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Evaluation of Genetic Variability and Viability Association of Eight Mungbean (*Vigna radiata* L.) Varieties under Different Level of Salinity Stress

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ABSTRACT

An investigation was carried to study the mean performance, variability and the associations between characters in mungbean. For this study eight mungbean genotypes viz., ADT 2, BM-2002-1, ML 5, AKM 4, Vishal, Vamban, TARM 1 and Utkarsh were used. The experiment was conducted in the Seed Science and Technology (SST) Laboratory and at Pot culture yard, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil nadu from September 2016 to December 2017 with four salt treatments (0, 2, 4 and 6 DSM^{-1}) in Ramdomized Block Design with three replications. Observation were on seven seedling characters such as germination percentage, seedling shoot length, seedling root length, seedling shoot fresh weight, seedling root fresh weight, seedling shoot dry weight, seedling root dry weight and seven quantitative characters such as days to first flowering, plant height, number of branches/plant, number of pods/plant, number of seeds/pod, 100 seed weight and seed yield/plant. Four elite genotypes were found to be superior based on the mean performance namely Utkarsh, TARM 1, Vamban and Vishal among the various levels of salinity.

Keywords Mungbean, Salt tolerant, Randomized Block Design, Germination percentage.

INTRODUCTION

Pulses are rich in proteins 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 (Singh *et al.* 2014). Despite developing several cultivars suitable for specific agro-climatic zones, mungbean crop is affected by a wide range of biotic and abiotic stresses. Mungbean is generally known as a salt sensitive crop and have found that when mungbean encounters cumulative adverse environmental effects such as insects, pests, high temperature, pod-shattering along with salinity causing high yield loss with significantly higher substantial growth reduction (Bindumadhava *et al.* 2016).

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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. Because of the 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 (Hasanuzzaman *et al.* 2013 and Sunil Kumar *et al.* 2012). This study, therefore, aimed at investigating the different agronomic performance of some accessions of mungbean to select for salt tolerance with the objectives of identifying salt stress tolerant accessions of mungbean based on their mean performance.

MATERIALS AND METHODS

The experiment was conducted in the Seed Science and Technology (SST) Laboratory and at Pot Culture Yard, Department of Genetics and Plant Breeding, Faculty of Agriculture Annamalai University, Annamalai Nagar, Tamil Nadu during January 2016 to December 2017. For this study, eight mungbean genotypes collected based on their geographical and ecological adaptations to semi-arid and semi-humid regions of India such as ADT 2, BM-2002-1, ML 5, AKM 4, Vishal, Vamban, TARM 1 and Utkarsh were used. The laboratory experiment was made to screen best performing accessions to assess the salt tolerance of mungbean at germination and seedling growth such as germination percentage, seedling shoot and root length, seedling shoot and root fresh weight and seedling shoot and root dry weight.

The pot culture experiment focused on evaluation of variation in morphological, yield and yield related traits such as days to flowering, plant height, number of branches/plants, number of pods/plants, number of seeds/pods, 100 seed weight and seed yield/plant of mungbean accessions. The design of both experiments was Randomized Block Design (RBD) with three replications for each treatment and control. The experiment was conducted using plastic earthen pot of 19 cm width at the base, 20 cm at the top and 18 cm height in pot culture yard, with four salt treatments (0, 2, 4 and 6 dSm^{-1}). The soil was analyzed in Soil Science Laboratory, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University sandy loam soil was sieved through a four mm sieve to get rid of large particles. The seeds were surface sterilized. The pots were filled with 3 kg sandy loam soil and seven sterilized seeds were sown in each pot and irrigated with tap water for three weeks at three days interval after sowing (Sunil Kumar *et al.* 2012). Then, each pot was treated with 360 ml of selected salt solutions for one month in three days intervals (Esechie *et al.* 2002). Equal amount of tap water was used to irrigate the control pots for the same duration.

