.69 cm when the genotypes are grown in presence of 130 mM NaCl, indicating a 48.87 percent reduction in presence of salinity stress as compared to control one (130mM NaCl). This reduction is consistent with earlier studies in blackgram [19,33 and 37]. Under non stress conditions, highest reduction was seen in DPU 968 (43.63%), followed by TMV1 (40.05%), while lower percent reduction was observed in the genotypes VBG18028 (11.78%) and VBG18032 (27.97%) respectively (Table 4).

Table 4. Shoot length and root length of blackgram genotypes in control and 130mM NaCl salt treatments (means).

S. No	Blackgram Genotypes	Shoot Length (cm)			Root Length (cm)		
		Control	Salt stress (130mM NaCl)	Percent Change	Control	Salt stress (130mM NaCl)	Percent Change
1	A31	9.65	3.40	-58.65	8.34	5.46	-34.47
2	DPU968	9.62	4.15	-55.16	8.65	4.87	-43.63
3	KU7720	9.34	6.72	-27.97	8.59	6.83	-20.45
4	LBG787	9.88	5.48	-44.46	7.36	5.90	-19.91
5	PLU621	9.50	4.88	-48.54	7.84	5.93	-24.32
6	TMV1	9.97	4.24	-57.46	7.61	4.56	-40.05
7	TUTIMINIMUM	9.12	4.49	-50.75	7.26	5.02	-30.90
8	VBG18028	10.15	4.47	-55.96	7.66	6.76	-11.78
9	VBG18032	9.20	6.07	-33.96	6.91	6.09	-11.79
10	VBN2	10.12	4.47	-55.79	7.25	5.43	-25.12
	Mean	**9.66**	**4.84**	**-48.87**	**7.75**	**5.69**	**-26.24**
		G	**T**	**G x T**	**G**	**T**	**G x T**
	SEd	0.277	0.124	0.392	0.246	0.110	0.348
	CD ($p<0.05$)	0.627	1.576	0.887	0.556	1.398	0.787

G = Blackgram genotypes; T = 130mM NaCl salt treatment; G x T = Interaction between Genotypes and 130mM NaCl salt treatment; SEd = Standard Error Difference; CD = Critical Difference

3.3.1 Fresh Weight (FW):

Biomass is an excellent indicator to study the variations and degree of salinity stress tolerance and comparison to salt sensitive and medium tolerant blackgram varieties, the shoot biomass of the tolerant varieties was least affected by salinity [38 and 39]. Salinity stress has significantly decreased the blackgram genotypes fresh weight (FW) (Table 5 and Figure 3). Result of the current study reveals that the salinity stress has reduced the FW by 40.87 percent as compared to control, which had the mean fresh weight of 0.39g (Table 5) as opposed to 0.23g under saline conditions (130mM NaCl). Salinity has significant impact on plant growth because of lower osmotic potential and altered nutrient absorption. Reduced water uptake by plants under stress led to physiological drought stress which negative impact on plants growth and development. Under stressful environment conditions, reduced rate of photosynthesis, stem growth and leaf expansion occur over time at slow pace even under stressful environment conditions. These factors along with reduced rate of stomatal conductance, relative water content, transpiration, results in reduced fresh weight [40 and 41]. In our investigation, genotypes, TMV1 (47.62%) and PLU621 (46.61%) showed substantial reductions in gain in FW in the presence of 130 mM NaCl as compared to the control, while the genotypes VBG18032 (30.94%) and KU7720 (33.83 %) showed the least reduction in the FW gain under similar conditions (Table 5).

3.2.1 Dry Weight (DW):

In the plant life cycle, dry matter production is recognized as a valuable measure for resource utilization and acquisition by plants [42]. Salinity stress has significantly reduced the DW of the Blackgram genotypes used in this study (Table 5). According to the observations of our study, salinity stress (130 mM NaCl) has reduced the DW accumulation by 35.48 percent as compared to that of control, with a mean dry weight of 0.0253g and 0.0162g under control and 130 mM NaCl stress conditions respectively (Table 5 & Figure 3). Baber *et al*., (2014) [43] observed a reduction in biomass accumulation when fenugreek plants were raised under salt stress, explaining that under high salinity, reductions in water imbibition occur due to changes in substrate-solute potential, and this process caused altered metabolism, which further reduced the plant growth and development. Salinity has significantly lowered the DW of the blackgram genotypes studied (Table 5). Under salinity, maximum decrease was observed in blackgram genotype DPU968 (57.69%), followed by TMV1 (48.39%), while the lowest reduction was observed in the genotype KU7720 (12.09%) followed by LBG787 (21.34%) (Table 5).

