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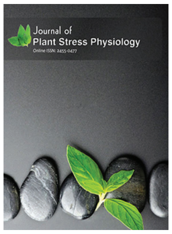

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Phenotyping of mungbean (*Vigna radiata* L.) genotypes against salt stress and assessment of variability for yield and yield attributing traits

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ABSTRACT

Salt tolerance is a complex polygenic trait that is genotype specific and tolerance can depend upon a plants developmental stage. To evaluate reproductive stage specific salt tolerance as well as investigate the inherent variability of mungbean (*Vigna radiata* L.) genotypes with respect to seed yields and yield-related traits, a pot culture experiment was conducted using 26 mungbean genotypes and exposure to salt stress (EC = 8.0 dS/m) applied at the reproductive stage, just before the opening of the first flowers. The experiment involved maintaining 100% field capacity for three weeks and used a randomized complete block design with three replicates. Data were collected, included days to maturity, plant height (cm), number of pod-bearing branches per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100-seed weight (g) and seed yield per plant (g). Salt stress led to a significant ($p<0.001$) decrease in seed yield per plant, with yields of the genotypes BMX 11116, BMX 11176, BMX 11140, BMX 11111 and BMX 11163 being the least impacted by exposure to salt. Principal component analysis revealed that the first two components explained 63.5% of the total variation among the mungbean genotypes. Seed yield per plant showed a significant positive correlation with days to maturity, number of pod-bearing branches per plant, number of pods per plant, pod length (cm), number of seeds per pod, and 100-seed weight (g). Cluster analysis grouped the 26 genotypes into five distinct clusters, where the tolerant genotypes placed in cluster I. Based on their stress tolerance indices BARI Mung-6, BMX 11176, BMX 11116, and BMX 11140 were categorized as tolerant genotypes, were selected for further study under direct field conditions and are recommended for the genetic improvement of salt stress tolerance in mungbean.

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KEYWORDS: Salt stress, mungbean, reproductive phase, stress tolerance indices, trait relationship

INTRODUCTION

Pulses are known as a "poor man's meat" and a "rich man's vegetable", because of their considerable protein content. Among the pulses, mungbean (also known as greengram or moong) is one of the most important crops that is extensively grown in tropical, subtropical and temperate zones of Asia [1]. From 2011-12, the total production of mungbean in Bangladesh was 19,972 metric tons (MT) from an area of 20,117 ha, with an average yield of about 0.98 ton/ha. It covers about 5% of the entire acreage under pulses in Bangladesh and approximately 4% of total legume production. Mungbean is 5th in acreage, 6th in production, 3rd in protein content (%) and 1st with regard to price [2]. Mungbean grains typically contain 22-28% protein, 60-65% carbohydrates, 1-1.5% fat, 3.5-4.5% fibers and content high levels of vitamins and minerals. Both their rapid life cycle short and nitrogen fixing ability make mungbean a valuable crop, with a simple cropping system and good sustainable agricultural production potential.

Salinity is a major abiotic constraint for crop production, which adversely affects the socio-economic condition in many developing countries. In Bangladesh, more than 30% of the net cultivable land area is located in the coastal regions, and approximately 53% of these areas affected by varying degrees of salinity [3]. As a result of primary (natural) or secondary/ (irrigation-associated) salinity, arable land is progressively being transformed into saline land (1-3% per year) and this is expected to result in up to 50% arable land loss by 2050 [4]. Around one-third of farmers in the coastal areas are now cultivating only one crop in a calendar year, i.e. aman rice during the monsoon season while most of the cultivable land remains almost barren in kharif-1 (mid March – mid July) and rabi season (mid November – mid March). Combating mono-cropping, mungbean contributes to the diversification of rice-based cropping system and overall system productivity in southern Bangladesh. The addition of a pulse, especially mungbean, to the cropping cycle of coastal areas offers great promise to avert food insecurity and improve the nutrition of poor households, as well as to improve the soil heath. Therefore, there is an urgent need to develop mungbean varieties tolerant to salt stress and management practices to exploit those saline areas for sustainable agricultural production and to increase cropping intensity and efficiency.

In the past systematic breeding approaches have led to the development of a many improved varieties of this mungbean. However, its true yield potential has yet to be achieved owing to several constraints [5]. One of the major constraints is the susceptibility of mungbean to abiotic and biotic stresses, including soil salinity. Additionally, the complex nature of salt stress and a lack of reliable techniques and suitable markers for screening for tolerance has restricted the development of salt tolerant cultivars of mungbean. Salt tolerance is a polygenic, genotype dependent and developmental stage-specific phenomenon, for which tolerance at one developmental stage may not be correlated with tolerance at other developmental stages [6]. Despite research have been conducted on plants at the seedling stage [7] very little attention has been devoted to the impacts of salinity at the reproductive stage, mainly because of the lack of a reliable reproductive-stage-specific phenotyping method [6]. With respect to productivity the reproductive stage is the most crucial as it ultimately determines grain yield. Therefore, to facilitate the development of suitable cultivars that can survive and give better performance under salt stress conditions, evaluation of local and exotic germplasm at various phases of plant development under saline environmental conditions is needed. This will provide suitable source for agronomic traits or genes that can be introduced into salt sensitive mungbean genotypes and hence enable breeding for increased yields [8].

Yield is the complex end product of many factors that jointly or singly influence seed number, size and quality. Therefore, considerable attention is needed for the selection of yield and yield related traits under favourable and salt stress conditions. In advanced plant breeding programs, the degree of association between characters, as indicated by correlation coefficients, help with the construction of selection indices that are helpful for the selection of desirable characters. Correlation and principal component analysis (PCA) estimate the mutual relationships between various plant characters and determine the component characters on which selection can be based for yield improvement. Therefore, the present study was undertaken to investigate the phenotypic performance of 26 diverse mungbean genotypes with respect to their variability for yield and yield-related traits and to select salt tolerant mungbean genotype that can produce substantial yields under saline environments following a reproductive-stage specific phenotyping technique.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The experiment was carried out in earthen pots filled with field soil at the pot-yard of the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during the period of 23rd March to 29th June, 2019. Twenty-six mungbean genotypes including four popular varieties *viz*., Binamoog-9, Binamoog-5, Binamoog-8, BARI Mung-6 and twenty-two promising advance lines *viz*., BMX 11159, BMX 11148, BMX 11165, BMX 11163, BMX 1141, BMX 11153, BMX 1137, BMX 11122, BMX 11107, BMX 11157, BMX 11170, BMX 11176, BMX 11116, BMX 11108, BMX 11106, BMX 11154, BMX 11140, BMX 11111, BMX 11144, BMX 1153, BMX 1131, BMX 1157 were used as plant material. To break seed dormancy and enhance germination, fresh seeds were kept in an incubator at 50ºC for 4-5 days. Ten seeds were initially placed in each pot containing 9 kg of field soil. Ten days after germination, five similar sized seedlings were retained in each pot. The seedlings were grown under natural field conditions.

