Seedling Vigor: An Important Criterion for the Selection of Salt Tolerant Lines for Mungbean [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Mungbean is an important food grain legume with high economic status and significant nutritive values. Productivity of this crop is rigorously limited due to prevailing environmental stress particularly, salinity stress. Climate changes raise the serious concern to develop improved mungbean cultivars with more adaptability and resistance towards salinity stress. Present study aimed to screen 15 mungbean accessions for salt tolerance under different salinity treatments i.e. $EC_{4.0}$, $EC_{7.0}$, $EC_{10.0}$ and $EC_{16.0}$ (dS/m) along with control (EC_0) at early seedling growth stage. The seed germination % and early seedling growth characteristics i.e. radical, plumule and total seedling length, dry weight of the seedlings, seedling vigor and salt stress index on 4th day after germination (DAG) were investigated. The result showed that all traits decreased gradually with increasing level of salt stress in all the genotypes. The genotypes showed variations for all the measured features within themselves and at different salt stress levels. The radical length was found to be more adversely affected due to salinity stress as compared to the plumule length during the first developmental stage of crop plants. The optimization of salt stress level was done below $EC_{10.0}$ dS/m for mungbean salt tolerance studies. The seedling vigor was found to be more desirable criterion on the basis of which available crop germplasm can be screened for salt tolerance. Highly divergent germplasm may provide promising salt resistance lines for breeding programs in near future.

Keywords: Mungbean, salinity, seed germination, seedling growth characteristics, salt stress index, seedling vigor

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INTRODUCTION

Mungbean (*Vigna radiate* (L.) Wilczek), is an important short duration pulse crop with short life span (60−90 days) domesticated in India (Kim *et al*., 2015). It is self−pollinated diploid crop with 2n = 2x = 22 chromosomes and small genome size (579 Mb) (Somta and Srinives, 2007). It belongs to the genus *Vigna*, subgenus *Ceratotropis* which consists of total 23 species (Tomooka *et al*., 2010; Aitawade *et al*., 2012). It provides rich source of protein, essential amino acids, fatty acids, fibers, vitamins and minerals for the vegetarian diet of Asians, particularly in South and South−east Asia where nearly 90% of the world's mungbean is produced annually (Tomooka *et al*., 2002; Fuller, 2007; Kang *et al*., 2014). Capacity to restore soil fertility by fixing atmospheric nitrogen makes it an excellent green manure crop and valuable in various cropping systems (Somta and Srinives, 2007; Algan and Çelen, 2011; Singh and Kaur, 2012). This crop can be used for both food and forage because it can produce a large amount of biomass and then recover after grazing to yield abundant seeds. India is the largest producer of pulses in the world and accounts for more than 50% of the global annual production of mungbean (Lambridge and Godwin, 2007; Nair *et al*., 2012). At global level, various mungbean research programs are being conducted for genetic improvement of mungbean against environmental stresses (AVRDC, 2015).