Data on morphological and agronomical characters such as Germination percentage (GP), Seedling shoot length (SSL), Seedling root length (SRL), Seedling shoot fresh weight (SSFW), Seedling root fresh weight (SRFW), Seedling shoot dry weight (SSDW), Seedling root dry weight (SRDW), Days to first flowering (days), Plant height (cm), Number of branches/plant, Number of pods/plant, Number of seeds/pod, 100 seed weight (g) and Seed yield/plant (g) were collected from three randomly selected plants per pot and their mean data was recorded for all observations. The data was analyzed using R studio software version 5594.2.3.0.

RESULTS AND DISCUSSION

Germination percentage (GP)

The germination percentage was calculated using the following formula

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

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 sa-

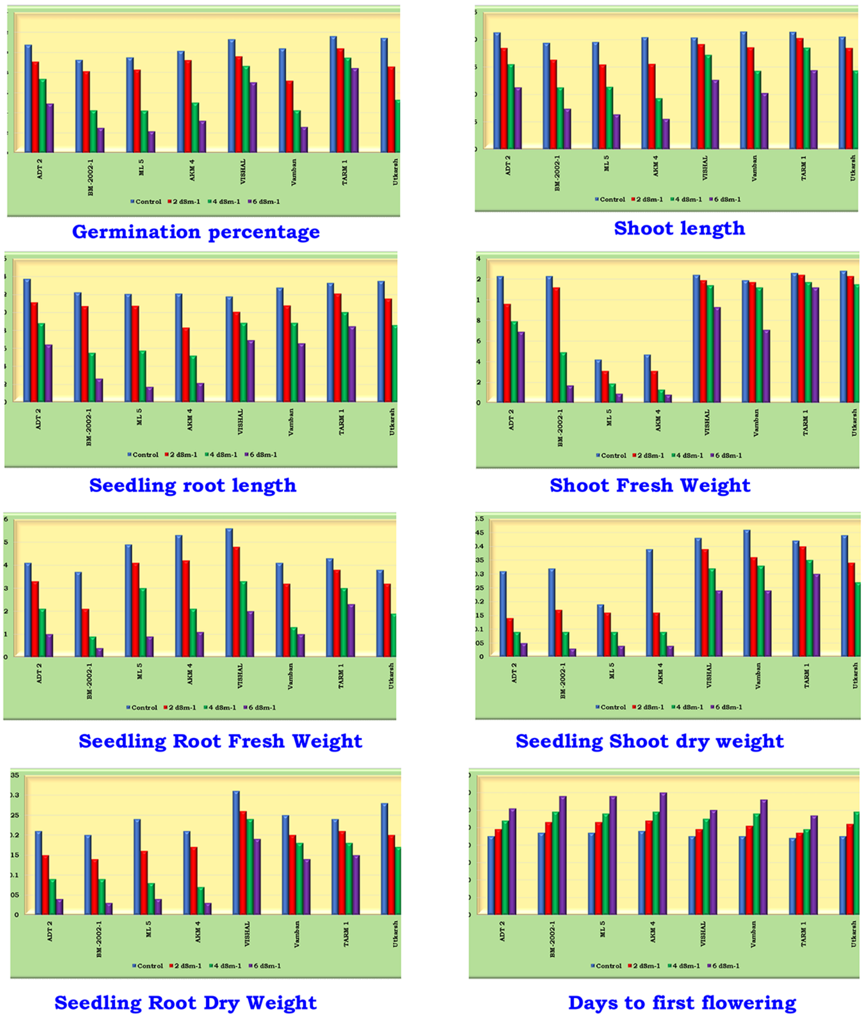

Fig. 1. Mean performance of elite genotypes among the various levels of salinity under germination percentage, shoot length, seedling root length, shoot fresh weight, seedling root fresh weight, seedling shoot dry weight, seedling root dry weight, days to first flowering.

linity levels, the germination percentage ranged from 96.67 (Vishal and TARM 1 at control) to 36.37 (BM-2002-1 at 6 dSm^{-1}) (Fig. 1). The genotypes Vishal, TARM 1 and ADT 2 exhibited consistent germination percentage across all the salinity levels. The present results were in line with the findings of Swarnakar (2016) and Keshtiban *et al.* (2015) who reported salt stress decreased germination percent. Sunil Kumar *et al.* (2012) 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 *et al.* (2010) who reported that germination percent of mungbean was reduced with increasing salinity.