Experimental Design and Salt Stress Treatments

The experiment was conducted following a randomized complete block design (RCBD) with three replicates, including two treatments a control and salt stress (8 dS/mNaCl). Salt stress was imposed at the reproductive stage (just before opening of the first flower) for each genotype through the application of saline irrigation water, while maintaining 100% field capacity, for three weeks. After 3 weeks of salt stress treatment the plants were grown under normal cultural practices and harvested at maturity.

Data Collection of Seed Yield and Yield-Related Traits

Data on eight yield attributing traits, such as days to maturity, plant height (cm), number of pod-bearing branches per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100-seeds weight and seed yield per plant (g) were recorded.

Estimation of Percent Yield Reduction

When comparing stressed plants with non-stressed control plants, the reduction in % grain yield was calculated by the following formula:

$$(\%)\ \text{Reduction} = \frac{\begin{array}{c}(\text{seed yield of streesed plants}) - \\ (\text{seed yield of control plants})\end{array}}{\text{seed yield of control plants}}\ \text{X } 100$$

Estimation of Stress Tolerance Indices

The stress tolerance indices were calculated by the following formula:

- Stress susceptibility index, SSI = $(1 - (Ys/Yp))/(1 - (\bar{Y}s/\bar{Y}p))$ [9]

- Stress tolerance, $TOL = Yp - Ys$ [10]
- Mean productivity, $MP = (Yp + Ys)/2$ [10]
- Stress tolerance index, $STI = (Yp \times Ys)/(\bar{Y}p)^2$ [11]
- Geometric mean productivity, $GMP = \sqrt{Y_p \times Y_s}$ [11]
- Yield index, $YI = Ys/\bar{Y}s$ [12]

In the above equations, Ys and Yp represent the seed yield of genotypes under saline (stress) and control (non-stress) conditions whereas $\bar{Y}s$ and $\bar{Y}p$ represent the mean seed yield over all genotypes under stress and non-stress conditions.

Statistical Analysis

Data recorded for different parameters were compiled and tabulated in proper form for statistical analysis which was carried out in Minitab 17 statistical software package (Minitab Inc. State College, Pennsylvania) and software R, version 3.3.2. The two-way analysis of variance was carried out using Minitab 17 software following RCBD design with two factors in mixed model, in which factors were fixed. Principal component analysis and phenotypic correlation co-efficient was done using Minitab 17 statistical software. Cluster analysis was done by statistical software R.

RESULTS

Analysis of Variance (ANOVA) for Seed Yield and Yield-Related Traits

The results of analysis of variance for all of the characters such as days to maturity, plant height (cm), number of pod-bearing branches per plant, number of pods per plant, pod length (cm), number of seeds per pod, 100-seed weight (g) and seed yield per plant (g) showed significant ($p<0.001$) variation among the genotypes (A) studied (Table 1). In the case of treatment (B), all of the characters also showed highly significant ($p<0.001$) differences among the genotypes (Table 1). In the genotype (A) x treatment (B) interaction, the characters which were highly significant ($p<0.001$) are days to maturity, plant height (cm), number of pod-bearing branches per plant, number of pods per plant, number of seeds per pod and seed yield per plant (g). The character 100-seed weight (g) was significant at $p<0.01$, whereas pod length (cm) was not significant (Table 1).

Effect of Salt Stress on Various Morphological Traits of Mungbean Genotypes

Days to maturity

The highest number of days to maturity (79.43 days) was recorded in the genotype BMX 11122, whereas the least (49.66 days) was recorded in the genotype BMX 11116, under control conditions. Under salt stress conditions, the highest number of days to maturity (69.50 days) was recorded in the genotype BMX 11140, whereas the least number of days (45.18 days) was found in the genotype BMX 11116. Salt stress led to a significant decrease in days to maturity. The greatest reduction (29.15%) in days to maturity was recorded for the genotype BMX 1157, followed by 29.09, 27.70 and 22.35% in BMX 11122, BMX 11106 and BMX 11107, respectively. The least reduction (6.00%) was found for the genotype Binamoog-9 (Table 2).

Plant height (cm)

The greatest plant height (47.58 cm) was recorded for the genotype BMX 11122, whereas the least plant height (14.66 cm) was recorded for the genotype BMX 11116, under control conditions. Under salt stress conditions, the greatest plant height (46.12 cm) was recorded in the genotype BMX 11165, whereas the least (4.56 cm) was found in the genotype BMX 11116. Salt stress caused a significant reduction in plant height for all the genotypes compared to control plants. The greatest reduction (33.52%) was recorded for the genotype BMX 11122, followed by BMX 11106 (31.38%), BMX 11154 (30.88%), Binamoog-9 (21.28%), BMX 11144 (21.28%), BMX 11116 (21.11%), BMX 11111 (20.70%), BMX 1131 (20.55%) and BMX 1157 (20.54%), with the least reduction (6.05%) found for the genotype BMX 11163 (Table 2).

Number of pod-bearing branches per plant

Under control conditions the greatest number of pod-bearing branches per plant (7.22) was recorded for the genotype BMX 11122, whereas the least (2.44) was recorded for the genotype BMX 11154. Under salt stress condition, the greatest number of pod-bearing branches per plant (3.89) was recorded for the genotype BMX 11140 whereas the least (1.46) was found for the genotype BMX 1157. Imposition of salt stress resulted in a significant decrease in the number of pod-bearing branches per plant, with the greatest reduction observed in the genotype BMX 1157 (59.50%), followed by BMX 11122, BMX 11157, BMX 11159, BMX 11154, BMX 1137, BMX 11131, Binamoog-5, BMX 11141, BMX 11106 and BARI Mung-6 (56.16, 43.25, 37.11, 34.65, 33.55, 33.22, 30.94, 28.86, 28.79 and 27.88%, respectively) and the least reduction observed for the genotype BMX 11176 (6.55%) as compared to control (Table 2).

