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# SCREENING MUNGBEAN (*VIGNA RADIATA* L.) LINES FOR SALINITY TOLERANCE USING SALINITY INDUCTION RESPONSE TECHNIQUE AT SEEDLING AND PHYSIOLOGICAL GROWTH ASSAY AT WHOLE PLANT LEVEL

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**ABSTRACT:** Mungbean (*Vigna radiata* L.) is an economically important food legume rich in nutrients. However, its productivity over the last few decades has been stagnant largely due to various biotic and abiotic stresses. Among the abiotic stresses, salinity stress is more damaging that limits functional plant growth and yield worldwide. Due to the complex nature of salinity stress and lack of suitable techniques for introgression of desirable agronomic traits, little progress has been made in developing salt tolerant lines in legumes, in general and mungbean in particular. In the present investigation, an attempt has been made to screen mungbean lines for salinity tolerance. Forty mungbean lines were screened and contrasting lines were identified based on Salinity Induction Response (SIR) technique at the seedling level. As tolerance is a developmental stage specific, we further subjected the identified nine tolerant and nine susceptible lines for physiology based whole plant growth and yield phenotyping assay under 150 and 300 mM NaCl stress in pots. The results shown a considerable reduction in growth and yield performances of both tolerant and susceptible lines, but a few lines displayed relatively a better biomass and pod yield on par with non-stressed control plants. Based on seedling and whole plant level tolerance, a few tolerant (EC 693357, 58, 66, 71 and ML 1299) lines were identified for further investigation. Efforts are underway to use these identified tolerant lines as donor source for salinity breeding program to introgress with high yielding popular varieties.

**Key words:** Mungbean, Salinity Induction Response, cellular level tolerance

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## INTRODUCTION

Pulses are rich in proteins, popularly known as "Poor man's meat" and "rich man's vegetable", contributing significantly to the nutritional security of the country serving as a main source of the essential component of nutrition, particularly for the predominant vegetarian population of India and adjacent countries [1]. India is the largest pulse producer, accounting for 25 per cent of world's pulses production. Among various pulse crops, chickpea dominates (> 40 % share) of total pulse production followed by pigeonpea (18-20 %), mungbean (11 %), urdbean (10-12 %), lentil (8-9 %) and other legumes (20 %). Mungbean is an important pulse crop due to its widespread consumption throughout the Indian subcontinent. In India, area under mungbean is 3.8 Mha with an annual production of 1.56 Mt and an average productivity of 413 kg ha$^{1}$[2].

Worldwide, a total of 43,027 mungbean lines are held *ex-situ* at different institutes [3,4]. It is increasingly becoming popular in other parts of the world in recent years due to its value added products that are rich in several nutrients. 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 [5,6]. Recently, [7] have reviewed that 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.

"Salinity has been a threat to agriculture in some parts of the world for over 3000 years; in recent times, the threat has grown" [8]. As the world population continues to increase, more food needs to be grown to feed the people. This can be achieved by an increase in cultivated land and by an increase in crop productivity per area. The former has brought agriculture to marginal, salt-affected lands. Moreover, salinity problem has been aggravated by surface irrigation in arid and semi-arid environments [5].

Salt stress was found to reduce seed germination, fresh and dry biomass, shoot and root length and yield attributes of mungbean [9,10,11]. 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 [7]. It also comprises multifaceted responses at molecular, physiological and plant canopy levels [5]. Because of this complex nature of salinity stress and the lack of appropriate techniques for introgression, little progress has been made in identifying and developing salt tolerant mungbean varieties over years [5,12].Possible strategies for the development of salt tolerant mungbean varieties depend on the gene transfer methods either transgenic approach or wide hybridization. Due to multigenic nature of this trait (salt tolerance), little genetic enrichment can be achieved using highly efficient transformation technique [13]. Conventional breeding through wide hybridization is more labour intensive and time consuming approach. The quantitative nature of salt tolerance traits and the problems associated with developing appropriate and replicable testing environments make it difficult to distinguish salt tolerant lines from sensitive ones. Therefore, rapid screening method should be employed for identification of potential parents in a breeding program [14]. Considerable improvement in salt tolerance of important crops (barley, rice, pearl millet, maize, sorghum, alfalfa and many grass species) have been achieved in the past, but not in legumes in general and mungbean in particular [12].