Seedling shoot length (SSL)

SSL was measured in cm from the seed to the tip of the leaf blade of five randomly selected seedlings using draftsman ruler. 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 (Fig. 1). 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 (Sen Kumar and Mandal 2018, Keshtiban *et al.* 2015, Hakim *et al.* 2011). Further, secondary cell wall appears sooner and cell wall becomes rigid, as a consequence of which the turgor pressure efficiency in cell enlargement decreases. This process may cause the SSL to remain small (Naser *et al*.2009). 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 were also in parallel to those of Sunil Kumar *et al.* (2012) in mungbean and Kandil *et al.* (2012) on chickpea. The finding of the present study indicated that as salinity level increases from 4 dSm^{-1} to 6 dSm^{-1}, the mean SSL was reduced highly in all mungbean accessions studied.

Seedling root length (SRL)

It was measured in cm from the seed to the tip of the root of five randomly selected seedlings using draftsman ruler. Significant differences between genotype, treatments and their interaction on seedling root length. The highest SRL (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 (Fig. 1). 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 was in line with the studies of Keshtiban *et al.* (2015) and Shitole and Dhumal (2012) 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 Swarnakar (2016), Arslan *et al.* (2016), Haleem (2015) in mungbean and Hakim *et al.* (2011) 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 (Yousofinia *et al.* 2012). In this study, with the increment of salt concentration, shoot 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.

Seedling shoot fresh weight (SSFW)

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. The mean seedling shoot fresh weight ranged from 0.42 to 1.28 dSm^{-1} in control, 0.31 to 1.24 in 2 dSm^{-1}, 0.13 to 1.17 in 4 dSm^{-1} and 0.08 to 1.12 in 6 dSm^{-1} salt concentrations (Fig. 1). 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 seemed that reduction in SSFW may be due to decreasing water uptake by seedling under salinity. Salt decreases the osmotic water potential, creating a water stress in seedlings. Reduction of SSFW 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 (Jamil *et al.* 2006). The present findings were in agreement with Swarnakar (2016), Arslan *et al.* (2016), Haleem (2015) in mungbean which indicated that there was a rapid decrease in SSFW of leguminous plants under saline conditions.

Seedling root fresh weight (SRFW)

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. As the salinity level increased, the seedling root fresh weight (SRFW) was gradually reduced. The mean SRFW ranged from 0.37 - 0.56 g in control, 0.21 - 0.48 g in 2 d m^{-1}, 0.21 - 0.48 gram in 4 dSm^{-1} and 0.04 – 0.23 g in 6 dSm^{-1} (Fig. 1). At control, Vishal (0.56 g) had the highest value of SRFW. Similarly, at 2 dSm^{-1} of NaCl also Vishal achieved the highest mean of SRFW with reduction compared to the control while accession BM-2002-1 had the lowest value with 56.67% reduction. At 4 dSm^{-1} salinity level, accession Vishal (0.33 g) followed by TARM 1 (0.30 g) and ML 5 (0.30 g) performed well compared to other genotypes, whereas the genotype BM-2002-1 performed poorly compared to the grand mean. At 6 dSm^{-1} salinity level, the genotype TARM 1 (0.23 g) and Vishal (0.20 g) had relatively higher values while, the genotypes BM-2002-1 and ML 5 had lower value of 0.04 and 0.09 g, respectively. Furthermore, increasing salinity levels to 2, 4 and 6 dSm^{-1} NaCl significantly reduced SRFW when compared with the control treatment. Okcu *et al.* (2005) indicated that decrease in seedling fresh and dry weights 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. Swarnakar (2016), Arslan *et al.* (2016), Haleem (2015) in mungbean, Heshmat *et al.* (2011) in cow-pea and Armin *et al.* (2010) 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 (Jamil *et al.* 2006).