Number of pods per plant

The greatest number of pods per plant (23.72) was recorded in the genotype BMX 11122, whereas the least (3.50) was recorded

Table 1: Analysis of variance (ANOVA) (mean square) for yield and yield-related traits

Source of variation	df	Days to maturity	Plant height (cm)	Number of pod-bearing branches per plant	Number of pods per plant	Pod length (cm)	Number of seeds per pod	100-seed weight (g)	Seed yield per plant (g)
Treatment (A)	1	2228.61***	1106.14***	35.169***	333.026***	20.579***	146.799***	68.086***	49.191***
Genotypes (B)	25	170.40***	238.68***	3.520***	32.466***	3.156***	8.540***	8.831***	1.261***
A×B	25	59.49***	17.90***	0.944***	17.484***	0.172	2.541***	0.662**	0.287***
Error	104	0.96	1.00	0.382	0.718	0.173	0.789	0.402	0.054

** and *** indicates significant at 1% and 0.1% level of probability respectively for the genotype BMX 1137 under control conditions. Under salt stress condition the highest number of pods per plant (7.56) was recorded in the genotype BMX 11122 whereas the least number of pods per plant (2.45) was found for the genotype BMX 1137. A significant decrease in the number of pods per plant in response to salt stress was found, with the greatest reduction (80.47%) for the genotype BMX 11159, followed by 68.14, 63.95, 61.37, 60.33, 53.23, 46.30, 45.69, 41.24, 41.22, 40.04 and 39.57% for the genotypes BMX 11122, BMX 1157, Binamoog-9, BMX 11144, BMX 11154, BMX 11106, BMX 1141, Binamoog-8, BMX 11111, BMX 11148 and BMX 11157, respectively. The smallest decrease (20.15%) was found for BARI Mung-6 (Table 2).

Pod length (cm)

Under control conditions the greatest pod length (8.73 cm) was recorded for the genotype BMX 1131, whereas the lowest (5.52 cm) was recorded for the genotype BMX 11108. Under salt stress conditions the greatest pod length (7.95 cm) was recorded for the variety BARI Mung-6, whereas the smallest pod length

Table 2: Combined effect of genotype and treatment interactions on seed yield and yield-related traits of mungbean

Genotype × Treatment		Days to maturity	Plant height (cm)	Number of pod-bearing branches per plant	Number of pods per plant	Pod length (cm)	Number of seeds per pod	100-seed weight (g)	Seed yield per plant (g)
BARI Mung-6	Control	56.30g-l	29.22j-n	3.44b-h	6.42c-j	8.47ab	7.73a-h	5.00c-i	3.33ab
	Salt	51.26pq	24.76p-u	2.48d-h	5.13e-p	7.95a-e	6.13f-m	3.22h-o	1.76i-r
Binamoog-9	Control	63.95c	29.61j-m	4.72bc	9.22c	8.03a-d	7.71a-h	5.15c-h	3.07abc
	Salt	60.11def	23.31r-u	3.56b-g	3.56j-p	6.88c-m	5.34g-m	3.58g-o	1.49n-u
Binamoog-5	Control	57.88fgh	31.55g-j	3.11c-h	6.37c-k	7.11b-l	7.22d-k	4.37d-k	2.29c-m
	Salt	54.14i-p	26.56m-r	2.15fgh	5.30e-p	6.30h-n	5.54f-m	3.30h-o	1.48n-u
Binamoog-8	Control	52.95m-q	24.72p-u	2.89c-h	4.66f-p	8.03a-d	7.50b-i	3.75f-o	1.86g-r
	Salt	49.66q	26.60stu	2.33e-h	2.74op	7.11b-l	5.12g-m	2.58j-o	0.79t-x
BMX 11159	Control	60.22def	39.91bc	5.39ab	12.63b	6.52f-n	10.43ab	2.40k-o	2.93a-d
	Salt	52.160pq	33.72fgh	3.39b-h	2.47p	5.52mno	5.50f-m	1.78o	0.49x
BMX 11148	Control	63.27cd	26.20n-r	2.81c-h	4.80e-p	6.56e-n	6.95d-k	6.45a-d	1.81h-r
	Salt	59.15efg	23.41r-u	2.50d-h	2.88nop	6.56e-n	4.82h-m	3.92f-o	0.68vwx
BMX 11165	Control	60.29def	46.12a	3.33b-h	4.73e-p	7.15b-k	8.02a-g	5.59b-g	2.48c-j
	Salt	55.19h-o	40.57bc	2.51d-h	3.83g-p	6.75d-n	4.28klm	4.42c-k	1.52m-u
BMX 11163	Control	57.72fgh	30.90h-k	2.67c-h	6.45c-i	7.37a-j	7.15d-k	4.32d-k	2.44c-j
	Salt	53.08l-p	29.03 j-o	2.44e-h	5.83d-m	6.73d-m	6.42e-l	3.86f-o	1.58l-t
BMX 1141	Control	61.56cde	35.23def	3.28c-h	5.84d-m	7.08b-l	6.07f-m	5.75b-f	2.54b-i
	Salt	56.48g-k	31.42g-j	2.33e-h	3.17m-p	6.71d-n	4.36j-m	3.59g-o	1.52m-u
BMX 11153	Control	69.19b	37.20cde	3.11c-h	6.02d-m	7.01c-l	7.67a-h	4.09e-m	2.50c-j
	Salt	56.06g-n	34.56efg	2.41e-h	4.06g-p	6.50f-n	5.47f-m	3.39h-o	0.74u-x
BMX 1137	Control	59.80ef	30.45h-l	3.03c-h	3.50k-p	7.78a-g	8.03a-g	7.41ab	2.48c-j
	Salt	56.04g-n	25.50p-t	2.01fgh	2.45p	6.65d-n	6.11f-m	6.21a-e	1.44o-v
BMX 11122	Control	79.43a	47.58a	7.22a	23.72a	6.28h-n	7.36c-j	2.88i-o	1.46o-v
	Salt	56.32g-l	31.63g-j	3.17c-h	7.56cde	5.74l-o	5.78f-m	1.99mno	0.65wx
BMX 11107	Control	72.33b	30.41h-l	3.30c-h	5.66e-n	6.15h-o	7.60b-i	3.53g-o	2.01e-q
	Salt	56.17g-m	25.69o-t	2.97c-h	4.78e-p	5.98j-o	6.16f-m	2.45j-o	1.28q-x
BMX 11157	Control	57.32f-j	30.18i-l	2.69c-h	4.70e-p	6.75d-n	9.43a-e	4.00f-m	1.74j-r
	Salt	53.18k-p	27.86k-p	1.53gh	2.84nop	6.11h-o	5.56f-m	2.89i-o	0.87s-x
BMX 11170	Control	57.74fgh	27.50l-q	3.11c-h	5.35e-o	7.51a-h	10.31abc	3.21h-o	2.42c-k
	Salt	54.04j-p	23.18r-u	2.39e-h	3.63i-p	6.40g-n	8.36a-f	2.33k-o	1.31q-w
BMX 11176	Control	57.85fgh	26.41m-r	2.44e-h	6.52c-h	7.81a-f	8.45a-f	3.76f-o	2.61a-g
	Salt	53.05l-p	24.41q-u	2.28e-h	5.50e-o	7.30b-k	7.55b-i	3.39h-o	1.88f-r
BMX 11116	Control	49.66q	14.66w	3.33b-h	6.45c-i	6.15h-o	5.76f-m	3.88f-o	2.56b-h
	Salt	45.18r	11.56w	3.09c-h	5.83d-m	5.41no	5.03g-m	3.29h-o	2.20d-o
BMX 11108	Control	56.33g-l	22.35tuv	2.89c-h	5.34e-o	5.52mno	6.35f-l	5.08c-h	1.40p-w
	Salt	52.12opq	19.12v	2.44e-h	3.39m-p	4.75o	4.62i-m	3.42h-o	0.76u-x
BMX 11106	Control	79.16a	35.87def	3.28c-h	6.62c-g	7.16b-k	10.61a	4.01f-m	1.77i-r
	Salt	57.23f-j	24.62p-u	2.33e-h	3.56j-p	6.11h-o	6.53e-l	1.81no	0.79t-x
BMX 11154	Control	57.43f-i	38.27cd	2.44e-h	6.29d-k	6.77d-n	5.85f-m	3.13h-o	2.67a-f
	Salt	53.17l-p	26.45m-r	1.60gh	2.94nop	5.91k-o	3.22m	2.48j-o	1.39p-w
BMX 11140	Control	69.50b	27.82k-p	4.28b-e	7.17c-f	7.95a-e	9.55a-d	4.55c-j	3.39a
	Salt	63.25cd	25.97n-s	3.89b-f	6.33d-k	6.64d-m	7.99a-g	3.94f-n	2.35c-l
BMX 11111	Control	57.41f-i	27.36l-q	4.00b-f	6.27d-l	6.66d-n	7.65a-h	4.14e-l	2.27d-n
	Salt	51.32pq	21.70u	3.06c-h	3.68h-p	6.50f-n	3.83lm	2.05l-o	1.48n-u
BMX 11144	Control	57.65fgh	42.52b	4.55bcd	8.61cd	6.63d-n	7.99a-g	3.34h-o	2.16d-p
	Salt	53.81k-p	33.47f-i	3.52b-h	3.42l-p	6.05i-o	7.19d-k	1.82no	0.74u-x
BMX 1153	Control	57.14f-j	26.56m-r	2.78c-h	4.83e-p	8.22abc	7.85a-g	8.12a	2.79a-e
	Salt	52.80n-q	23.24r-u	2.20e-h	3.32m-p	7.44a-i	7.85a-g	5.64b-g	1.21r-x
BMX 1131	Control	61.37cde	34.45efg	3.00c-h	5.48e-o	8.73a	7.42c-i	5.22c-h	2.63a-g
	Salt	55.04h-o	27.37l-q	2.00fgh	4.15g-p	7.46a-h	7.42c-i	3.80f-o	1.63k-s
BMX 1157	Control	79.35a	34.77efg	3.61b-g	7.32c-f	7.01c-l	8.43a-f	6.52abc	2.30c-m
	Salt	56.22g-m	27.62k-q	1.46h	2.64op	6.15h-o	8.43a-f	4.14e-l	0.64wx