Salinity stress is a major constraint in the production of this crop where 50 mM NaCl can cause yield losses up to 80% (Salim and Pitman, 1988; Saha *et al*., 2010; Sehrawat *et al*., 2013a; Sehrawat *et al*., 2013b; Sehrawat *et al*., 2014a). Sodium ions in saline soils are toxic to plants because of their adverse effects on potassium ion nutrition, cytosolic enzyme activities, photosynthesis, and metabolism (Jacoby, 1999; Hasanuzzaman *et al*., 2012). Nearly 20% of the irrigated land is affected by salinity which is increasing day by day (Munns and Tester, 2008; Qadir *et al*., 2014). The increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of the twenty−first century (Mahajan and Tuteja, 2005). The domestic consumption of mungbean has increased because of the rising popularity in Indian ethnic foods and perceived health benefits. Low productivity of mungbean emphasizes that there is a great need to genetically improve this crop with better adaptations and yield production in salt−affected soils. But very little work has been reported to develop mungbean cultivars adapted to salinity stress until recently (Singh and Singh, 2011; Sehrawat *et al*., 2013b; 2014b; 2016). Moreover, the non−availability of an appropriate technique and reliable parameter for screening further restrict the development of salt tolerance in the target crop. Salt tolerance is a polygenic, highly intricate trait dependent on genotype and plant developmental stage. Hereditarily diverse germplasm resistant to salt stress within *Vigna* genotypes may help to study the mechanism governing salt tolerance and for the delivery of genetic resources for salinity in breeding program (Win *et al*., 2011; Sehrawat *et al*., 2015; Sehrawat *et al*., 2016). Various cultural practices have been implemented for the evaluation of salt tolerance in legumes with plant material ranges from germinating seeds to seedlings to mature plants (Ashraf and Waheed, 1990; Bayuelo−Jimenez *et al*., 2012; Sehrawat *et al*., 2013a; Sehrawat *et al*., 2014c). The degree and nature of damage to the crop plants and the impact on yield depends upon the nature, extent, duration of the stress and the plant growth stage at which a crop comes across (Sehrawat *et al*., 2013c; 2014d). Development of salt tolerant mungbean cultivars is only the promising alternate to reduce the lethal effects of soil salinity on crop production (Epstein *et al*., 1980; Sehrawat *et al*., 2015; Sehrawat *et al*., 2016). The ability of seeds to germinate under stressed environment can be correlated to plant growth, survival and production during later stages (Ashraf and Harris, 2004; Kandil *et al*., 2012; Sehrawat *et al*., 2014c). Keeping the importance of all these facts in consideration, the present study was undertaken with the following objectives were to determine the part of mungben seedling greatly influenced by variable saline conditions during early seedling growth stage, determine the most significant parameter that can be used as an effective selection criteria for mungbean genotypes under salinity stress and optimize the salinity stress level for mungbean under which the crop can produce significant yield.

MATERIALS AND METHODS

Plant Material and Salinity Stress Levels

The seeds of 15 genotypes of mungbean (MGG 295, MGG 351, MGG 348, PLM 707, PLM 891, WGG 37, PDM 11, LGG 407, MGG 336, ET 52194, LGG A60, LGG 410, LGG 450, LAM M2 and TM 96−2) were procured from core collection at National Bureau of Plant Genetic Resources (NBPGR), New Delhi, for the present study. A total of five different salinity levels were prepared as EC_0, $EC_{4.0}$, $EC_{7.0}$, $EC_{10.0}$ and $EC_{16.0}$ (dS/m) in irrigation water from mixture of salts (NaCl, $Na_2SO_4^{2-}$, $CaCl_2$, and $MgSO_4^{2-}$) in defined proportions i.e. Na:Ca, Mg (2:1) and Cl: SO_4 (3:1) for EC = 1.0 dS/m, for imposing the salinity stress in mungbean genotypes. The solution of 10 m Eq/L = $EC_{1.0}$ dS/m which was prepared by mixing of NaCl (5 m Eq/L); $Na_2SO_4^{2-}$ (1.75 m Eq/L), $CaCl_2$ (2.5 m Eq/L) and $MgSO_4^{2-}$ (0.75 m Eq/L).

