EVALUATION OF MUNG BEAN ACCESSIONS FOR SALT TOLERANCE BASED ON GERMINATION AND SEEDLING TRAITS

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Abstract

Ten mung bean accessions collected from Bangladesh Agricultural Research Institute were exposed to two salinity levels (control and 8 dS m^{-1}) to observe the sensitivity of their germination and early seedling traits. The observations were recorded on germination percentage, rate of germination, coefficient of germination, shoot length, root length and seedling dry weight. The accessions, salinity levels and their interaction exhibited significant differences for all the traits. The mean performance of all the traits reduced under salt stress as compared to control. Salt tolerant accessions showed less reduction in their germination and seedling traits under salinity than the other accessions tested. Considering salt tolerance index (STI) based on seedling dry weight, the order of tolerance was BD-6882 > BD-10022 > BD-10024 > BD-10026 > BD-10023 > BD-10027 > BD-6885 > BD-6887 > BD-6884 > BD-6875. Based on relative total dry weight (RTDW), three accessions viz. BD-6882, BD-10022 and BD-10024 were identified as salt tolerant and other seven as moderately salt tolerant. The identified salt tolerant accessions could be a useful stock for further breeding programs to develop salt tolerant mung bean genotype of considerable economic value.

Introduction

Mung bean (*Vigna radiata* L.) is the most important pulse crops of the world with great value for food, feed, fodder, fuel (straw), green manure and as a cover crop. It is also known as moong bean, mug dal or green gram, one of the popular pulse crops in Bangladesh. Being a short duration (about 60 days) crop, it is an ideal legume for catch cropping, intercropping, and relay cropping. Mung bean has become the popular legumes in the tropic and sub-tropics for its rich nutritional components (Thomas *et al.*, 2004) and easily digestible protein source for human especially for poor people where meat is scarce (Bangar et al., 2019). It is an excellent source of protein (24.5%) with high quality of lysine (460 mg g⁻ ¹) and tryptophan (60 mg g^{-1}), fat (0.6%), fiber (0.9%) and ash (3.7%) (Potter and Hotchkiss, 1998). It also maintains soil fertility through biological nitrogen fixation in soil and thus plays a vital role in sustainable agriculture (Kannaiyan, 1999).

Despite an economically important crop, the annual overall production (41000 MT) as well as average yield (0.93 MT ha[−]) of mung bean (BBS, 2022) is rather low in Bangladesh. Scarcity of quality seed of improved varieties, poor crop management practices as well as biotic and abiotic constraints are responsible for the low productivity of the crop (Pratap *et al.*, 2019).

Among different abiotic stresses, salinity is undoubtedly a major constraint for the production of mung bean as it is a salt sensitive crop (Ashraf and Rasul, 1988), where 50 mM NaCl can cause up to 70% yield loss (Saha *et al.*, 2010). Salinity increases the concentration of sodium and chloride ions which adversely affects the process of germination, seedling establishment, nutritional balance, plant growth and yield in almost all the cultivated crops by lowering the osmotic potential of water or by causing specific ionic toxicity, or both in the growing medium (Zahedi *et al.*, 2012; Alom *et al.*, 2016; Kazal *et*

al., 2017). Higher accumulation of salt causes oxidative stress ultimately leads to plant death as a consequence of growth arrest and metabolic damage (Hasanuzzaman *et al.*, 2012).

It is predicted that 50% of the world's arable land will be salinized by 2050 (Jamil *et al.*, 2011; Hasanuzzaman *et al.*, 2013). In Bangladesh, salinity has increased to 26% in the last 35 years (Mahmuduzzaman *et al.*, 2014) and the saline affected area is increasing continuously due to the effect of sea level rise, coastal subsidence, increased tidal effect and continuous reduction of river flow, particularly during dry period. It is a bitter truth that the agricultural land of the country is decreasing day by day and it is quite impossible to expand land horizontally. To increase the total food production of the country, using of marginal land like salt affected soil of the coastal areas for production purpose may be one of the effective strategies. Therefore, screening and adaptation of salt tolerant crop varieties will be feasible and economical approach to increase the cropping intensities in the saline areas as well as to ensure food and nutritional security of the country like Bangladesh.

Seed germination is largely affected by the toxicity of Na⁺ and Cl[−] ions, and the ability to withstand this toxicity vary from species to species and even from plant to plant (Atak *et al.*, 2006). Keeping in view the yield losses due to low germination by salinity, it is very crucial to tackle salinity problem at germination level. In that case, to improve global crop productions, seed germination criteria could be used as quicker and early-stage screening approaches against salinity to identify salt-tolerant genotypes. Current investigation was aimed to perform quick and effective screening of mung bean accessions at seed germination and early seedling stage against salinity stress for early selection of tolerant accessions.