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 hrs and the average data was recorded. The reduction in seedling shoot dry weight (SSDW) 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 SSDW reduction percentage comparing with control (Fig. 1). This result was in close conformity with the finding of Karimi *et al.* (2011) who claimed that increasing salinity levels remarkably decreased SSDW. This may be a result of a combination of osmotic and specific ion effect of Cl^- and Na^+ (Taffouo *et al.* 2010). The reduction in SSDW 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 *et al.* (2011) claimed that increased salinity levels caused remarkable decrease in SSDW. The present study was also in line with earlier stud-ies in mungbean by Swarnakar (2016), Arslan *et al.* (2016), Haleem (2015) and in safflower by Ghazizade *et al.* (2012) reporting that adding NaCl resulted in decrease in seedling shoot dry weight.

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 hrs and weighing them using sensitive balance and the average data was recorded. The results of this study showed that the SRDW 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 (Fig. 1). The mean SRDW decreased as salinity level increased. However, the reduction varied among the accessions and salinity levels studied. The present investigation is in agree-ment with previous reports by Kinfemichael (2011) in haricot bean and Akram *et al.* (2007) 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 Swarnakar (2016), Arslan *et al.* (2016), Haleem (2015) in mungbean and Taghipour and Salehi (2008) claimed that SRDW of Iranian barley decreased as the result of salt stress which reported that decrease in SRDW 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.

Days to first flowering (days)

Number of days taken for first flowering of the plants to flower from the date of sowing was calculated for each genotype in each replication. The mean days to first flower (DFF) variation ranged from 44 – 48 days, 47 – 54 days; 49 – 59 days and 57 – 68 days in control, 2 dSm^{-1}, dSm^{-1} and 6 dSm^{-1}, respectively (Fig. 1). The highest DFF (68 days) was found for the genotypes BM-2002-1 and ML 5 at 6 dSm^{-1} salinity level and the lowest for the genotype TARM 1 (44 days) in the control. On the other hand, genotype Vishal, Vamban and ADT 2 showed intermediate values and the genotype TARM 1 showed the smallest mean value of 57 days. This result indicates accession TARM 1 was found to be salt tolerant with respect to its control. Generally, in greenhouse experiment, the increment of salinity level delayed DF of mungbean accessions. In view of the above result the lower value for DF showed that accessions were earlier flowering with respect to their respective controls. On the other hand, accessions with higher values for DF showed delayed DF compared to their respective controls. The present finding agrees with Amador and Dieguez (2007) and Hadi *et al.* (2012) who reported that increase in salinity levels delayed days to first flowering in cowpea. It may be due to the fact that high salinity increased the duration for vegetative and reproduction growth due to salt stress and high osmotic pressure of salts in the cells of plants.

Plant height (cm)

The height of the plant was measured at the time of harvest from the ground level to the tip of the panicle and expressed in centimeters. Mean values for plant height ranged from 32 - 36 cm in control, 30 - 34 cm in 2 dSm^{-1}, 20- 31 cm in 4 dSm^{-1} and 12 - 28 cm in 6 dSm^{-1} (Fig. 2). The accession TARM 1 exhibited good performance regarding plant height in all salinity levels than all the other genotypes and may be considered as salt tolerant whereas accession Utkarsh showed moderate to weak performance among the different salinity levels. The reductions of plant height in these accessions as compared to their respective controls were more pronounced at 4 and 6 dSm^{-1} NaCl concentrations.

The present result is in close conformity with the findings of Hadi *et al.* (2012) in cowpea and Hussain *et al.* (2008) in mungbean. The present results were also in line with the findings of Mahmood *et al.* (2009) who reported linear reduction in plant height in rice with increasing NaCl salinity. The reduction in PH of the mungbean accessions correlated with increased NaCl concentration in growth medium but, the reduction level was low in the tolerant and more in the sensitive accessions. The reason behind this may be under soil salinity, the osmotic pressure in soil solution exceeds the osmotic pressure in plants cells due to the presence of higher concentrations of salts, and thus reduces the ability of plants to take up water and minerals. Plants need to regulated ion concentration in various organs and within the cells by synthesis of organic solutes for osmoregulation or protection of macromolecules for maintenance of membranes integrity (Maghsoudi and Maghsoudi 2008).

