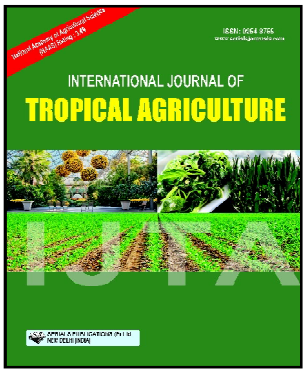

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Screening of Mungbean Genotypes for Salinity Stress During Germination and Early Seedling Growth

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Abstract: Reproducible and reliable evaluation methods are the basis of any successful breeding programme especially for abiotic breeding. In this study, For this study eight mungbean genotypes were used. They were ADT 2, BM-2002-1, ML 5, AKM 4, Vishal, Vamban, TARM 1 and Utkarsh. The genotypes were collected based on their geographical and ecological adaptations to semi-arid and semi-humid regions of India. The experiment was caried out in SST laboratory to screen best performing accessions to assess the salt tolerance of mungbean at germination and seedling growth with three replications and four salt treatments (0, 2, 4 and 6 dSm^{-1}). Among the genotypes under investigation, the genotypes Utkarsh, TARM 1, Vamban and Vishal were found to express tolerance and the remaing enetypes were sensitive for various seedling characters.

Keywords: Mungbean, Salinity, Seedling Characters.

INTRODUCTION

Pulses are rich in proteins, popularly known as "Poor man's meat" and "rich man's vegetable", contributing significantly to the nutritional security of the country serving as a main source of the essential component of nutrition, particularly for the predominant vegetarian population of India and adjacent countries [1]. India is the largest pulse producer, accounting for 25 per cent of world's pulses production. Among various pulse crops, chickpea dominates (> 40 % share) of total pulse production followed by pigeon pea (18-20 %), mungbean (11 %), urdbean (10-12 %), lentil (8-9%) and other legumes (20%). Mungbean is an important pulse crop due to its widespread consumption throughout the Indian subcontinent.

Salinity has been a threat to agriculture in some parts of the world for over 3000 years; in recent times, the threat has grown" As the world population continues to increase, more food needs to be grown to feed the people. This can be achieved by an increase in cultivated land and by an increase in crop productivity per area. The former has brought agriculture to marginal, salt-affected lands. Moreover, salinity problem has been aggravated by surface irrigation in arid and semi-arid environments. Salt stress was found to reduce seed germination, fresh and dry biomass, shoot and root length and yield attributes of mungbean. Salt tolerance is a polygenic, genotype dependent and developmental stage-specific phenomenon, therefore, tolerance at initial developmental stage may not be correlated with tolerance at later developmental stages. It also comprises multifaceted responses at molecular, physiological and plant canopy levels. Because of this complex nature of salinity stress and the lack of appropriate techniques for introgression, little progress has been made in identifying and developing salt tolerant mungbean varieties over years [2 and 3].

MATERIALS AND METHODS

The experiment was conducted in the Seed Science and Technology (SST) Laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture Annamalai University, Annamalai Nagar, Tamil Nadu. For this study eight mungbean genotypes were used. They were ADT 2, BM-2002-1, ML 5, AKM 4, Vishal, Vamban, TARM 1 and Utkarsh. The genotypes were collected based on their geographical and ecological adaptations to semi-arid and semi-humid regions of India.

The design of the study involved laboratory experiments. The laboratory experiment was made to screen best performing accessions to assess the salt tolerance of mungbean at germination and seedling growth using high grade germination paper (Pleated method). The design of the experiment was Randomized Block Design (RBD) with three replications and four salt treatments (0, 2, 4 and 6 dsm) for each treatment and control.

In order to screen the different genotypes, five seed of each genotype was sown to 100 ml paper cups containing nutrient medium (control) and nutrient medium supplemented with different levels of NaCl, each in triplicate. The NaCl solutions of different molarity (0, 2, 4 and 6 dsm) were prepared in the nutrient medium to represent different salinity levels [4]. These were kept at room temperature. Fifteen days later the following observations was measured.

Germination percentage (GP)

The germination percentage was calculated using the following formula

$$Germination\ Percentage = \frac{No.\ of\ seeds\ germinated}{Total\ number\ of\ seed\ sown} \times 100$$

Shoot length (SL)

SSL was measured in cm from the seed to the tip of the leaf blade of five randomly selected seedlings using draftsman ruler.

Root length (RL)

It was measured in cm from the seed to the tip of the root of five randomly selected seedlings using draftsman ruler.