Seed Germination and Seedling Growth Characteristics

The seeds of all fifteen mungbean accessions were allowed to germinate on filter paper moistened with 10 ml of saline solutions of different concentrations i.e. $EC_{4.0}$, $EC_{7.0}$, $EC_{10.0}$, and $EC_{16.0}$ (dS/m) along with their respective control (EC_0) in petriplates consisting of 10 seeds / genotype / treatment. The experiment was carried out with four replicates per salinity treatment. The germination % and the seedling growth feature (radical, plumule and total seedling length) and dry weight of the seedling were measured on 5th day after seed germination. Dry weights were measured after drying the samples in a hot air oven at 80°C for three days as per the method of Veli *et al*. (1994). Salt stress index (SSI) [(dry weight of salt stressed seedling/dry weight of control seedling) x 100] as per the method of Sopha *et al*. (1991) and seedling vigor (seed germination percentage × total seedling length) were calculated. All the observations were mean of four replications. The seedlings of the genotypes WGG−37, PLM−891, LGG−A60 and LAM−M2 were transferred to pots containing soil and farmyard manure (3:1) for further evaluation of the optimum salinity stress level for mungbean. Scheduled irrigation was maintained with the similar saline solutions during the crop season to check the salinity stress level under which the genotypes could survive and can produce significant yield. The visual effects of the salinity treatment on growth (plant height, number of trifoliate, leaf size and tip burning) and survival of genotypes were observed. The electrical conductivity of the soil saturation paste (EC_e) was observed by Conductivity Bridge (EC meter) at regular period of time according to the method of Jackson (1973). The data calculated was further subjected to statistical analysis, ANOVA appropriate to the experimental design using OPSTAT program (HAU, Hisar, India). F−test was carried out to test the significance of the treatment differences and the least significant difference (LSD) was computed to test the significance of different treatment at 5% level of probability.

RESULTS AND DISCUSSION

The result revealed significant variability in response towards salt stress for all the observations examined in this study. A clear genetic difference was observed among all the genotypes due to salinity treatments for seed germination %, seedling growth and biomass features over the control.

Germination Assay

The results showed that the germination percentage was gradually decreased with increasing salinity stress levels in all the mungbean genotypes but the response was variable (Figure 1). Seed germination is the first stage of crop development during which salinity stress can be encountered. All seeds of the genotypes PLM−891 and PDM−11−mung were germinated (100%) even at high salinity level on first day followed by WGG37 under all the salinity treatments showing their resistance towards salt stress. However the greater reduction in seed germination was observed in LGG A60 followed by LGG 410 and TM−96−2 genotypes as compared with their respective control plants showing their highly sensitive behavior.

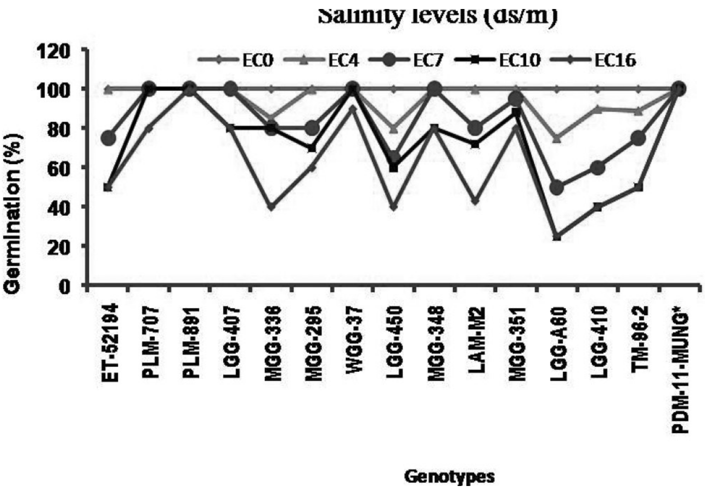


Figure 1 Effect of different salinity treatments on germination % in mungbean genotypes

Seedling Growth Characteristics

The radical and plumule length of 5−day old seedlings revealed marked changes in all the genotypes due to salinity stress and there was increase in decline percentage with increasing salinity treatments. However, the effect was significantly varied among all the genotypes and under different salinity levels. As a result of decrease in radical and plumule length, the percentage decline in total seedling length was found to be increased with the increasing salinity treatments in all the genotypes (Figure 2). The genotype PLM−707 showed minimum reduction of 18.52%, 31.82% and 24.49% for radical, plumule and total seedling length respectively under high salinity stress level ($EC_{16.0}$ ds/m) whereas the maximum reduction in all the measured seedling growth characters was observed for ET−52194 (100%) followed by LGG−A60 (89.29%). The genotypes PLM−707 and WGG−37 maintained less reduction in seedling growth showing their more resistance towards salt stress than the susceptible accessions LGG−A60, LGG−407, MGG−351 and TM−96−2, which showed reverse response (Figure 3A to 3C). The genotype PDM−11−mung tried to maintain less reduction in all over seedling growth even under high salinity level showing its ability to adapt in stressed environment. The genotypes MGG−351and ET−52194 showed greater reduction in radical length as compared to the plumule length but on an average the total seedling length was decreased more that showed their salt susceptibility. The radical growth was more adversely affected under high salinity (≥ $EC_{10.0}$ dS/m) with an average reduction of 89.29% (at $EC_{10.0}$) up to 100% (at $EC_{16.0}$) in the genotype ET−52194 over control than the plumule length which showed an average reduction ranged from 74.76 % (LGG−A60) up to 100% (ET−52194) at $EC_{10.0}$ and $EC_{16.0}$ (dS/m) respectively.