(4.75 cm) was found for the genotype BMX 11108. Salt stress led to a significant reduction in pod length, as compared to controls, the greatest reduction (27.03%) was for genotype BMX 11159 followed by BMX 11122 (21.70%), BMX 11140 (18.99%), BMX 1131 (17.72%), BMX 11116 (16.10%), BMX 11106 (15.82%), BMX 11165 (14.73%), Binamoog-9 (14.66%), BMX 1157 (13.37%), Binamoog-8 (13.33%). The least reduction (6.17%) was recorded for the genotype BMX 1153 (Table 2).

Number of seeds per pod

The greatest number of seeds per pod (10.61) was recorded for the genotype BMX 11106, whereas the lowest (5.76) was recorded for the genotype BMX 11116, under control conditions. Under salt stress conditions the greatest number of seeds per pod was recorded for the genotype BMX 1157, whereas the least was found in the genotype BMX 11154. Imposition of salt stress resulted in a significant decrease in number of seeds per pod for all the genotype as compared to control. The greatest percentage of reduction (49.91%) in number of seeds per pod was recorded for the genotype BMX 11111, followed by BMX 11159, BMX 11165, BMX 11154, BMX 1153, BMX 11157, BMX 11106, Binamoog-8, BMX 11148, Binamoog-9 (47.22, 46.59, 44.87, 43 61, 41.05, 38.45, 31.73, 30.66 and 30.12%, respectively) and least percentage reduction (9.93%) was recorded for genotype BMX 11144 (Table 2).

100-seed weight (g)

Under control conditions the greatest 100-seed weight (8.12 g) was recorded for the genotype BMX 1153, whereas the least (2.40 g) was recorded in the genotype BMX 11159. Under salt stress condition, the greatest 100-seed weight (6.21 g) was recorded for the genotype BMX 1137, whereas the least (1.78 g) was found in the genotype BMX 11159. A significant reduction in 100-seed weight was observed in response to salt stress, the greatest reduction (54.90%) was recorded for the genotype BMX 11106 followed by 50.60, 45.41, 39.14, 37.41, 36.48, 35.53, 32.70, 31.26, 31.02, 30.62, 30.60 and 30.53% for the genotypes BMX 11111, BMX 11144, BMX 11148, BMX 1141, BMX 1157, BARI Mung-6, BMX 11108, Binamoog-8, BMX 11122, BMX 11107, BMX 1153 and Binamoog-9, respectively. The least reduction (9.85%) was recorded for the genotype BMX 11176 (Table 2).