Shoot fresh weight (SFW)

It was measured in grams by weighing the mass of shoots of five randomly selected seedlings in gram using sensitive balance and recording the average.

Root fresh weight (RFW)

It was measured in grams by weighing the mass of roots of five randomly selected seedlings for RFW using sensitive balance and recording the average.

Seedling shoot dry weight (SSDW)

It was measured in grams using sensitive balance after oven-drying the shoots of the five seedlings selected for SFW at 70°C for 48 hours and the average data was recorded.

Seedling root dry weight (SRDW)

It was measured in grams by oven-drying the roots of the five seedlings picked for RFW at 70°C for 48 hours and weighing them using sensitive balance and the average data was recorded.

RESULTS AND DISCUSSION

The ANOVA for the seedling characters was studied at the various salinity levels *viz*., Control, 2 dS m^{-1}, 4 dS m^{-1} and 6 dS m^{-1}. The 't' values were highly significant for all the seedling characters studied.

Germination percentage

Increase in NaCl concentration reduced germination percentage and significantly decreased percent seed germination. Low germination was recorded in 6 dsm^{-1} NaCl treatment for BM-2002-1 and ML 5 accessions. The results obtained from the germination studies showed that the ten accessions responded differently to the different levels of salinity. As the concentration increased, there was corresponding decrease in germination percentage. Among the salinity levels, the germination percentage ranged from 96.67 (Vishal and TARM 1 at control) to 36.37 (BM-2002-1 at 6 dSm^{-1}). Among the genotypes, Vishal, TARM 1 and ADT 2 showed consistent germination percentage across all the salinity levels. Their mean values were greater or equal to the grand mean values. The present result is in line with the findings of Swarnakar [5]; Keshtiban [6] and Nair [7] who reported salt stress decreases germination percent. Sunil Kumar [3] and Naher and Alam [8] also reported that highest germination percent was observed in the control of all accessions compared with different treatment in mungbean. The present study was also similar to the work of Pandiya [9] who reported that germination percent of mungbean was reduced with increasing salinity. The present study also agrees with the finding of Fery [10] who reported increase in salinity inhibits germination percentage of mungbean (Table 1).

Table 1

Effect of salinity (NaCl) stress on seed germination among various genotypes

Sl. No.	*Genotypes*	*Germination (%)*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
G1	**ADT 2**	86.67	83.33	73.33	66.67**
G2	**BM-2002-1**	93.33	83.33	63.33	36.67
G3	**ML 5**	93.33	83.33	63.33	43.33
G4	**AKM 4**	93.33	83.33	76.67**	53.33
G5	**VISHAL**	96.67	93.33**	83.33**	73.33**
G6	**Vamban**	93.33	83.33	66.67	53.33
G7	**TARM 1**	96.67	86.67	76.67**	73.33**
G8	**Utkarsh**	93.33	83.33	73.33	66.67**
Grand Mean		93.33	85.00	72.08	58.33
SE	1.74	1.40	0.95	0.76	
CV (%)	3.23	2.82	2.28	2.25	
CD (P=0.05)		5.30	4.22	2.88	2.30
CD (P=0.01		7.35	5.85	4.10	3.19

Values are an average mean of 3 replications + standard deviation following 11 days of exposure to different NaCl solutions.

Analysis of variance for germination percentage

Source	df	MSS			
		Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}
Replication	2	2.62	1.05	0.56	0.11
Genotype	7	25.57**	151.82**	38.11**	571.49**
Error	14	9.12	2.70	5.78	1.72

**Significant at P<0.05

Shoot length (cm)

All the genotypes responded differently to salt stress with respect to mean seedling shoot length. The seedling shoot length ranged from 19.40 (BM-2002-1) to 21.45 cm (Vamban) under control; 15.42 (ML 5) to 20.25 cm (TARM 1) at 2 dSm^{-1}; 9.32 (AKM 4) to 18.50 cm (TARM 1) at 4 dSm^{-1} and from 1.72 (ML 5) to 8.45 cm (TARM 1) at 6 dSm^{-1}, respectively. It was observed that for the genotype TARM 1 had superior shoot length and ML 5 was drastically affected with an increase in salinity levels. It is an established fact that salt tolerant accessions have a higher mean seedling shoot length under saline environment than sensitive accessions in mungbean [6, 11, 12, 13]. Naseer [14] reported that the reduction in SL may be due to excessive accumulation of salt in the cell wall, which modifies the metabolic activities and limits the cell wall elasticity. Further, secondary cell wall appears sooner, and cell wall becomes rigid, because of which the turgor pressure efficiency in cell enlargement decreases. The possible reason for the reduced shoot development could also be due to the toxic effects of the NaCl used as well as the unbalanced nutrient uptake by the seedlings. The present findings are parallel to those of Sunil Kumar [3] in mungbean, Jamil [15] on vegetables species, Kandil [16] on chickpea and Rahman [17] on wheat. The finding of the present study indicated that as salinity level increases from 4 dS/m to 12 dS/m, the mean SL was reduced highly in all mungbean accessions studied. Similar outcomes were reported by El-Hendawy [18]; Khajeh-Hosseini [19] in soybean and wheat, respectively (Table 2).