Therefore, it is continuously raising the concern to enhance the agricultural productivity of the nutritious staple food crop to meet the demand of increasing population world-wide especially in the underdeveloped and developing countries. Towards that end mungbean, a protein rich legume has no exception. In this context, the present study was designed to screen mungbean germplasm (breeding lines) for salt tolerance at seedling level and observe physiological growth /yield (phenotypic) performance of selected lines under saline conditions at whole plant level.

## MATERIAL AND METHODS

### Sourcing seed material

Forty mungbean lines were sourced from the World Vegetable Centre, South Asia, for salinity screening assay (Appendix-1). These lines have good agronomic attributes including, some of them resistance to mungbean yellow mosaic disease [15].

### Standardising Salinity Induction Response (SIR) protocol in Mungbean

Salinity Induction Response technique developed and standardized earlier in several cereal and pulse crops at the Department of Crop Physiology, University of Agricultural Sciences, Bengaluru, has been used to screen mungbean lines for cellular level tolerance. In this technique, young seedlings were initially exposed to a mild saline concentration (sub lethal or induction stress) subsequently these seedlings were exposed to relatively a high salinity concentration for a specific period of time and then allowed to recover by transferring them back to water and recovery growth of seedlings is determined as a measure of tolerance [16].The same technique was employed to screen mungbean lines for cellular level tolerance (CLT).

### Screening for cellular level tolerance

Using SIR technique, 40 mungbeanlines were phenotyped for CLT. The seeds were soaked in distilled water for 3h and subsequently kept for germination in the incubator (28 °C and 60 % RH) for 12h. The uniformly grown seedlings were selected for screening under control (0 mM), induced (gradual increase from 100 to 350 mM at 3h interval) and non-induced (350 mM) saline treatment in petri plates. After stress imposition, seedlings were allowed to recover in water for 72 h. At the end of recovery period, per cent survival, per cent reduction in recovery growth (%RRG) was analysed.

Per cent survival = (No. of seedlings survived after recovery/No of seedlings taken) × 100

Per cent RRG = $\{(RGc-RGt)/RGc\}\times 100$

Where,

RGc is the recovery growth of absolute control plants,
RGt is the recovery growth of treated plants.

Three replications for each treatment were maintained. Initially, seedlings were exposed to sub-lethal salinity concentration for a known period of time, and recovery growth was determined. A schematic representation of SIR protocol for CLT is provided in (Figure 1).