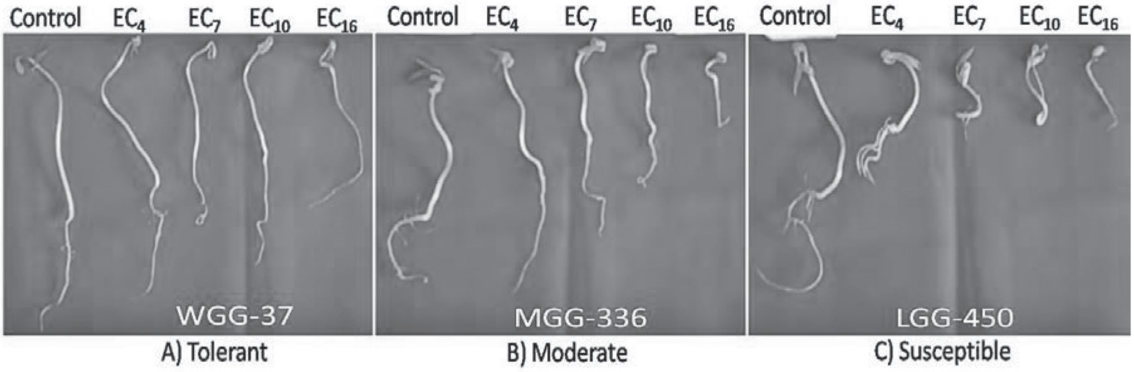
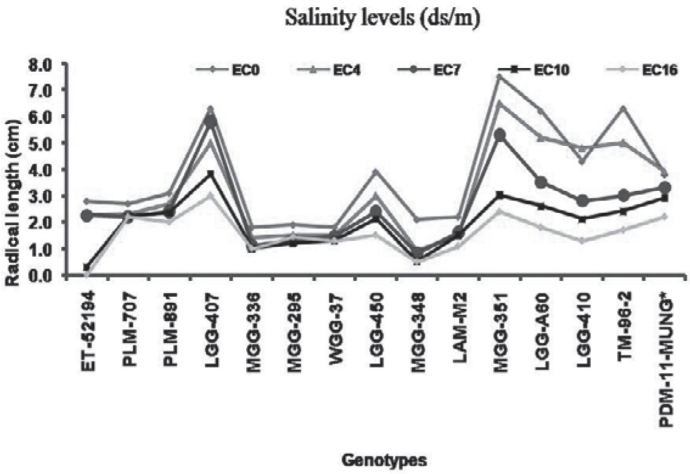
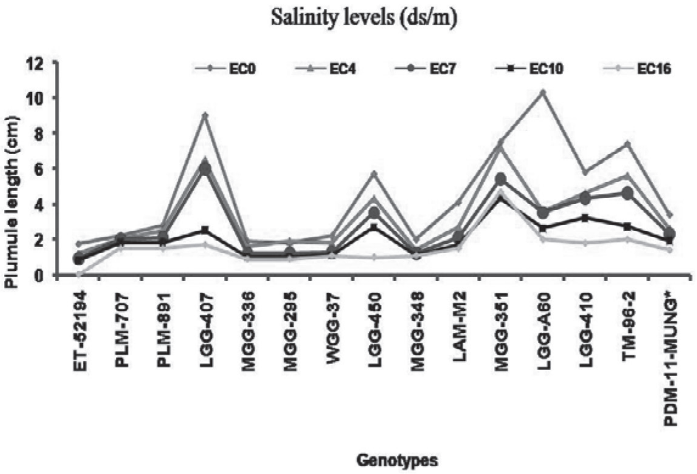