Table 5. Seedling fresh and dry weight of blackgram genotypes in control and 130mM NaCl salt treatments (means).

S.No	Blackgram Genotypes	Fresh Weight (g)			Dry Weight (g)		
		Control	Salt stress (130mM NaCl)	Percent Change	Control	Salt stress (130mM NaCl)	Percent Change
1	A31	0.39	0.25	-36.37	0.029	0.016	-44.79
2	DPU968	0.39	0.21	-46.59	0.029	0.012	-57.69
3	KU7720	0.36	0.24	-33.83	0.023	0.020	-12.09
4	LBG787	0.37	0.23	-39.12	0.026	0.020	-21.34
5	PLU621	0.41	0.22	-46.61	0.027	0.016	-40.37
6	TMV1	0.46	0.24	-47.62	0.023	0.012	-48.39
7	TUTIMINIMUM	0.41	0.26	-37.22	0.026	0.016	-38.65
8	VBG18028	0.37	0.20	-44.52	0.025	0.016	-34.38
9	VBG18032	0.37	0.26	-30.94	0.023	0.015	-33.41
10	VBN2	0.37	0.20	-45.84	0.024	0.018	-23.69
	Mean	**0.39**	**0.23**	**-40.87**	**0.0253**	**0.0162**	**-35.48**
		G	**T**	**G x T**	**G**	**T**	**G x T**
	SEd	0.012	0.005	0.016	0.001	0.001	0.001
	CD ($p<0.05$)	0.027	0.064	0.036	0.002	0.013	0.002

G = Blackgram genotypes; T = 130mM NaCl salt treatment; G x T = Interaction between Genotypes and 130mM NaCl salt treatment; SEd = Standard Error Difference; CD = Critical Difference

Figure 3: Seedling growth of blackgram genotypes in control (0 mM NaCl) and salt stress (130mM NaCl)

3.4 STRESS TOLERANT INDICES

In order to evaluate salt tolerance, we used the Stress Susceptibility Index (SSI) and Stress Tolerance Index (STI) in accordance with quantitative standards suggested for genotype selection based on yield performances under non-stress (control) and 130 mM NaCl (Salinity stress) conditions. By comparing the rate of change in yield to stress with the control conditions we may identify the stress tolerant genotypes, which results in improved yield stability [17]. According to Fernandez *et al*., 1992) [17], STI will help to identify genotypes with a high production potential and tolerance towards stresses. Actually, greater STI reflects maximum yield and stress tolerance. Others have demonstrated that STI is more effective than SSI in differentiating the genotypes that have higher production potential across various conditions [17 and 44]. However, SSI enables the selection of blackgram genotypes with more consistent yield under salinity-related conditions (lower changes). Actually, genotypes with lower values for SSI will have a lower yield differential between the stress and control conditions, which indicates stronger yield stability. In Table 6, the SSI and STI tolerance indices are given. The results show that under salinity stress, blackgram genotype DPU 968 (1.599) and TMV1 (1.341) had the higher SSI values compared to other genotypes, while the genotype KU7720 (0.335) and LBG 787 (0.592) had the lower SSI values compared to other genotypes (Table 6). On the other hand, STI index values were highest for the genotype KU7720 (0.722), followed by VBG 18032 (0.831), and it was lowest for the genotype TMV1 (0.425), then LBG 781 (0.541). So, genotype KU7720and LBG 787 are higher production potential and consistent yield under 130mM NaCl salt stress condition based on STI and SSI respectively.

Table 6. Tolerance indices of blackgram genotypes in control and 130mM NaCl salt treatments.

S.No	Blackgram Genotypes	SSI	STI
1	A31	1.241	0.706
2	DPU968	1.599	0.541
3	KU7720	0.335	0.722
4	LBG787	0.592	0.831
5	PLU621	1.119	0.662
6	TMV1	1.341	0.425
7	TUTIMINIMUM	1.071	0.640
8	VBG18028	0.953	0.646
9	VBG18032	0.926	0.559
10	VBN2	0.657	0.657
	Mean	**0.983**	**0.639**

3.5 CLUSTER HEATMAP ANALYSIS

Due to difficulties in deciphering the statistical significance of variations, it is challenging to examine the salinity tolerance for all ten tested genotypes based on individual criteria (Tables 2,3,4 and 5). All the parameters were compared among themselves by cluster heat map analysis. In this analysis, all the ten blackgram genotypes were grouped in to two clusters i.e., as Control and Salinity Stress. Within the control cluster, two major and within four minor clusters were formed. Genetic differences, particularly in MTG and all growth factors are responsible for these groupings. It has been attributed that these variances are due to genotypic difference among each genotype used in this study [45].