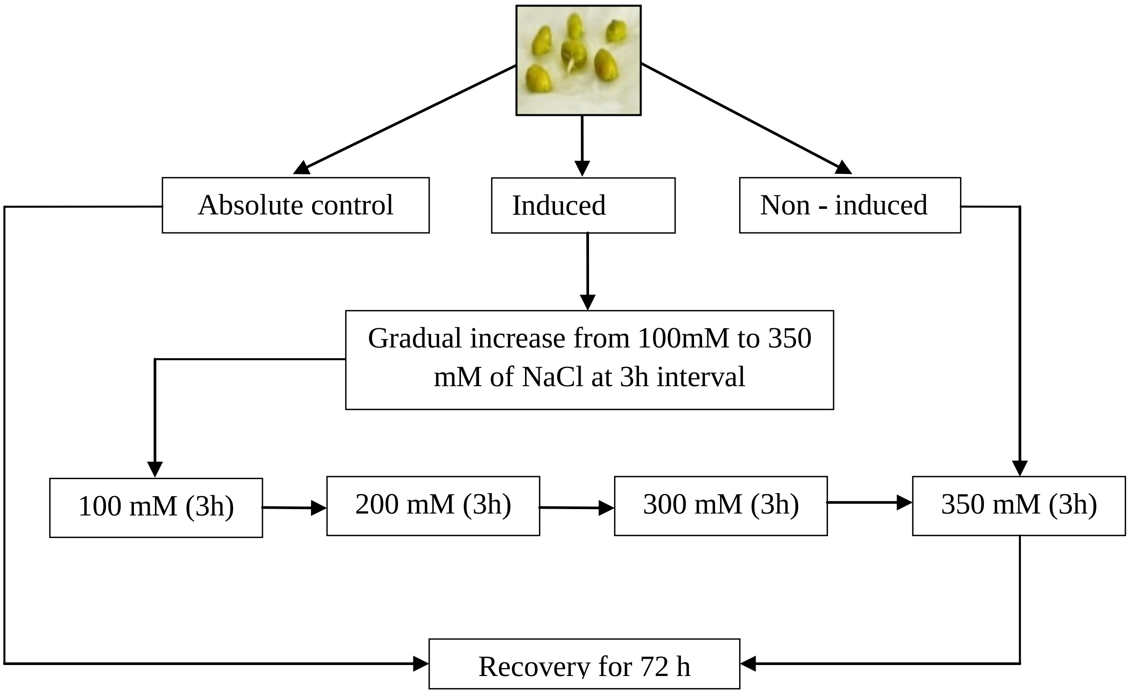


**Figure 1: Salinity protocol for cellular level tolerance**

### Assessment of the physiological growth and yield parameters at whole plant level (pot experiment)

A managed salinity screening in pots was conducted at greenhouse facility of the Department of Crop physiology. All pots were filled with equal proportions of soil, sand and compost. After sowing, seedlings were provided with normal irrigation until 20 DAS. After this period, salinity stress was imposed by fertigation technique. Two levels of stress was given *i.e.*, 150 and 300 mM of NaCl by adding 500ml of NaCl solution for stressed plants based on the field capacity of the soil to avoid leaching, whereas, on other hand, plants were irrigated with plain water, serving as control. After normal irrigation for 7 days, again stress was imposed as earlier. Likewise, stress was imposed thrice, and 45 DAS, the following growth and yield parameters were recorded. The protocol was as per [7], with slighter modifications that suits to Bengaluru weather conditions.

### Total dry matter (g/plant)

After harvesting the seeds, leaves and stem were separated and dried. Total dry weight was obtained and expressed in grams. The total dry matter consists of leaf weight, stem weight, root weight and pod weight

### Pod yield per plant (g)

The total quantity of pods obtained after harvesting from selected plants were dried completely, weighed and expressed in grams.

### Seed yield per plant (g)

After harvesting, pods were separated from selected plants and seeds were dried completely, weighed and expressed in grams per plant.

## RESULTS

### Salinity screening at seedling level

A significant variability in the parameters associated with CLT was observed. Per cent seedling survival was ranged from as low as 34 % to as high as 86 % with a mean survivability of 63 % for induced seedlings. However, for non-induced (lethal) seedlings, the per cent seedling survival ranged from as low as 0 % to as high as 44 % with a mean survivability of 26.35 % (Figure 2). Similarly, the per cent reduction in the recovery growth of induced seedlings was ranged from 56.84 % to 90.81 % with a mean value of 75.23 % (Figure 3).

### Identification and selection of contrasting mungbean lines differing in CLT for salinity stress.

Lines which had lower per cent Relative reduction in recovery growth (% RRG) and higher % survival were considered as tolerant types whereas lines with higher % RRG and lower % survival considered susceptible. To recheck the consistency of these lines for other parameters, a standardized normal distribution (Z-distribution) was plotted between absolute recovery growth and % RRG, lines grouped in first quadrant were selected as tolerant and lines in third quadrant as susceptible (Figure 4A). Z-distribution was also plotted between % survival and absolute recovery growth, lines in fourth and second quadrants were selected as high and low types respectively (Figure 4B).

In spite many lines were found tolerant and susceptible from Z- distribution, we selected the lines which were having highest and least % RRG. The lines found tolerant were EC 693365, EC 693366, VC 6173 B-10, VC 6368 (46-40-4), EC 693369, VC 6372 (45-8-1), ML 818, ML 1299, EC 693371 (Figure 5) and susceptible KPS-2, EC693361, NM92, EC 693367, EC 693368, EC 693370, PAU 911, NM 94, IPM 99-125 (Figure 5) (Table 1.) at seedling level were taken forward for assessing physiological responses at whole plant level.

**Table 1. Tolerant and susceptible lines along with per cent survival and % RRG values**

| **With Induction** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **High SIR lines** | | | | **Low SIR lines** | | | |
| **Line no** | **Mungbean lines** | **% survival** | **% RRG** | **Line no** | **Mungbean lines** | **% survival** | **% RRG** |
| 14 | EC 693365 | 74 | 69.25 | 6 | KPS-2 | 43 | 82.73 |
| 15 | EC 693366 | 85 | 56.84 | 7 | EC693361 | 38 | 81.43 |
| 16 | VC 6173 B-10 | 73 | 67.07 | 8 | NM92 | 35 | 85.68 |
| 19 | VC 6368 (46-40-4) | 76 | 64.59 | 17 | EC 693367 | 36 | 90.81 |
| 22 | EC 693369 | 70 | 64.52 | 20 | EC 693368 | 34 | 88.14 |
| 23 | VC 6372 (45-8-1) | 79 | 65.32 | 24 | EC 693370 | 57 | 81.25 |
| 25 | ML 818 | 80 | 64.42 | 29 | PAU 911 | 58 | 81.99 |
| 26 | ML 1299 | 82 | 64.07 | 30 | NM 94 | 59 | 81.14 |
| 35 | EC 693371 | 86 | 64.54 | 39 | IPM 99-125 | 48 | 90.27 |