Figure 2 Effect of different salinity level on seedling growth on 4th DAG in mungbean genotypes



(A)



(B)

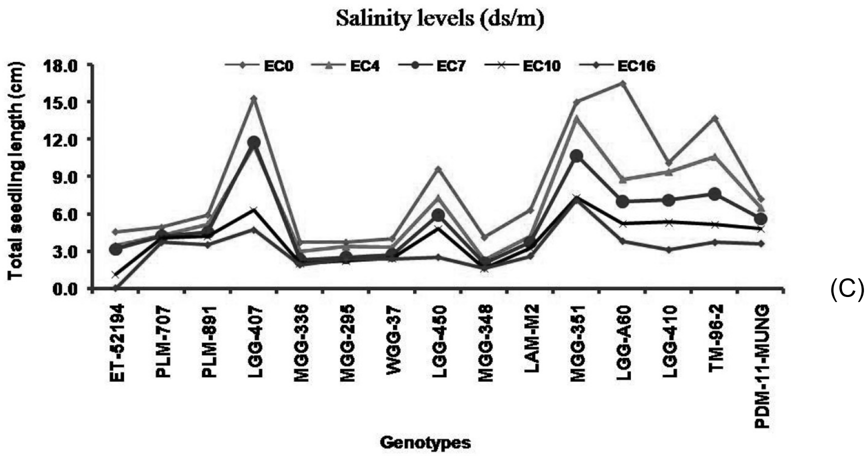


Figure 3 Effect of different level of salinity on seedling growth characteristics (radical, plumule and total seedling length) in different genotypes of mungbean (A, B and C respectively)

Dry Weight and Salinity Stress Index (SSI)

Generally dry weight decreased with increasing level of salinity as a result of decrease in total seedling growth. In this study, the dry weight of the seedlings was significantly decreased in all the genotypes under all salinity treatments but the genotypes MGG−351, WGG−37, and TM−96−2 showed less reduction in dry weight where greater biomass was maintained by WGG−37 as a result of less decrease in water content due to salinity (Figure 4). The genotype LGG−407 showed greater reduction in dry weight even under low salinity treatment followed by PDM−11−mung, where decrease in water content was more showing their high sensitivity towards salt stress. Salinity treatments decreased salinity stress index (SSI%) which is an important characteristic when selecting a variety for salinity tolerance but the genotypes as TM−96−2, WGG−37, and PLM−707 showing less decrease in dry weight maintained higher value of SSI (%) showing their more resistance towards salt stress (Figure 5). However, the genotypes LGG−A60, LGG−450, and MGG−336 showed greater decline in SSI even under low salinity level leading to their greater susceptibility towards salt stress.

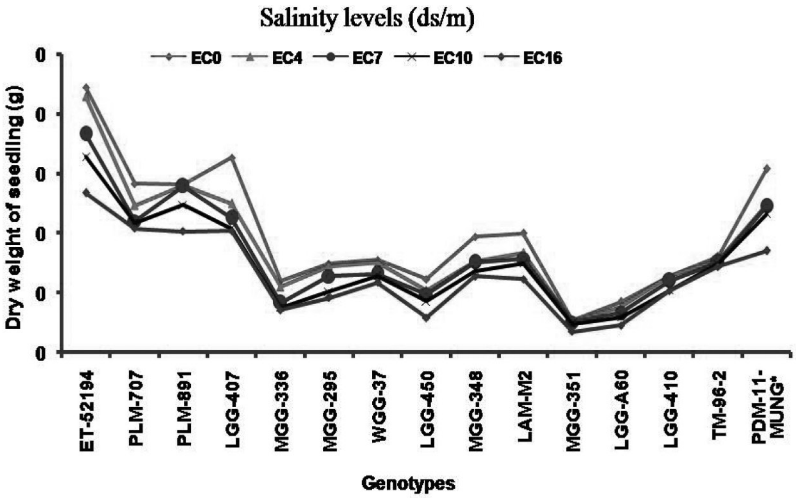


Figure 4 Effect of different salinity treatments on seedling dry weight in mungbean genotypes