Table 2

Effect of salinity (NaCl) stress for shoot length and root length among various genotypes

Sl. No.	Genotypes	Shoot length (cm)				Root length (cm)			
		Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}	Control	2 dSm^{-1}	4 dSm^{-1}	6 dSm^{-1}
G1	**ADT 2**	21.29*	18.44	15.51**	11.29**	13.73**	11.10*	8.80**	6.42**
G2	**BM-2002-1**	19.40	16.33	11.27	7.43	12.24	10.69	5.52	2.65
G3	**ML 5**	19.55	15.42	11.37	6.36	12.06	10.73	5.76	1.72
G4	**AKM 4**	20.43	15.56	9.32	5.57	12.08	8.30	5.18	2.14
G5	**VISHAL**	20.36	19.14**	17.19**	12.63**	11.76	10.06	8.84**	6.92**
G6	**Vamban**	21.45*	18.53	14.28	10.27	12.76	10.76	8.85**	6.57**
G7	**TARM 1**	21.40*	20.25**	18.50**	14.38**	13.26*	12.08**	10.03**	8.45**
G8	**Utkarsh**	20.54	18.46	14.36	10.27	13.47*	11.52**	8.58*	6.77**
Grand Mean		*20.55*	*17.77*	*13.98*	*9.78*	*12.67*	*10.66*	*7.70*	*5.21*
SE		0.37	0.28	0.18	0.18	0.20	0.14	0.13	0.06
CV (%)		3.14	2.70	2.26	3.27	2.68	2.20	2.74	1.83
CD (P=0.05)		1.13	0.84	0.55	0.56	0.60	0.41	0.39	0.17
CD (P=0.01		1.58	1.17	0.77	0.78	0.83	0.57	0.54	0.23

Values are an average of 3 replications 15 days after initiation of the salinity stress.

Analysis of variance for germination percentage

Source	*df*	*MSS*							
		Shoot length				*Root length*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*	*Control*	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
Replication	2	0.38	0.13	0.13	0.02	0.09	0.07	0.06	0.01
Genotype	7	1.77**	29.64*	9.45*	28.73**	1.65**	7.68*	3.81**	20.28**
Error	14	0.42	0.10	0.23	0.10	0.12	0.05	0.06	0.01

** Significant at P<0.05

Root length (cm)

Significant differences between genotype, treatments and their interaction on seedling root length. The highest RL (13.73 cm) was found in the control and the lowest (1.72 cm) in 6 dSm^{-1} salinity level. The genotypes *viz*., TARM 1, Vishal, Utkarsh, Vamban and ADT 2 had relatively higher values of 8.54, 6.92, 6.77, 6.57 and 6.42 cm of SRL, respectively. Generally, increasing salinity levels to 2, 4 and 6 dSm^{-1} NaCl significantly reduced shoot root length when compared with the control treatment. The present study is in line with Keshtiban [6] and Shitole and Dhumal [20] who reported that seedling root length was reduced with increasing NaCl concentration in mungbean. The results of the study are also in agreement with the observations of Hakim [13] in rice. The reduction in root lengths may be due to decreased physiological activities resulting from water and nutrient stress occurring under high salinity stress [21]. In this study, with the increment of salt concentration, the root length gradually decreased in all accessions of mungbean, but the effect of the treatments varied from one genotype to other genotypes. Therefore, different genotypes responded differently to different salinity levels (Table 2).

Shoot fresh weight (g)

The mean shoot fresh weight ranged from 0.42 to 1.28 in control, 0.31 to 1.24 in 2 dSm^{-1}, 0.13 to 1.17 in 4 dS m^{-1} and 0.08 to 1.12 in 6 dSm^{-1} salt concentrations. The genotypes TARM 1 (1.12 g) and Vishal (0.93) were significant and had higher values than the grand mean value, whereas AKM 4 and ML 5 achieved low value of SSFW with relatively higher reduction from that of control.