Materials and Methods

Experimental site and period

The experiment was conducted at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in the month of March, 2023.

Experimental design and treatments

Ten mung bean accessions were evaluated under two growing conditions, control and NaCl induced salt stress (8 dS m[−]¹) following completely randomized design (CRD) with three replications. Saline solution was prepared by dissolving calculated amount of NaCl (640 mg liter[−]¹ water for 1 dS m⁻¹) in tap water. Normal tap water was used as control treatment.

Seed placement and data collection on germination and seedling traits

Twenty seeds of each accession were placed on filter paper soaked with treatment solution and tap water according to treatment in 11 cm diameter sterilized Petri dish. Before sowing, seeds were surface sterilized in 0.1% mercuric chloride solution for 30 seconds, and then were washed thoroughly with tap water followed by distilled water. Each Petri dish containing the seeds was irrigated with respective treatment solution and water throughout the experimental period. Germination was counted at 24 hours interval and continued up to $6th$ day. A seed was considered germinated when plumule and radicle came out and was more than 2 mm long. The percentage of germination was counted on 6th day of placement of seeds. The rate of germination was calculated according to Krishnasamy and Seshu (1990) and co-efficient of germination was calculated using the formulae of Copeland (1976).

The length of shoot and root of seedling was recorded by centimeter scale on 8th day after seed placement. Five seedlings from each Petri dish were sampled for measuring shoot and root length. Then the seedlings were dried separately at 70° °C for 72 hours in an electric oven, and weight was recorded with an electrical balance. The mean of length and dry weight were calculated for each treatment combination.

Calculation of stress tolerance index and relative total dry weight

Stress tolerance index (STI) was calculated according to Goudarzi and Pakniyat (2008) using the following formulaStress tolerance index $=\frac{Variable \text{ measured under stress condition}}{Variable \text{ measured under normal condition}}$

Relative total dry weight (RTDW) was calculated according to Ashraf and Waheed (1990) and Aziz *et al.* (2016) using the following formula-

> Relative total dry weight $=\frac{Ory}{Orr}$ weight of salt treated seedling $\frac{y}{y}$ weight of sait treated seedling \times 100

All the accessions were categorized as tolerant, moderately tolerant, susceptible and highly susceptible using a range of 0-9 scale following Ashraf and Waheed (1990).

Statistical analyses of data

The experimental data were analyzed by partitioning the total variance using Statistix 10 program and the means were compared by Tukey's Test at 5% level of probability.

Results and Discussion

Germination characteristics

Germination characteristics (percentage of germination, germination rate, and coefficient of germination) of mung bean were significantly varied due to individual effect of different mung bean accessions and salinity levels (Table 1). Different accessions showed statistically significant difference in germination percentage, germination rate and co-efficient of germination. The maximum germination percentage (94.74%) was recorded in BD-10026 which was nearly followed by BD-10022 (92.90%) and the minimum germination percentage (75.47%) was recorded in BD-6884 which was statistically similar with that of BD-6885 (75.62%) followed by BD-6887 (79.80%).

Other accessions showed moderate performance in respect to germination percentage (81.92% to 88.23%). The maximum and statistically similar germination rate was recorded in BD-10026 (93.23%) and BD-10022 (92.16%) followed by BD-6875 (85.76%) and BD-10024 (86.14%), whereas the minimum and statistically similar germination rate was observed in BD-6884 (72.98%) and BD-6885 (73.03%) followed by BD-6887 (77.07%). The maximum and statistically similar co-efficient of germination was found in BD-10022 (39.42), BD-10023 (39.18) and BD-10026 (38.97), while the minimum and statistically similar value of the trait was recorded in BD-6884 (31.70) and BD-6887 (31.86).

Salt stress significantly inhibited the germination capacity of mung bean accession which was represented by lower germination percentage (76.55%), germination rate (74.17%) and co-efficient of germination (33.58) compared to that of control (92.02, 91.18, and 38.15%, respectively).

Table 1. Individual effect of mung bean accessions and salinity levels on germination characteristics of mung bean

In a column, values having same letter(s) did not differ significantly by Tukey at $p \le 5$ % level.