Seed yield per plant (g)

Under control conditions the highest seed yield per plant 3.39 (g) was recorded in the genotype BMX 11140, whereas the lowest 1.40 (g) was recorded in the genotype BMX 11108. Under salt stress condition the highest seed yield per plant (2.35 g) was recorded for the genotype BMX 11140, whereas the lowest (0.79 g) was found in the genotype BMX 11159. Imposition of salt stress resulted in a significant decrease in seed yield per plant for all the genotypes compared to control. The greatest reduction in seed yield per plant was recorded for the genotype BMX 11159 (83.05%), followed by BMX 1157 (72.03%), BMX 11153 (70.53%), BMX 11144 (65.59%), BMX 11148 (60.70%), Binamoog-8 (57.53%), BMX 1153 (56.46%), BMX 11106 (55.47%), BMX 11122 (55.38%), Binamoog-9 (51.30%), BMX 11157 (50.10%). The lowest reduction was recorded for the genotype BMX 11116 (18.01%) (Table 2).

Principal component analysis

A PCA of eight seed yield and yield-related traits, and their relative contributions towards the total genetic divergence was carried (Table 3). Data were considered in each component with Eigen values more than 1, as it determines a minimum 10% of the variation [13]. Superior Eigen values are considered as the best attributes in PCs. In the present study, two components indicated Eigen values of greater than one. The first two PCs with Eigen values explained 63.5% of the total variation among 26 mungbean genotypes for the 8 quantitative traits studied. PCA revealed that PC1 accounted for more than 41% of total variance where days to maturity had the highest positive loadings (0.420) followed by the number of pods per plant (0.403), number of pod-bearing branches per plant (0.394), number of seeds per pod (0.369), seed yield per plant (g) (0.375) and plant height (cm) (0.343). The PC2 accounted for more than 22% of total variance whereby the number of pod-bearing branches per plant (0.358), number of pods per plant (0.384) exhibited the highest positive loadings and 100-seed weight (g) (-0.523) showed the highest negative loading, followed by pod length (cm) (-0.495) and seed yield per plant (g) (-0.340). (Table 3).

Estimation of correlation coefficient among yield and yield-related traits

Phenotypic correlation between different yield-related components revealed that seed yield per plant showed a highly significant ($p<0.001$) positive correlation with days to maturity, number of pod-bearing branches per plant, number of pods per plant, pod length, number of seeds per pod and 100-seed weight (g) (Table 4). Days to maturity showed a highly significant positive ($p<0.001$) correlation with plant height, number of pod-bearing branches per plant, number of pods per plant, number of seeds per pod and 100-seed weight. Pod length was positively and significantly ($p<0.05$) correlated with days to maturity. Plant height showed a significant ($p<0.001$) positive correlation with the number of pod-bearing branches per plant, number of pods per plant and the number of seeds per

Table 3: Principal components (PCs) for nine seed yield and yield-related traits in 26 mungbean genotypes from Principal Component Analysis with Eigen vectors (loadings) of the first two principal components

Variables	PC1	PC2
Days to maturity	0.420	0.121
Plant height (cm)	0.343	0.224
Number of pod-bearing branches per plant	0.394	0.358
Number of pods per plant	0.403	0.384
Pod length (cm)	0.271	-0.495
Number of seeds per pod	0.369	-0.163
100-seed weight (g)	0.196	-0.523
Seed yield per plant (g)	0.375	-0.340
% Variation explained	41.3%	22.2%

pod. Plant height also showed a positive, but non-significant, phenotypic correlation with pod length and 100-seed weight. The number of pod-bearing branches per plant showed a positive and highly significant ($p<0.001$) correlation with the number of pods per plant and the number of seeds per pod. In contrast, the number of pod-bearing branches per plant showed a positive, but non-significant, correlation with pod length and a negative but non-significant correlation with 100-seed weight. The number of pods per plant showed a significant ($p<0.001$) positive correlation with the number of seeds per pod, but it showed positive, but a non-significant, correlation with pod length. A non-significant negative relationship was recorded between the number of pods per plant and 100-seed weight. Pod length showed highly significant ($p<0.001$) positive correlation with number of seeds per pod and 100-seed weight. 100-seed weight was found to be positively correlated with seed yield per plant, days to maturity, plant height, pod length and number of seeds per pod. In contrast, 100-seed weight was negatively correlated with the number of pod-bearing branches per plant and the number of pods per plant.

Cluster analysis of the genotypes based on morphological traits

The 26 mungbean genotypes were grouped into 5 distinct clusters in this study using Euclidean distance, following Ward's method based on D^2 (Table 5). Generally, genotypes from the same cluster group shows similarity in their performance and were highly divergent from other cluster groups. The distribution pattern revealed that cluster II was the largest one, containing 8 genotypes, while cluster III contained only 3 genotypes. Clusters I, IV and V contained 4, 6 and 5 genotypes, respectively. The average intra and inter-cluster distances are presented in Table 6 and a cluster diagram is presented in Figure 1. The intra-cluster distance for all the 5 clusters ranged from 9.81 to 14.22. The intra-cluster distance of cluster III was 14.22, which was the highest value. Cluster II, the largest group containing the highest number of genotypes (8), had the second highest (12.13) intra cluster distance. However, the intra-cluster distance was lowest in cluster I (9.81). The range of inter-cluster distances was between 12.35 to 19.79. The maximum inter-cluster genetic distance was observed between clusters III and II (19.79), followed by clusters III and II (18.20) and clusters V and III (16.25), whereas the minimum distance was found between clusters V and I (12.35), followed by the genetic distance between clusters II and I (12.42) (Table 6).