Generally Increasing salinity levels to 2, 4 and 6 dSm^{-1} NaCl significantly reduced shoot fresh weight when compared with the control treatment. It seems that reduction in SFW may be due to decreasing water uptake by seedling under salinity. Salt decreases the osmotic water potential, creating a water stress in seedlings. Reduction SFW in mungbean under salt stress may be resulted from combination of ions toxicity and altered water relation that caused large accumulation of sodium and magnesium ions and reduced calcium and potassium concentration, transpiration, stomatal conductance and hydraulic conductance decreased as salinity increased [15]. The present findings were in agreement with Noreen [22] who indicated that there was a rapid decrease in SSFW of leguminous plants under saline conditions (Table 3).

Root fresh weight (g)

As the salinity level increased, the root fresh weight (RFW) was gradually reduced. The mean RFW ranged from 0.37 - 0.56 gram in control, 0.21 - 0.48 gram in 2 d m^{-1}, 0.21 - 0.48 gram in 4 dS m^{-1} and 0.04 – 0.23 gram in 6 dSm^{-1}. At control, Vishal (0.56 gram) had the highest value of SRFW. Similarly, at 2 dSm^{-1} of NaCl also Vishal achieved the highest mean of RFW with reduction compared to the control

Table 3
Effect of salinity (NaCl) stress for shoot and root fresh weight among various genotypes

Sl. No.	*Genotypes*	*Shoot fresh weight (g)*				*Root fresh weight (g)*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*	*Control*	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
G1	**ADT 2**	1.23**	0.96	0.79	0.69	0.41	0.33	0.21	0.10
G2	**BM-2002-1**	1.23**	1.12	0.49	0.17	0.37	0.21	0.09	0.04
G3	**ML 5**	0.42	0.31	0.19	0.09	0.49	0.41**	0.30**	0.09
G4	**AKM 4**	0.47	0.31	0.13	0.08	0.53	0.42**	0.21	0.11
G5	**VISHAL**	1.24**	1.19*	1.14**	0.93**	0.56**	0.48**	0.33**	0.20**
G6	**Vamban**	1.19**	1.17	1.12**	0.71	0.41	0.32	0.13	0.10
G7	**TARM 1**	1.26**	1.24*	1.17**	1.12**	0.43	0.38*	0.30**	0.23**
G8	**Utkarsh**	1.28**	1.23*	1.15**	0.63	0.38	0.32	0.19	0.13
Grand Mean		1.04	0.94	0.77	0.55	0.45	0.36	0.22	0.13
SE		0.02	0.75	0.05	0.07	0.02	0.01	0.00	0.01
CV (%)		2.51	13.24	10.35	20.23	8.07	2.83	2.51	4.28
CD (P=0.05)		0.05	0.23	0.15	0.20	0.07	0.02	0.01	0.01
CD (P=0.01		0.06	0.32	0.21	0.28	0.09	0.02	0.01	0.01

Values are an average of 3 replications 15 days after initiation of the salinity stress.

Analysis of variance for shoot and root fresh weight

Source	*df*	*MSS*							
		Shoot fresh weight				*Root fresh weight*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*	*Control*	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
Replication	2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Genotype	7	0.41**	0.64**	0.53**	0.50**	0.01**	0.02**	0.02**	0.01**
Error	14	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01

**Significant at P<0.05

while accession BM-2002-1 had the lowest value with 56.67 % reduction. At 4 dSm^{-1} salinity level, accession Vishal (0.33 gram) followed by TARM 1 (0.30 gram) and ML 5 (0.30 gram) performed well compared to other genotypes, whereas the genotype BM-2001-1 performed poorly compared to the grand mean. At 6 dS m^{-1} salinity level, the genotype TARM 1 (0.23 gram) and Vishal (0.20 gram) had relatively higher values while, the genotypes BM-2002-1 and ML 5 had lower value of 0.04 and 0.09 gram, respectively. Furthermore, increasing salinity levels to 2,4 and 6 dSm^{-1} NaCl significantly reduced SRFW when compared with the control treatment.