** indicates significantly different at 1% level of probability. SE indicates standard error.

The interaction effect of different mung bean accessions and salinity levels was also significant on germination characteristics of mung bean (Table 2). Among the different treatment combinations, the maximum germination percentage (99.19%) was observed in BD-10023 and BD-10026 under control condition, whereas the minimum value of the trait was recorded in BD-6885 (63.63%) under salt stress. Salt stress reduced the germination percentage by 2.24, 10.58, 14.84, 27.37, 31.73, 9.21, 28.00, 3.80, 8.97 and 29.26% in BD-6875, BD-6882, BD-6884, BD-6885, BD-6887, BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively. Mung bean accession BD-10026 also showed highest germination rate (99.13%) under control which was statistically similar to that of BD-10023 (99.10%) and BD-10027 (98.43%) germinated under control condition. The lowest germination rate (60.63%) was recorded in BD-6885 under salt stress which was statistically at par with that of BD-6887 (62.66%) under salt stress.

The degrees of reduction in germination rate due to salt stress were 5.85, 10.52, 14.38, 29.02, 31.50, 12.31, 32.06, 3.68, 11.91 and 32.52% in BD-6875, BD-6882, BD-6884, BD-6885, BD-6887, BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively. The maximum co-efficient of germination (43.96) was found in BD-10023 under control but BD-6887 showed the minimum value (27.33) of the trait under salt stress. The extents of lowering of co-efficient of germination due to salt stress were 11.89, 8.06, 6.94, 2.15, 24.88, 13.42, 21.75, 3.84, 11.63 and 15.83% in BD-6875, BD-6882, BD-6884, BD-6885, BD-6887, BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively.

Development at the germination stage have been adopted a suitable growth stage for testing the stress tolerance in crop. In the present study salt stress considerably reduced the germination traits of mung bean accessions at different magnitudes as compared to control.

Mung bean accessions	Salinity levels	Germination (%)	$\%$ change over control	Rate of germination (%)	$\frac{0}{0}$ change over control	Co-efficient of germination	% change over control
BD-6875	Control Salt stress	87.33 ef 85.37 g	-2.24	88.34 c 83.17 f	-5.85	37.84 d 33.34 g	-11.89
BD-6882	Control Salt stress	86.49 fg 77.34 i	-10.58	$85.30 d-f$ 76.33 g	-10.52	38.33 d 35.24 f	-8.06
BD-6884	Control Salt stress	81.52h 69.42 k	-14.84	78.63 g 67.32 h	-14.38	32.84 g 30.56h	-6.94
BD-6885	Control Salt stress	87.61 ef 63.631	-27.37	85.42 c-f 60.63 i	-29.02	34.43 f 35.17f	-2.15
BD-6887	Control Salt stress	94.85 c 64.751	-31.73	91.47 b 62.66 i	-31.50	36.38 e 27.33 i	-24.88
BD-10022	Control Salt stress	97.39 ab 88.42 de	-9.21	98.20 a 86.11 c-f	-12.31	42.26 _b 36.59 e	-13.42

Table 2. Interaction effect of mung bean accessions and salinity levels on germination characteristics of mung bean

In a column, values having same letter(s) did not differ significantly by Tukey at $p \le 5\%$ level.

** indicates significantly different at 1% level of probability. SE indicates standard error.

Differential degree of germination capacity of mung bean accessions under control and salt stress condition might be due to some genetic factors as well as heredity dissimilarities (Win *et al.*, 2011). It could be assumed that the NaCl induced osmotic stress during the growth of germination stage inhibits the developmental traits and survival of mung bean as seedling survival of crop on saline soils may depend on tolerance to low osmotic potential (Roundy, 1983). These adverse effects in germination might be due to reduction in water absorption by seeds due to high salinity which decreased osmotic pressure and create abnormalities in normal water uptake mechanisms (Bayuelo-Jiménez *et al.*, 2002). Decreasing germination rate and percentage of seed germination was due to excessive toxic cations and anions (Na⁺ and Cl[−]) produced in seeds at saline media (Sanchez *et al.*, 2014; Sehrawat *et al.*, 2013), where salt tolerant genotypes show relatively higher germination due to accelerated absorption of Na⁺ (Zhang *et al.*, 2010). Sehrawat *et al.* (2014) and Kamrul *et al.* (2018) also observed salt stress caused reduction in germination traits of mung bean which corroborates with our present findings. Germination and seedling characteristics were decreased with increasing salinity levels and widely reported in mung bean (Swarnakar, 2016); in lentil, chickpea and faba bean (Arslan *et al.*, 2016) and in cowpea (Haleem, 2015). These results are in consistent with the results of our study.