Stress tolerance indices based on seed yields of 26 mungbean genotypes obtained from control and salt stress

The different stress tolerance indices of mungbean genotypes estimated from seed yield per plant obtained from normal and salt stress conditions are presented in Table 7. The highest SSI was obtained for the genotype BMX 11159 (1.76), followed by BMX 1157 (1.53), BMX 11153 (1.49), BMX 11144 (1.39) and the lowest was for the genotype BMX 11116 (0.30). The highest TOL (2.43) was observed for the genotype BMX 11159 followed by 1.76, 1.66, 1.57, 1.57, 1.42 and 1.42 for the genotypes BMX 11153, BMX 1157, BARI Mung-6, Binamoog-9, BMX 1153, BMX 11144, BMX 11154, respectively and the lowest (0.36) was observed for the genotype BMX 11116. The MP was highest for the genotype BMX 11140 (2.87) followed by BARI Mung-6 (2.54), BMX 11116 (2.38), Binamoog-9 (2.28), BMX 11176 (2.25), BMX 1131 (2.13) and the lowest was recorded for the genotype BMX 11122 (1.05). Maximum STI was obtained by BMX 11140 (1.41) followed by BARI Mung-6, BMX 11116, BMX 11170, Binamoog-9 and BMX 1131 (1.03, 1.00, 0.87, 0.81 and 0.76, respectively) and the lowest for the genotype BMX 11122 (0.17). The GMP was the highest for the genotype BMX 11140 (2.82) followed by BARI Mung-6 (2.42), BMX 11116 (2.37), BMX 11176(2.21), Binamoog-9 (2.14), BMX 1131 (2.07) and the lowest for the genotype BMX 11122 (0.97). Finally, the highest YI (1.87) was obtained for the genotype BMX 11140 followed by 1.75, 1.49, 1.40, 1.30 and 1.25 for the genotypes BMX 11116, BMX 11176, BARI Mung-6, BMX 1131, BMX 11163, respectively and the lowest (0.40) was found for the genotype BMX 11159.

DISCUSSION

Mungbean is salt-sensitive pulse and significant reduction in yield and yield attributing traits was recorded for mungbean plants in response to salt stress [14, 15]. Under high-salinity conditions plants are unable to uptake adequate water for metabolic processes or maintain cell turgidity due to low external osmotic potentials [16]. Initially soil salinity inhibits plant growth through osmotic stress, but this is then followed by ion toxicity [17]. In the initial stage, osmotic stress causes various physiological alterations, such as disruption of membranes, nutrient imbalance, impaired ability to detoxify reactive oxygen species (ROS), differences in antioxidant enzymes and lower photosynthetic activity [18, 19, 20]. In salt-stressed plants, the reduction in osmotic potential can be due

Table 4: Simple phenotypic correlation coefficient among seed yield and yield-related traits of mungbean genotypes

Characters	Days to maturity	Plant height (cm)	Number of pod-bearing branches per plant	No. of pods per plant	Pod length (cm)	Number of seeds per pod	100-seed weight (g)
Seed yield per plant (g)	0.300***	0.178*	0.341***	0.327***	0.566***	0.459***	0.446***
Days to maturity		0.539***	0.483***	0.529***	0.179*	0.452***	0.266***
Plant height (cm)			0.401***	0.464***	0.154	0.266***	0.064
Number of pod-bearing branches per plant				0.786***	0.072	0.307***	-0.053
Number of pods per plant					0.058	0.308***	-0.072
Pod length (cm)						0.400***	0.440***
Number of seeds per pod							0.220**

*, **and *** indicates significant at and 5%, 1% and 0.1% level of probability, respectively

to alteration of inorganic ion (Na^+, Cl^-, and K^+) levels at the cellular level. At the whole plant level, the toxic effect of salt stress is the reduction plant productivity or in some cases the death of the plant. In the present study, efforts were made to investigate the effect of salt stress on seed yield and yield-related traits for twenty-six mungbean genotypes at the reproductive phase. The association among characters was also studied by correlation coefficient analysis. Principal component analysis and cluster analysis were also used to explore the variability of the studied characters and genotypes, respectively. Salt tolerance indices were also estimated to identify salt tolerant and salt susceptible genotypes.

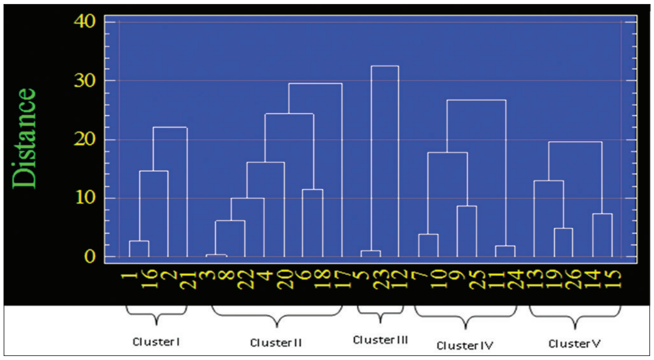

Figure 1: Dendrogram showing differentiation among 26 mungbean genotypes for yield components, according to Ward's method

Table 5: Number, percent of the genotypes in different clusters for yield-related traits

Cluster number	Number of genotypes	Percent (%)	Name of varieties
I	4	15. 38	V_{1} (BARI Mung-6), V_{2} (Binamoog-9), V_{16} (BMX 11176) and V_{21} (BMX 11140)
II	8	30.77	V_{3} (Binamoog-5), V_{4} (Binamoog-8), V_{6} (BMX 11148), V_{8} (BMX 11163), V_{17} (BMX 11116), V_{18} (BMX 11108), V_{20} (BMX 11154) and V_{22} (BMX 11111)
III	3	11.54	V_{5} (BMX 11159), V_{12} (BMX 11122) and V_{23} (BMX 11144)
IV	6	23.08	V_{7} (BMX 11165), V_{9} (BMX 1141), V_{10} (BMX 11153), V_{11} (BMX 1137), V_{24} (BMX 1153) and V_{25} (BMX 1131)
V	5	19.23	V_{13} (BMX 11107), V_{14} (BMX 11157), V_{15} (BMX 11170), V_{19} (BMX 11106) and V_{26} (BMX 1157)

Table 6: Average intra and inter-cluster distance among 26 mungbean genotypes for seed yield and yield-related traits

Cluster	I	II	III	IV	V
I	**96.26** **(9.81)**	154.21 (12.42)	331.28 (18.20)	158.44 (12.59)	152.57 (12.35)
II		147.03 (12.13)	391.61 (19.79)	212.53 (14.58)	216.52 (14.71)
III			202.33 (14.22)	227.10 (15.07)	264.18 (16.25)
IV				139.22 (11.80)	155.23 (12.46)
V					121.79 (11.04)