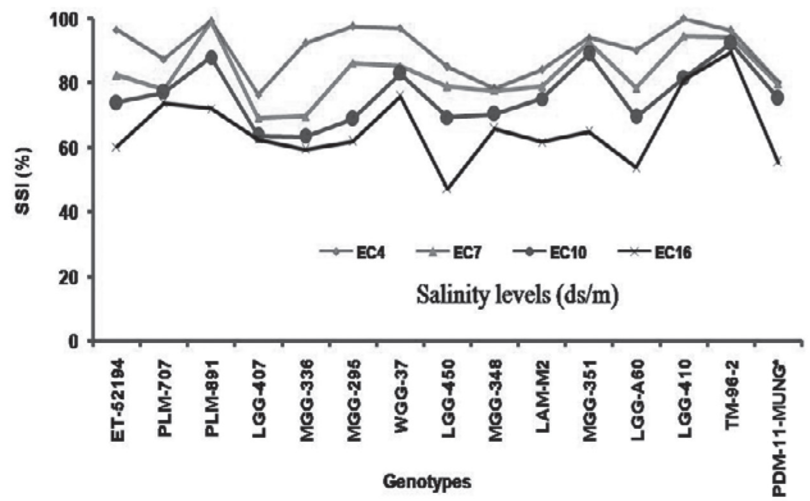


Figure 5 Salt susceptibility index (SSI) in investigated mungbean accessions at different salinity treatments

Seedling Vigor

The seedling vigor decreased with increase in salinity stress levels in all the genotypes. The result revealed that the genotypes PLM−707, PLM−891, WGG−37 and PDM−11−mung, showed less reduction in vigor index as compared with the genotypes i.e. ET−52194, LGG−A60, LGG−450, LGG−410 and TM−96−2, which showed major reduction under high salinity resulted in their highly sensitivity for saline environment (Figure 6).

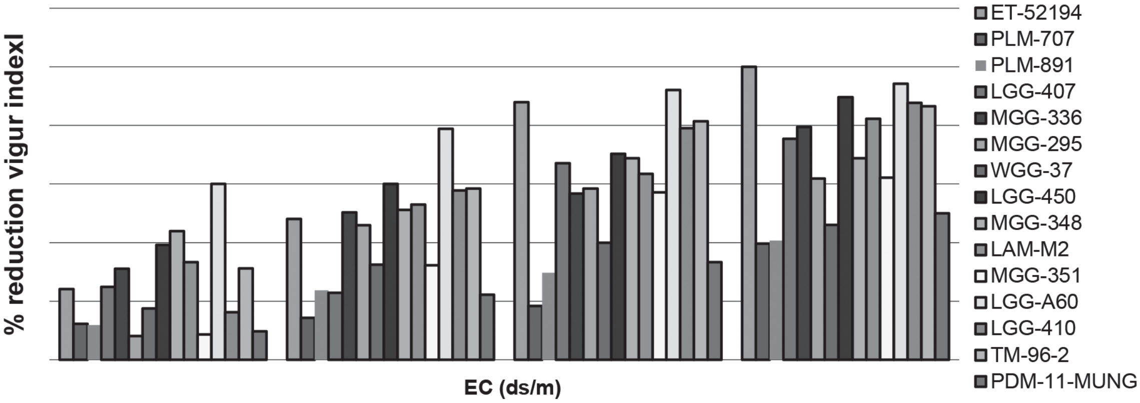


Figure 6 Percent reduction in vigor index under different salinity treatments in different mungbean genotypes