It has been found that the decrease in seedling fresh and dry weights was due to the restricted provider of metabolites to younger growing organs, since metabolic performance was considerably disturbed at higher levels of salinity and also due to the low water absorption and toxicity of NaCl. Armin [23] in watermelon reported that the seedling growth was ceased by increase in the NaCl levels. The roots were more sensitive to salt stress than the

shoots which might be because of the direct contact of root with soil that results in the accumulation of higher salt ions in the root cells [15] (Table 3).

Shoot dry weight (g)

The reduction in seedling shoot dry weight (SDW) was significantly higher at higher NaCl concentration compared to the control. The outcome of the result indicated that as the concentration of NaCl level increased there was significant reduction of SSDW in all the genotypes. The genotypes ADT 2 is considered to be salt tolerant and TARM 1 as salt sensitive with respect to SDW reduction percentage comparing with control.

This result is in close conformity with the finding of Karimi [25] who claimed that increasing salinity levels remarkably decrease in SDW. This may be a result of a combination of osmotic and specific ion effect of Cl^- and Na+ [24]. The reduction in SDW could also be associated with reduced rate of leaf production, hence low number of leaves leading to reduced photosynthesis and accumulation of dry matter. Karimi [25] claimed that increased salinity levels caused remarkable decrease in SSDW. The present study is also in line with earlier studies in safflower by Ghazizade [26] reported that adding NaCl resulted in decrease in seedling shoot dry weight (Table 4).

Table 3
Effect of salinity (NaCl) stress for shoot and root dry weight among various genotypes

Sl. No.	*Genotypes*	*Shoot dry weight (g)*				*Root dry weight (g)*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*	*Control*	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
G1	**ADT 2**	0.31	0.14	0.09	0.05	0.21	0.15	0.09	0.04
G2	**BM-2002-1**	0.32	0.17	0.09	0.03	0.20	0.14	0.09	0.03
G3	**ML 5**	0.19	0.16	0.09	0.04	0.24	0.16	0.08	0.04
G4	**AKM 4**	0.39*	0.16	0.09	0.04	0.21	0.17	0.07	0.03
G5	**VISHAL**	0.43**	0.39**	0.32**	0.24**	0.31**	0.26**	0.24**	0.19**
G6	**Vamban**	0.46**	0.36**	0.33**	0.24**	0.25*	0.20**	0.18**	0.14**
G7	**TARM 1**	0.42**	0.40**	0.35**	0.30**	0.24	0.21**	0.18**	0.15**
G8	**Utkarsh**	0.44**	0.34**	0.27**	0.21**	0.28**	0.20**	0.17**	0.10*
Grand Mean		0.37	0.27	0.20	0.14	0.24	0.19	0.14	0.09
SE		0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01
CV (%)		3.10	3.80	3.62	3.99	2.64	2.99	4.38	3.93
CD (P=0.05)		0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01
CD (P=0.01		0.03	0.02	0.02	0.01	0.02	0.01	0.01	0.01

Values are an average of 3 replications 15 days after initiation of the salinity stress.

Analysis of variance for shoot and root dry weight

Source	*df*	*MSS*							
		Shoot dry weight				*Root dry weight*			
		Control	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*	*Control*	*2 dSm^{-1}*	*4 dSm^{-1}*	*6 dSm^{-1}*
Replication	2	1.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Genotype	7	0.03**	0.05**	0.04**	0.04**	0.01**	0.01**	0.01**	0.01**
Error	14	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

** Significant at P<0.05

Root dry weight (g)

The results of this study showed that the RDW was affected by salinity and their effect varied depending on salinity level and genotype. At 2 dSm^{-1} and 4 dSm^{-1} salinity level and TARM 1 had relatively high values of seedling root dry weight. The mean RDW decreased as salinity level increased. However, the reduction varied among the accessions and salinity levels studied. The present investigation was in line with previous reports by Asfaw Kinfemichael [27] in haricot bean and Akram [28] in sunflower which indicated that salinity stress decreased the mean SRDW considerably. Reduction in root and seedling growth under saline conditions may be due to decrease in the availability of water or increase in sodium chloride toxicity. The present result is also in line with the previous studies of Taghipour and Salehi [29] claimed that RDW of Iranian barley decreased as the result of salt stress. These authors reported that decrease in RDW was due to the restricted provision of metabolites to younger growing organs, since metabolic performance was considerably disturbed at higher levels of salinity and also due to the low water absorption and toxicity of NaCl. The present result is also in line with the finding of Karaköy [30] in chickpea; Aydin°akir [31] in corn and Kondetti [32] in soybean (Table 4).

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