Results indicate that enormous phenotypic variability was present among the genetic material studied and genotypes displayed distinct variation in salt tolerance, which is very much desirable to the breeder for identification of suitable high-yielding genotypes for use in crop improvement programs, to enhance the grain yield of mungbean [21, 22, 23, 24]. Salinity caused significant reductions in plant growth, as compared to control plants during all growth stages (vegetative, flowering, and pod-filling) and it prevented plants from expressing their full genetic potentials. Salt stress caused a reduction in intra-cellular water potential and water scarcity around the root zone, with roots unable to absorb sufficient water and nutrients for adequate plant growth [25]. Under salt stress, the genotype BMX 11116 was found to be an early maturing genotype that was less affected by salt stress, whereas BMX 11122 was found to be late maturing genotype and more affected by salt stress (Table 2). The genotype BMX 11116 showed better performance than the other genotypes studied. In response to salt stress, genotype BMX 11116 showed the lowest plant height than the other studied genotypes importantly the genotypes had the lowest plant height under control condition also. A reduction in plant height due to salinity has also been found in other studies [6, 2, 26]. Under salinity stress, plant height decreased due to a loss of turgor in the meristematic tissues resulting from reduced water movement from the root zone [27]. In the present study, salt stress significantly reduced the number of pod-bearing branches per plant at harvesting stage. The reduction in the number of pod-bearing branches per plant was found to be higher in genotype BMX 1157 (59.50%) and lower in the genotype BMX 11176 (6.55%) (Table 3). Similar results of lower numbers of

Table 7: Stress tolerance indices in mungbean genotypes, estimated from seed yields obtained in a control and salt stress conditions

Genotypes	SSI	TOL	MP	STI	GMP	YI
BARI Mung-6	1.00	1.57	**2.54**	**1.03**	**2.42**	**1.40**
Binamoog-9	1.09	1.57	**2.28**	**0.81**	**2.14**	1.19
Binamoog-5	**0.75**	**0.81**	1.88	0.60	1.84	1.18
Binamoog-8	1.22	1.07	1.33	0.26	1.21	0.63
BMX 11159	1.76	2.43	1.71	0.26	1.21	0.40
BMX 11148	1.32	1.12	1.25	0.22	1.11	0.54
BMX 11165	**0.82**	**0.95**	**2.00**	0.67	1.94	1.21
BMX 11163	**0.75**	**0.86**	**2.01**	0.68	1.96	**1.25**
BMX 1141	**0.85**	1.02	**2.03**	0.68	1.96	1.21
BMX 11153	1.49	1.76	1.62	0.33	1.36	0.59
BMX 1137	**0.88**	1.03	1.96	0.63	1.89	1.15
BMX 11122	1.17	**0.81**	1.05	0.17	0.97	0.52
BMX 11107	**0.77**	**0.73**	1.65	0.46	1.61	1.02
BMX 11157	1.06	**0.87**	1.30	0.27	1.23	0.69
BMX 11170	0.97	1.11	1.87	0.56	1.78	1.04
BMX 11176	**0.60**	**0.74**	**2.25**	**0.87**	**2.21**	**1.49**
BMX 11116	**0.30**	**0.36**	**2.38**	**1.00**	**2.37**	**1.75**
BMX 11108	**0.97**	**0.64**	1.08	0.19	1.03	0.61
BMX 11106	1.18	0.98	1.28	0.25	1.18	0.63
BMX 11154	1.01	1.28	**2.03**	0.65	1.93	1.11
BMX 11140	**0.66**	1.05	**2.87**	**1.41**	**2.82**	**1.87**
BMX 11111	**0.73**	**0.79**	1.88	0.59	1.83	1.18
BMX 11144	1.39	1.42	1.45	0.28	1.27	0.59
BMX 1153	1.20	1.57	**2.00**	0.60	1.84	0.97
BMX 1131	**0.80**	1.00	**2.13**	**0.76**	**2.07**	**1.30**
BMX 1157	1.53	1.66	1.47	0.26	1.22	0.51

pod-bearing branches per plant for mungbean was also reported by others [28, 29, 30].

The number of pods per plant, pod length, number of seeds per pod, number of seeds per plant and seed yield per plant were also noted to significantly decrease in response to salt stress in most of the genotypes examined (Table 2). In support of our findings, decreases in yield and its associated characters in response to salt stress were reported in mungbean [31, 32, 33, 34, 28]. Low yield observed under salinity was probably due to the reduction of assimilate production, reduction of the mobilization of photosynthetic products in addition to altered cytosolic metabolism [35, 36, 37, 38, 39]. However, few genotypes showed better performance under salt stress conditions indicating their salt tolerance capabilities probably through the modulation of stress responsive pathways.

The progress in breeding programs for yield-related characters mainly depends on the availability of a large germplasm bank with a high level of genetic variation. In order to ensure the efficient and effective use of crop germplasm, characterization of this germplasm is imperative and multivariate analysis provides a good method of evaluation of landraces, by providing a rapid means of isolating phenotypes that should be further evaluated at the genetic level [40]. Subdividing the variance into its components contributes to genetic resource conservation and utilization and assists in planning for the use of appropriate gene pools in crop improvement for specific plant attributes [41]. In the present study, PCA indicated two principal components with Eigen values more than unity and accounted for 63.5% of the total variance in the data (Table 3) Here, a criterion was chosen to determine the cut off limit for the coefficients of the proper vectors, where this criterion treated coefficients greater than 0.3 as having a large effect and so to be considered important [42]. Traits having a lesser coefficient value than 0.3 were considered not to have important effects on the overall variation observed. Here, PC1 differentiated those plant/genotypes having more days to maturity, and greater plant heights (cm), number of pod-bearing branches per plant, number of pods and seeds with larger sizes and higher yielding plants. In PC2 the genotypes were differentiated according to the positive and negative values of the traits. However, the negative values only suggest the direction of the correlation between the component and the variable. So, the larger values of 100-seed weight (g), pod length (cm), number of pods per plant, number of pod-bearing branches per plant and seed yield per plant (g) play a stronger role in explaining the variation in PC2. Similar results were also reported by other researchers [43, 44, 45].