Note: SIR – Salinity Induction Response, RRG – Reduction in Recovery Growth

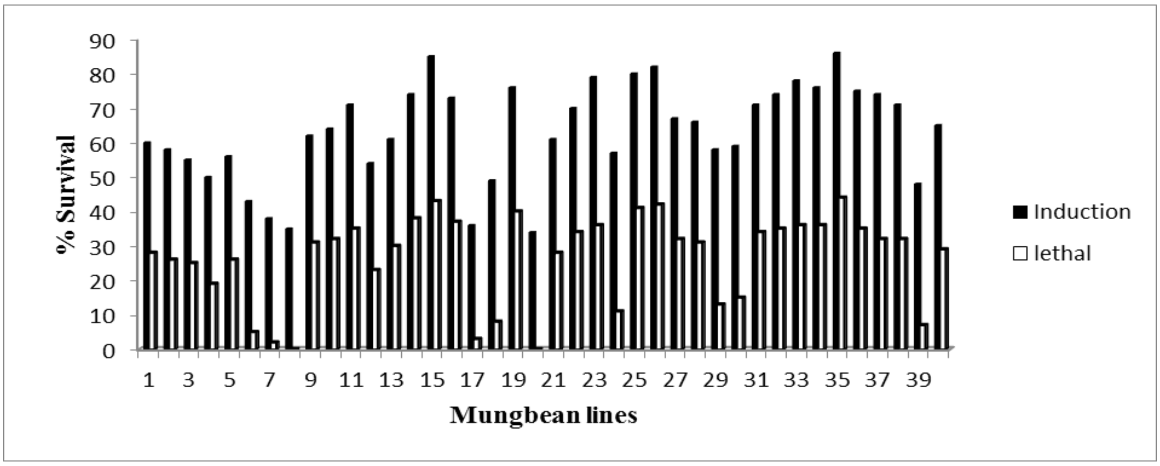


**Figure 2: Per cent survival of 40 mungbean lines for induction and lethal concentrations**

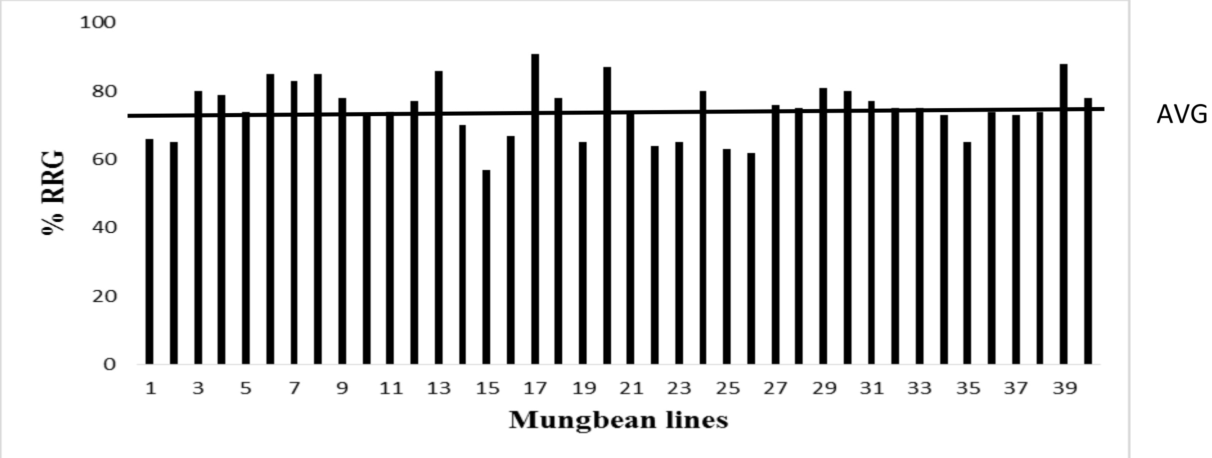


**Figure 3: Variability in per cent RRG of 40 mungbean lines exposed to induction salinity stress with overall average**

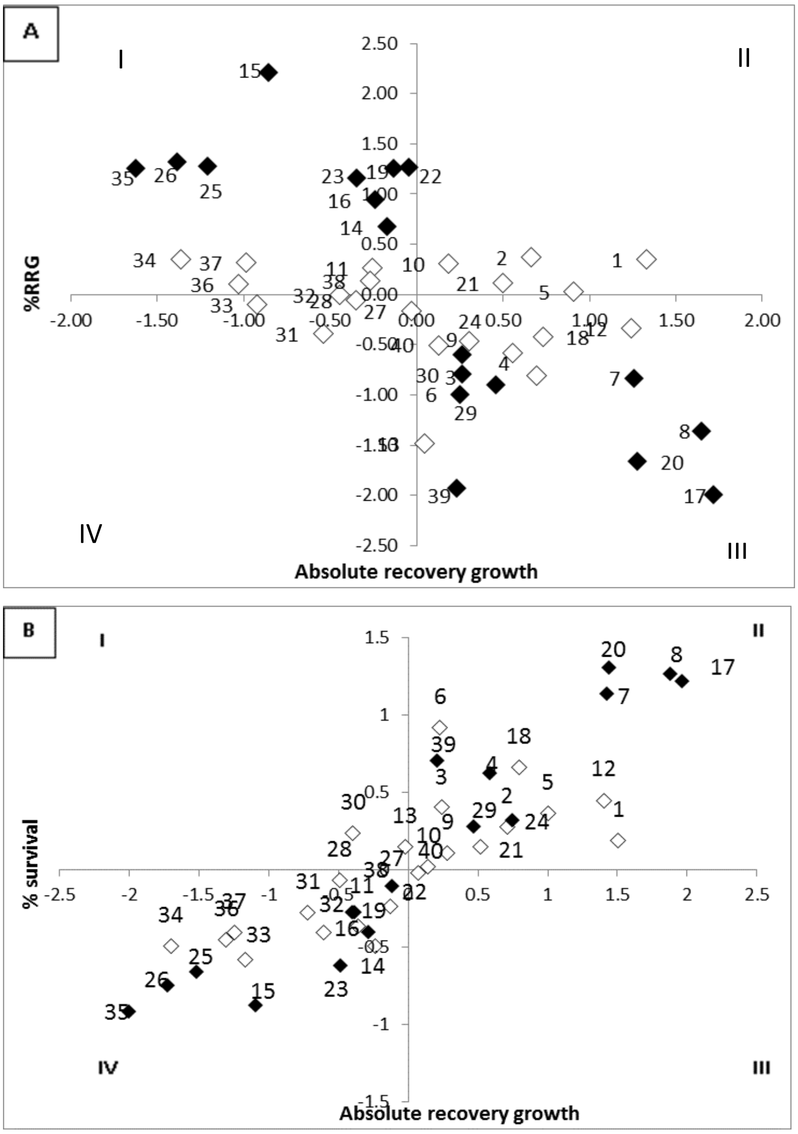


**Figure 4: (A) Normal Z-distribution based on absolute growth after recovery period and per cent reduction in recovery growth over control. Quadrant I: salt tolerant lines, and Quadrant III: susceptible lines. Tolerant and susceptible lines in solid black were selected. (B) Normal Z-distribution of selected mungbean lines based on absolute growth after recovery period and per cent survival Quadrant II: Salt sensitive lines and Quadrant IV: Salt tolerant lines. Tolerant and susceptible lines in solid black were selected.**