Early seedling traits

Early seedling traits (shoot length, root length and seedling dry weight) of mung bean were significantly influenced by individual effect of mung bean accessions and salinity levels (Table 3). Among the 10 mung bean accessions, the tallest shoot (10.89 cm) was observed in BD-6882 closely followed by that of BD-10024 (9.86 cm). Mung bean accession BD-10026 produced the longest root (5.85 cm) which was statistically similar to root length of BD-6875 (5.49 cm). The maximum accumulation of dry matter in seedling (14.97 mg plant⁻¹) was recorded in BD-10024 which was followed by BD-6884 (13.28 mg plant⁻¹) and BD-10023 (13.08 mg plant⁻¹). The shortest shoot (6.08 cm) and the minimum accumulation of dry matter in seedling (10.16 mg plant⁻¹) were recorded in BD-6887, whereas the shortest root was found in BD-6884 (2.54 cm) which was statistically similar to that of BD-6885 (2.62 cm).

Table 3. Individual effect of mung bean accessions and salinity levels on early seedling traits of mung bean

In a column, values having same letter(s) did not differ significantly by Tukey at $p \le 5\%$ level.

** indicates significantly different at 1% level of probability. SE indicates standard error.

The combined effect of mung bean accessions and salinity levels significantly interacted on early seedling traits of mung bean (Table 4). Among different interactions, BD-6882 produced maximum shoot length (11.44 cm) when grown under control condition which was followed by BD-10024 (10.60 cm) under control and BD-6882 (10.33 cm) under salt stress. The longest root (6.33 cm) was observed in BD-10026 under control followed by root length produced by BD-6875 (5.63 cm) under control, whereas the shortest root (2.50 cm) was recorded in BD-6884 under salt stress which was statistically at par with root length of BD-6884 (2.58 cm) under control and root length of BD-6885 (2.52 cm) under salt stress. Among different treatment combinations, the maximum dry matter plant⁻¹ (15.69 mg plant⁻¹) was recorded in BD-10024 under control condition followed by that of BD-6884 (14.45 mg plant⁻¹) under control, while the minimum value of the trait (9.46 mg plant⁻¹) was found in BD-6887 under salt stress. Salt stress significantly reduced the seedling traits in all mung bean accessions but the degrees of reduction were different in different accessions. The degrees of reduction were 13.24, 9.70, 18.19, 9.23, 10.59, 13.45, 8.36, 13.96, 10.16 and 19.37% in shoot length; 5.15, 5.07, 3.10, 7.01, 11.38, 9.57, 9.94, 9.95, 15.32 and 15.73% in root length and 17.78, 6.77, 16.19, 12.04, 12.81, 8.45, 10.85, 9.18, 10.50 and 11.05% in seedling dry weight in BD-6875, BD-6882, BD-6884, BD-6885, BD-6887, BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively.

Table 4. Interaction effect of mung bean accessions and salinity levels on early seedling traits of mung bean

Mung bean accessions	Salinity levels	Shoot	% change	Root length	% change	Seedling dry	% change
		length (cm)	over	(cm)	over	weight (mg	over
			control		control	$plan-1$)	control
BD-6875	Control	7.78 gh	-13.24	5.63 ab	-5.15	13.50d	-17.78
	Salt stress	6.75 jk		5.34 bc		11.10 hi	
BD-6882	Control	11.44a	-9.70	$4.93\;{\rm b\text{-}d}$	-5.07	$10.93 h-i$	-6.77
	Salt stress	10.33 b		4.68 $c-f$		10.19k	
BD-6884	Control	8.74 de	-18.19	2.58j	-3.10	14.45 _b	-16.19
	Salt stress	7.15 ij		2.50 i		12.11 ef	
BD-6885	Control	7.37 hi	-9.23	2.71 ij	-7.01	11.88 fg	-12.04
	Salt stress	6.69 ik		2.52 i		10.45 jk	
BD-6887	Control	6.42k	-10.59	3.78f gh	-11.38	10.85 ij	-12.81
	Salt stress	5.741		$3.35g$ h-j		9.461	
BD-10022	Control	9.44c	-13.45	5.33 bc	-9.57	12.55 e	-8.45
	Salt stress	8.17 fg		$4.82b-e$		11.49 gh	

In a column, values having same letter(s) did not differ significantly by Tukey at $p \le 5\%$ level.

** and * indicate significantly different at 1% and 5% level of probability. SE indicates standard error.

Salt stress caused a significant reduction in early seedling growth and associated developmental parameters which was due to reduction in root and shoot lengths with considerable genotypic variations. Growth inhibition under salt stress may be due to the diversion of energy from growth to maintenance (Greenway and Gibbs, 2003). Salt stress caused low intra-cellular water potential and water scarcity around the root zone due to which roots failed to absorb sufficient water and nutrients for adequate plant growth (Sunil *et al.*, 2012).