On the basis of the above observed response under salt stress, seedling vigor was found more significant observation for salt tolerance; therefore; on the basis of percent reduction in seedling vigor, all 15 genotypes were categorized in three groups as tolerant, moderate and susceptible genotypes (Table 1). The average value for all the measured seedling characteristics under salt stress is given in Table 2. The correlation studies showed that seed germination percentage and seedling vigor index positively and significantly correlated with all the parameters except salt susceptibility index (SSI) which was negatively correlated. However, seedling length characteristics positively and significantly correlated with each other and negatively correlated with SSI. However the seedling growth parameters were negatively and non−significantly correlated with seedling dry weight (Table 3).

Table 1 Grouping of all the 15 mungbean genotypes on the basis of reduction percentage in vigor index under salt stress

Groups	Reduction % at $EC_{16.0}$ ds/m	Response for salt tolerance	Name of the Accessions
1	(40−60)%	Tolerant	PLM 707, PLM 891, WGG 37, PDM 11
2	(60−80)%	Moderate	MGG 295, MGG 351, MGG 348, LGG 407, MGG 336
3	≥ 80%	Susceptible	ET 52194, LGG A60, LGG 410, LGG 450, LAM M2, TM 96−2

Table 2 Average value of all the measured observations at different salinity levels in the 15 mungbean genotypes

Characters	Salinity stress levels (dS/m)					Critical Difference (CD) *		
	Control	$EC_{4.0}$	$EC_{7.0}$	$EC_{10.0}$	$EC_{16.0}$	Treatment	Genotypes	Interaction (T × G)
Germination (%)	100.00	94.60	84.00	70.33	63.20	1.6200	2.810	6.290
Radical length (cm)	3.82	3.12	2.61	1.95	1.56	0.0630	0.109	0.245
Plumule length (cm)	4.53	3.29	2.77	2.01	1.54	0.0780	0.136	0.305
Total Seedling length (cm)	8.30	6.46	5.38	3.96	3.10	0.1160	0.202	0.451
Dry weight (mg)	80.00	72.00	66.00	60.00	52.00	0.0014	0.002	0.005
Seedling vigor index	830.30	604.80	449.50	286.10	208.50	11.9600	20.720	46.340
SSI (%)	100.00	90.24	82.89	75.94	65.57	0.0190	0.032	0.074

Note: *Significant value and P ≤ 0.01)

Table 3 Correlation study of investigated characteristics under salinity stress among 15 mungbean genotypes

Traits	Seed Germination (%)	Radical Length (cm)	Plumule Length (cm)	Total Seedling Length (cm)	Dry Weight (mg)	Salt susceptibility index (%)	Seedling vigor index
Seed Germination (%)	1.000	0.364**	0.284*	0.329**	0.313**	−0.219	0.624**
Radical Length (cm)		1.000	0.886**	0.964**	−0.002	−0.248*	0.150
Plumule Length (cm)			1.000	0.978**	−0.155	−0.298**	−0.018
Total Seedling Length (cm)				1.000	−0.091	−0.284*	0.057
Dry weight (mg)					1.000	−0.129	0.907**
Salt susceptibility index (%)						1.000	−0.196
Seedling vigor index							1.000

Note: *Significance value at 5% and ** Significance value at 1%

Optimization of Salinity Stress Level

The genotypes transferred to pots and irrigated with saline solutions of different concentrations. Visually observations demonstrated that the investigated mungbean genotypes showed reduction in plant survival and height, number of trifoliate, leaves size and symptoms of tip burning and low grain yield than the control plants. The genotype, WGG37 followed by PLM 891 maintained higher values for the measured features even under high salinity showing their great adaptability under saline environment however; all other genotypes showed significantly greater reduction for all the investigated traits at EC ≥ 10 dS/m. The EC_e values measured for soil samples were 1.89, 3.78, 6.88, 9.02 and 15.45 for the salinity treatment EC_0, $EC_{4.0}$,

$EC_{7.0}$, $EC_{10.0}$ and $EC_{16.0}$ (dS/m) respectively which were mean of 4 replications. Therefore, the EC level below 10 dS/m was selected as optimum level for mungbean that can be effectively used in future study of salt tolerance of this crop.