Number of branches/plant (NBP)

The number of branches/plants were counted at the time of maturity. The number of branches /plants ranged from 4 - 6 in control, 3 -5 in 2 dSm^{-1}, 2 - 4 in 4 dSm^{-1} and 1 - 3 in 6 dSm^{-1} (Fig. 2). The result of the present study indicated that as salinity levels increased number of branches/plants decreased considerably. The present result showed that increasing NaCl concentration led to reduction in number of branches in all the genotype tested. This result is in agreement with the finding of (Tarinejad *et al.* 2013) in canola who reported a decrease in NBP by increasing salinity. It seemed that the cultivars with more branches have higher number of secondary pods, higher number of main pods and more biomass weight, so that they enhance yield.

Number of pods/plants

The total number of pods/plants was counted at the time of harvest. The number of pod/plants performed well under control and five genotypes out performed than the grand mean of 4.88 under control, the number of pods/plants ranged from 6 (TARM 1 and Utkarsh)

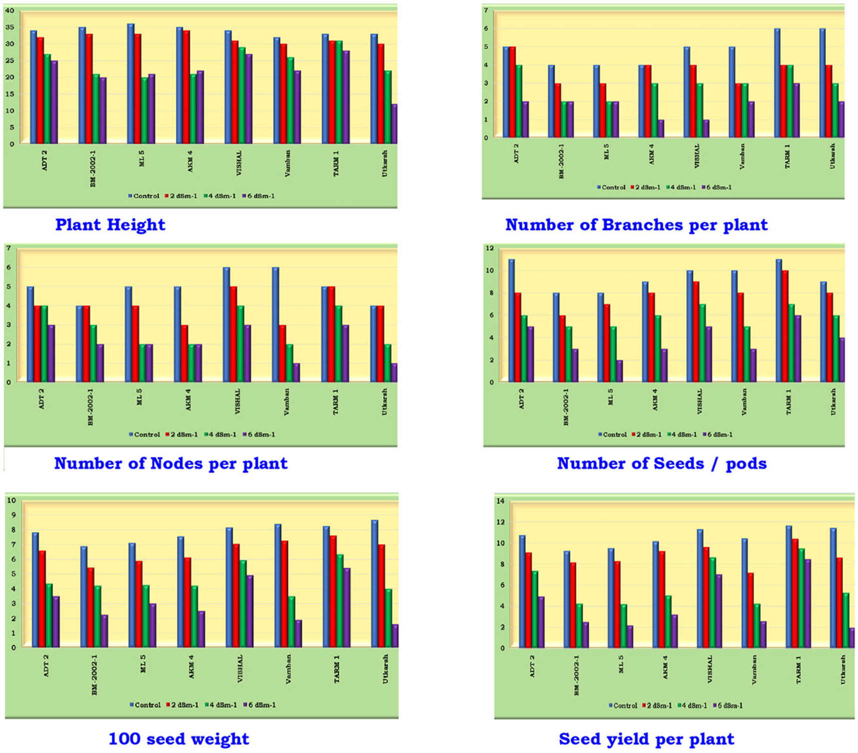

Fig. 2. Mean performance of elite genotypes among the various levels of salinity under plant height, Number of branches per plant, Number of nodes per plant, Number of seeds / pods, 100 seed weight seed yield per plant.