Salinity suppressed the uptake of essential nutrients like P and K (Nasim *et al.*, 2008), which could adversely affect seedling growth and vigor. Significant reduction in term of shoot length, root length and seedling dry weight among the mung bean accessions might be attributed to their differential response in term of sensitivity to salt stress. Reduction in seedling dry weight also might be due to inability to accumulate respiratory product to mung bean seedlings. The results are in accordance of findings of Aziz *et al.* (2016), Kumawat and Gothwal (2020) and Sarkar and Kundagrami (2021) who concluded that seedling dry weight of mung bean accessions was affected to a greater extent under salt stress condition.

Correlation analysis among different germination and early seedling traits

Correlation analysis among different germination and early seedling traits of different mung bean accessions presented in Table 5 designates that all the traits maintained a significant positive correlation with each other.

Table 5. Correlations (Pearson) among the different germination and seedling traits of mung bean accessions

** and * indicate significant at the 1% and 5% probability level, respectively.

Stress tolerance index based on germination and seedling traits

Stress tolerance index of mung bean accessions based on different germination and early seedling traits (Table 6) indicates different levels of salt tolerance of mung bean under NaCl induced salt stress.

The accession with high STI showed more tolerance to stress condition than the other accessions. The order of stress tolerance index of mung bean accessions was BD-6875 > BD-10024> BD-10026 > BD-10022 > BD-6882> BD-6884 > BD-6885> BD-10023> BD-10027> BD-6887based on germination percentage, BD-10024 >BD-6875 > BD-6882 > BD-10026 > BD-10022 > BD-6884 >BD-6885 > BD-6887 > BD-10023> BD-10027 based on rate of germination, BD-6885> BD-10024> BD-6884> BD-6882> BD-10026> BD-6875> BD-10022> BD-10027> BD-10023> BD-6887 based on coefficient of germination, BD-10023> BD-6885> BD-6882 > BD-10026 > BD-6887 > BD-6875 > BD-10022 > BD-10024 > BD-6884 > BD-10027 based on shoot length, BD-6884 > BD-6882 > BD-6875 > BD-6885 > BD-10022> BD-10023 > BD-10024 > BD-6887 > BD-10026 > BD->10027 based on root length and BD-6882 > BD-10022 BD-10024 > BD-10026 > BD-10023 > BD-10027 > BD-6885> BD-6887> BD-6884>BD-6875 based on seedling dry weight. Other researchers (Hooshmandi, 2019; Haque *et al.*, 2021; Pramanik *et al.*, 2022a and Pramanik *et al.*, 2022b) also used STI as an important tolerance criterion for plant in stress conditions.

Salt tolerance based on relative total dry weight seedling[−]¹

The relative total dry matter (TDM) per seedling (% TDM to control conditions) was significantly reduced in different degrees due to salt stress in all the accessions evaluated (Table 7).

Table 7. Comparative salt tolerance group of mung bean accessions based on relative total dry weight of seedling

The accessions tested in this study were considered to be classified into ten groups using 0-9 scale and then categorized as tolerant (RTDW > 90%), moderately tolerant (RTDW is in the range of 70- 90%), susceptible (RTDW is in the range of 50-70% respectively) and highly susceptible (RTDW < 50%) (Ashraf and Waheed, 1990). There were three accessions (BD-6882, BD-10022 and BD-10024) that produced greater than 90% RTDW and were identified as tolerant to salinity. Other seven accessions had RTDW in the range of 70-90% and were categorized as moderately tolerant to salinity.

In the third (susceptible) and fourth (highly susceptible) group, there was no accession. Among the 10 accessions, 30% accession was found as tolerant and other 70% was found as moderately tolerant based on RTDW seedling⁻¹. The relative total dry weight is an important indicator for evaluating salt tolerance in plants which is most usefully presented in terms of relative production over a range of salinities (Sarkar and Kundagrami 2021 and Uddin *et al*., 2017). Aziz *et al.* (2016) also considered the percent of relative total dry weight for calculating salt tolerance levels of 50 mung bean genotypes that shows conformity with the findings of our study.

Conclusion

The essential requirement of a successful breeding program of any crop is collection and evaluation of germplasm having desirable variability for economically important and environmentally sustainable characters. The response to salt stress is a complex phenomenon and according to genetic variability of the genotypes the response of genotypes to stress varied. From this study, it is observed that salt tolerant accession was found to be less affected by the salt stress and produced better TDM compared to other accessions. Based on RTDW, three accessions viz. BD-6882, BD-10022 and BD-10024 were screened to be the tolerant to salt stress, whereas other seven accessions were found as moderately tolerant. These accessions may be useful resources for future breeding programs to develop salt tolerant mung bean genotype.

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