Estimation of the correlation coefficient is important to know the nature of relationships between and among these yield-related traits in order to improve yields. This will help breeders to develop effective breeding programs. Results from the phenotypic correlation coefficients (Table 4) revealed that seed yield per plant had a positive and significant correlation with days to maturity, plant height, number of pod-bearing branches per plant, number of pods per plant, pod length, number of seeds per pod, 100-seed weight, indicating the importance of these phenotypes for increasing yields. The significant and positive associations between these characters suggested that an additive genetic model was less affected by environmental fluctuation. Also, other positive and significant correlations were found for days to maturity with plant height, number of pod-bearing branches per plant, number of pods per plant, pod length, number of seeds per pod, 100-seed weight, which shows that early maturing genotypes had a significant contribution to increasing the traits; plant height with the number of pod-bearing branches per plant, the number of pods per plant, the number of seeds per pod, indicating that increasing plant height resulted in more pod-bearing branches that accommodated more pods per plant and significantly increased the number of seeds per pod; pod length with number of seeds per pod and 100-seed weight revealed that seed weight and the number of seeds increased with increased pod length. These results agreed with those of previous workers [46, 47, 48, 49]. Positive, but non-significant associations were for the number of pod-bearing branches per plant with pod length; the number of pods per plant with pod length which referred information of inherent relation among the pairs of combination. The negative and non-significant association referred a complex linked of relation among the pair of combinations. Overall the results of the present study indicate that selection of high yielding mungbean genotypes would be possible with positive increase in characters such as days to maturity, plant height, number of pod-bearing branches per plant, number of pods per plant, pod length, number of seeds per pod, 100-seed weight. These traits could be utilized via indirect selection so as to improve seed yields per plant. Therefore, correlation analysis revealed the relationship of the dependent variable yield with its independent variables, thus association of various mungbean traits would determine their relative significance to improve yields.

Suitable parent selection plays an important role in any successful plant breeding program. Parents having more genetic diversity are expected to exhibit higher genetic gains from selection. Based on D^2-values, genotypes were grouped into five clusters (Table 5). The distribution pattern revealed that cluster II contained 8 genotypes and was the largest, while cluster III had the smallest number of genotypes (3 genotypes) suggesting the existence of greater diversity among the genotypes in cluster II than in cluster III where they are more closely related. These results support the results of others [50, 51]. Results from intra and inter-cluster distances (Table 6) indicated that maximum inter-cluster distance was observed between genotypes of clusters II and III (19.79) followed by clusters I and III (18.20), indicating the more diversity between the genotypes present in these clusters. Hybridization between members of these clusters might be beneficial for producing heterotic responses with high variability in segregating generations. The closer distance between the genotypes of the other cluster groups indicated genetically closer genotypes. Similar results were reported by others [52, 50 53]. The non-hierarchical Euclidean clustering grouped the genotypes into five distinct clusters shown in the dendrogram (Figure 1), where salt tolerant mungbean genotypes were separated in cluster I. These genotypes could be marked for selection of salt tolerant genotypes and may be used in a breeding program to develop high-yielding salt-tolerant mungbean varieties.

Several selection criteria are proposed for selecting genotypes, with consideration to plant yield, based upon their performances under normal and salt stress environments. The SSI study (Table 7) demonstrated that genotype BMX 11159 had the highest value, whereas the lowest value was recorded for the genotype BMX 11116. Higher values of SSI indicate higher sensitivity to and a greater reduction in yield under salt stress conditions [54]. In contrast, lower values indicate a lower susceptibility to salt stress. Therefore, based on SSI, BMX 11116, BMX 11176, BMX 11140, BMX 11111, Binamoog-5, BMX 11163 genotypes could be considered as tolerant genotypes. Results of the TOL index showed that BMX 11159 genotype had the highest TOL value with the lowest value obtained by the genotype BMX 11116. A low TOL index value indicates higher tolerance to salt stress [55]. Selections made on this criterion are specific for genotypes with low yield potential under non-stress conditions and better yield potentials under stress conditions [11]. So, this criterion does not help us to separate genotypes yielding well under stress conditions, from genotypes yielding well under both stress and unstressed conditions. Based on the result of MP, the genotype BMX 11140 had the highest value followed by BARI Mung-6, BMX 11176, BMX 11116, Binamoog-9, BMX 1131, BMX 11154, BMX 1141, BMX 11163 and BMX 11165. It was reported that a positive correlation was present between MP and Ys (stressful environment), therefore selection based on MP will improve average yields under both stress and non-stress conditions [10]. Therefore, high MP can be used in the genotype selection process. A higher STI value for a genotype in a stressful environment means higher stress resilience and greater yield potential [56]. Thus, BMX 11140 followed by BARI Mung-6, BMX 11116, BMX 11176, Binamoog-9 and BMX 1131 genotypes that have higher values of STI, indicate tolerance to salt stress. The study of GMP showed that the genotype BMX 11140 had the highest value. Based on this index, genotypes with higher values were considered tolerant and had high yields under both normal and stress conditions [55]. In the present study, YI discriminated the genotypes BMX 11140, BMX 11116, BMX 11176, BARI Mung-6, BMX 1131 and BMX 11163 as the most tolerant genotypes. YI was particularly relevant to differentiate tolerant and sensitive varieties under saline conditions [57]. According to our results salinity significantly reduced the seed yield of some genotypes, while others were tolerant to salt stress. This indicates that sufficient genetic variability was present for salinity tolerance among the genotypes studied. Based upon the stress tolerance indices it was found that BARI Mung-6, BMX 11176, BMX 11116 and BMX 11140 were potential salt tolerant genotypes as they performed well under salt stress conditions.

In conclusion, significant variation in yield attributing traits was observed among the genotypes, treatments and their interactions. Imposition of salt stress resulted in a significant decrease in yield and yield-related traits, but the genotypes BMX 11163, BMX 11176, BMX 11116, BMX 11140 and BMX 11111 showed greater salt tolerance. PCA indicated that the days to maturity, number of pods per plant, number of pod-bearing branches per plant, number of seeds per pod and seed yield per plant are the most important characters contributing to the total genetic divergence. Correlation analysis revealed that seed yield per plant showed a significant positive correlation with days to maturity, the number of pods per plant, the number of pod-bearing branches per plant, the number of seeds per pod, pod length and 100-seed weight. Cluster analysis grouped the tolerant genotypes into cluster I and based on stress tolerance indices BARI Mung-6, BMX 11176, BMX 11116, BMX 11140 were categorized as tolerant genotypes. Therefore, these genotypes can be used for further testing in saline affected areas of Bangladesh for the development of salt tolerant and high yielding mungbean varieties.

AUTHOR'S CONTRIBUTION

Mohammad Anwar Hossain conceived the idea. Israt Jahan, Mohammad Anwar Hossain and Shamsun Nahar Begum contributed in designing of the experiment. Israt Jahan, Md. Musfiqur Rahman, Mst. Fatema Tuzzohora carried out the experiment and collected the data. Israt Jahan and Md. Anwar Hossain analysed the data and developed figures and tables. Israt Jahan and Md. Musfiqur Rahman wrote the draft manuscript and Mohammad Anwar Hossain and David J. Burritt critically read and evaluated the manuscript and rewrote the MS as required. All authors provided critical feedback and helped to shape the research, analysis and manuscript.

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