to 4 (BM-2002-1, ML 5, AKM 4 and Utkarsh) (Fig. 2). At 2 dSm^{-1}, the genotypes ADT 2 followed by AKM 4, Vishal, TARM 1 and Utkarsh produced the maximum number of pods/plants with the same values of 5 and 4, respectively and they showed reduction as compared to the control. Genotypes with high mean number of pods/plants were considered as salt tolerant and those with low mean NPP considered as salt sensitive. All accessions responded differently to 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} NaCl salinity levels. The result of the present investigation regarding the effect of salinity on the mungbean accessions indicated that as salt concentration increased NPP decreased linearly. Reduction in crop yield as a result of salt stress has been reported by Manasa *et al.* (2017) in mungbean and Sohrabi *et al.* (2008) for chickpea. The present study which is the reduction in NPP affected by salinity stress is consistent with the findings of (Waheed *et al.* 2006) in pigeon pea, (Hadi *et al.* 2012) in cowpea and (Tarinejad *et al.* 2013) in canola. Similar result was obtained by Ahmed (2009) who reported that salt stress was more severe at vegetative, flowering and seed filling stages compare to seed development stage in mungbean accessions.

Number of seeds/pods

The total number of seeds/pods was counted at the time of harvest. The highest number of seeds/pods was recorded for accession TARM 1 and ADT 2 with the value of 11 in the control whereas the lowest number of seeds/pods was recorded for the genotypes BM-2002-1, AKM 4 and Vamban with the value of 3 in 6 dS m^{-1} NaCl salinity level (Fig. 2). Ahmed (2009) reported reduced flowers, pods and seeds under salinity stress. Gradual increase in salinity levels not only linearly reduced the number of pods but also reduced the number of seeds. The present result was also in close conformity with the finding of Hadi *et al.* (2012) in cowpea and Hossain *et al.* (2008) in mungbean.

100 seed weight (g)

One hundred randomly selected seeds from each genotype was weighed and expressed in grams. The mean 100 seed weight of tested genotype varied with the different NaCl salinity levels. 100 seed weight of tested genotype ranged from 6.89 to 8.67, 5.42 to 7.61, 4.00 to 6.33 and 1.59 to 5.41 g in control, 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1} salt concentrations, respectively (Fig. 2). The highest 100 seed weight was recorded in the control (8.67 g for Utkarsh) whereas, the lowest value of 100 seed weight was recorded at 6 dSm^{-1} (1.59 g for Utkarsh). Increasing salinity levels caused remarkable decreases in 100 seed weight, although all genotype showed different responses to each salinity level. Manasa *et al.* (2017) and Ghassemi-Golezani *et al.* (2009) reported that grain filling duration decreased with increasing salinity which resulted in decreasing final grain weight. The effect of salt might lead to shriveled seeds and consequent lower yield. Thus, it may be concluded that reduced yield under salt stress may be due to reduced efficiency per day of plant to fill the developing seeds, which may lead to reduced number of seeds per pod/or plant and dry matter yield of individual seeds.

Seed yield/plant (g)

Seeds from five randomly selected plants were harvested from each genotype in each replication. They were cleaned and dried to a constant moisture level and weighed in grams. Salinity causes reduction of seed yield /plant in mungbean accessions at all salinity levels which varied among accessions. Seed yield/plant varied from 9.26 to 11.36 g, 7.18 to 10.38 g, 4.21 to 9.48 g and 1.19 to 8.43 g in control, 2 dSm^{-1}, 4 dSm^{-1} and 6 dSm^{-1}, respectively (Fig. 2). Salinity can severely limit crop production because high salinity lowers water potential and induces ionic stress and results in a secondary oxidative stress. Reductions in grain yield as a result of salt stress have also been reported for some other crop species (Manasa *et al.* 2017 and Sohrabi *et al.* 2008). The depressive effect of salinity on yield may be attributed to the inhibitory effect of salinity on vegetative growth. Moreover, Taffouo *et al.* (2009) reported that, the significant decrease of yield components observed under salt stress in cowpea would be partly related to a significant reduction of foliar chlorophyll contents (more than 50%) and K^+ concentration in saline medium. These results were in close conformity with the work of Tarinejad *et al.* (2013)) on canola.

CONCLUSION

The present experiments have generated considerable quantum of information on the mean performance. Four elite genotypes were found to be superior based on the mean performance namely Utkarsh, TARM 1, Vamban and Vishal among the various levels of salinity which could be recommended as parents in salinity breeding program.

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