Similarly with respect to genotypes under salinity stress cluster, two major clusters are formed. Two minor clusters were formed in first major clusters. The minor clusters are Cluster1: Tutiminumum, Cluster 2: DPU 968, and TMV1; Cluster 2: VBTG 18032 and A31. Four minor clusters were formed in second major clusters. The minor clusters are Cluster 1: VBG 18032, Cluster 2: A31 AND VBN2, Cluster 3: KU7720 and Cluster 4: LBG 787, PLU 621 and VBG 18028 (Figure 4). These findings allow us to classify the blackgram genotype Tutiminimum along with the DPU 968 and TMV 1 which are considered to be sensitive for salinity stress. On the other hand, addition to LBG 787 and KU 7720, we may also consider PLU 621 and VBG 18028 as salt-tolerant genotype because of cluster together for all measured parameters. (Figure 4).

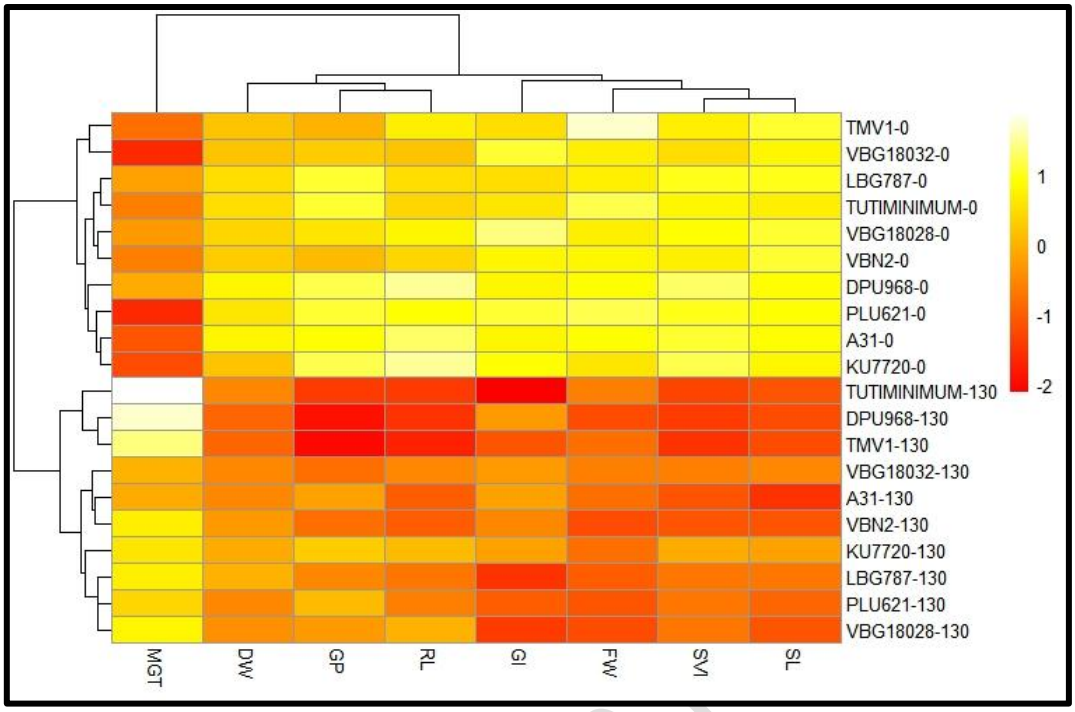

Figure 4. Cluster heat map (Standardized data) of blackgram genotypes in control and 130mM NaCl salinity conditions. GP = Germination Percentage; GI = Germination Index; MGT = Mean Germination Time; SL = Shoot length of seedling; RL = Root length of seedling; SVI = Seedling vigour index; FW = Fresh weight of seedling; DW = Dry weight of seedling; A31-0, A31-130 - A31 denotes genotype, 0 denotes - Salinity level (0 mM NaCl) while, 130 denotes - Salinity level (130 mM NaCl).

4. CONCLUSION

In the current work, we demonstrated the presence of substantial differences among the blackgram genotypes against salinity stress both at germination and seedling phases. These genetic variations are an excellent starting point for evolving blackgram genotypes that can be yielded well under salt-affected locations, and this can be used to improve the salt tolerance in various crop breeding program. Finally, it can be concluded that compared to other blackgram genotypes tested, the genotypes, LBG 787, KU 7720, PLU 621, and VBG 18028 found to possess a higher tolerance towards salinity stress.

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