DISCUSSION

Increasing salinity levels significantly reduced germination with varying response for all genotypes studied and the results are in agreement with the earlier reports (Misra and Gupta, 2006; Naher and Alam, 2010; Kandil *et al.*, 2012; Sehrawat *et al.,* 2014c). Delay or reduction in germination may be due to toxic Na^+ and Cl^- ions which produced an outside osmotic potential that avoids water uptake or due to increased dormancy of seeds under salinity stress. It causes poor activation of the hydrolytic enzymes under saline environment resulted in reduced seed germination (Khajeh−Hosseini *et al.*, 2003; Mahdavi and Modarres Sanavy, 2007). Germination potential of seeds in saline environments may be interrelated with the tolerance at later growth stages of the crop species (Mass, 1985; Ashraf and Harris, 2004; Sehrawat *et al.*, 2014c). The differences between mungbean cultivars in germination might be due to the genetically factors and heredity variations (Uddin *et al.*, 2009; Win *et al.,* 2011).

The result showed that seedling growth characteristics significantly reduced with increase in salinity levels, where radical growth was more adversely affected than the plumule length under high salinity (≥ $EC_{10.0}$ ds/m) which are in accordance with the findings of Ahmad *et al.* (2005); Babbar and Dhingra (2007); Sehrawat *et al.* (2014c). The radical length is one of the most important character for salinity stress tolerance because roots absorb water and nutrients from soil therefore, radical length provides significant clue to the response of plants to salinity stress (Kaya *et al.,* 2003; Sehrawat *et al*., 2014c). As a result of reduction in seedling dry weight, the SSI (%) was found to be affected due to salinity treatments but the genotypes having higher value of salt stress index resulted in higher resistance to the salt stress and the low value of SSI resulted in salt susceptibility which showed similarity with the findings of Veli *et al*. (1994), Ozdemir and Engin, (1994), and Konak *et al*. (1999).

The result indicated that the increasing levels of salinity caused pronounced decrease in seedling vigor which corroborates with the earlier findings (Misra and Dwivedi, 2004; Sehrawat *et al*., 2014c). The seedling vigor was found to be more convenient test than the seed germination percentage alone; therefore, it can be used as an effective criterion for selection of tolerant mungbaen genotypes under salt stress (Mass and Hoffman, 1977; Sehrawat *et al*., 2014c). This study revealed that the early growth period of mungbean genotypes was significantly influenced by different concentrations of salinity treatments. The variable response observed in investigated genotypes for the measured traits under saline and non−saline environments also corroborates with the findings of Prakash *et al*. (1998); Yilmaz and Konak (2000); Sehrawat *et al*. (2014c). The control treatment clearly exhibited the existence of inherent variation between the varieties (Yermanos *et al*., 1964; Kandil *et al*., 2012; Sehrawat *et al*., 2014c). The genotypes showed <30% reduction in all the measured seedling characteristics were recorded as highly tolerant genotypes and those showed >70% reduction as highly susceptible genotypes under high salinity. Salt tolerant or resistant genotypes can be used in breeding program for genetic improvement of mungbean for salinity affected regions (Win *et al*., 2011; Sehrawat *et al*., 2013c; 2014b; 2016). This may also provide suitable source to study plant defense mechanisms and associated pathways regulating salt tolerance.

CONCLUSIONS

The present study concludes that the entire seedling growth characteristics are affected due to salinity and the seedling vigor can be used as a major criterion for the rapid selection of mungbean genotypes from the core for the desired agronomic trait like salt tolerance. The selected accessions can be used in further studies to identify the promising salt tolerant lines for breeding program and to study salt tolerance mechanisms and major genes responsible for salt tolerance. This will also help in the genetic improvement of mungbean for saline areas in near future.

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