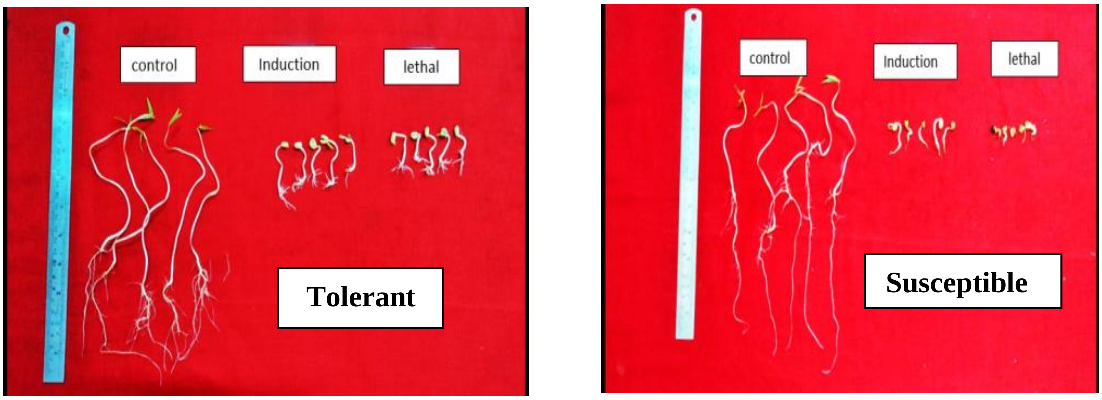


**Figure 5: Variability in salinity induction response for tolerant and susceptible lines screened at seedling level**

## Salinity screening at whole plant level

The plants were raised in pots and salinity stress treatment was imposed to 20 days old seedlings at every 7 days interval. Biometric and yield parameters were assessed in both tolerant and susceptible lines selected previously from seedling level experiment.

## Effect of salt stress on growth parameters and total biomass

Reduction in growth biometric parameters (plant height, number of leaves, root length, root volume and relative water content) along with total biomass was observed with increasing salinity levels (Table 2). Total biomass (g/pl) values ranged from 7.92 to 24.3 for control plants, 5.10 to 15.32 for 150 mM and 3.32 to 12.73 for 300 mM (Table 3). Significant differences were found between lines at two stress levels. When we looked individual parameters which constituted total biomass, a significant difference was found between lines and treatments. Leaf weight values ranged from 1.63 to 6.39 for control, and 1.45 to 4.92 and 1.32 to 4.32 g for 150 and 300 mM respectively. Stem weight values were ranged from 2.55 to 6.97, 1.53 to 3.16, 1.44 to 2.45 g for control 150 and 300mM, respectively.

## Effect of salt stress on yield parameters

When examined the effect of salt stress on yield parameters, *viz.*, number of fruiting points per plant, pod number, pod yield, seed yield, etc., as the NaCl concentrations increased, yield parameters decreased at both levels of stress compared to untreated control (Table 4). Significant variability in fruiting points was observed with a mean value of 4.39 in control and 4.12 and 3.62 in 150 and 300 mM NaCl stress respectively. For pod number, values ranged from 4 to 20.33 for control 3.67 to 16.67 for 150 mM and 1.50 to 10 for 300 mM. Pod weight ranged from 3.28 to 7.76 for control plants, 1.88 to 5.58 and 0.41to 4.57 g for 150 and 300 mM stress. For seed weight it ranged from 2.67 to 5.73, 1.31 to 4.35, and 0.28 to 3.04 g for control, 150 and 300 mM NaCl respectively. Significant differences were found between the genotypes and two stress levels for all the parameters.

## Identification of tolerant and susceptible lines at whole plant level

Yield attributing traits like biomass and pod/seed yield are considered most important in any sort of stresses. Hence from total original 40 lines, 18 tolerant and susceptible lines were identified based on their biomass and pod yield values. To achieve these, biometric values of control, 150 (S1) and 300 mM (S2) NaCl were pooled to deduce average value in all the lines for total biomass and pod yield. Firstly, a line graph was plotted to get tolerant and susceptible types for biomass (Figure 6-A and B) pod yield (Figure7-A and B). Lines which were resting above the average values were considered as tolerant whereas lines rested below the pooled average as susceptible. From this analysis, NM 92, EC 693366, EC 693367, VC 6372 (45-8-1), ML 818, ML 1299, EC 693371 were found to be tolerant and EC 693365, VC 6173 B-10, VC 6368 (46-40-4), EC 693369, PAU 911, NM 94 were found susceptible for pod yield. However, for biomass, KPS-2, EC693361, EC 693366, EC 693367, ML 1299, EC 693371 were found tolerant and NM92, VC 6173 B-10, VC 6368 (46-40-4), EC 693369, EC 693370, NM 94 were found susceptible (Figure 8).

## Identification of consistent tolerant and susceptible lines both at whole plant and seedling levels

Based on comprehensive assay on growth, survival response and recovery (seedling stage) both during and after salinity stress and physiological function/maturity coupled with biomass accumulation and yield (pod and seed) responses, the lines EC 693366, ML 1299, EC 693371were identified as tolerant and NM 94 as susceptible.

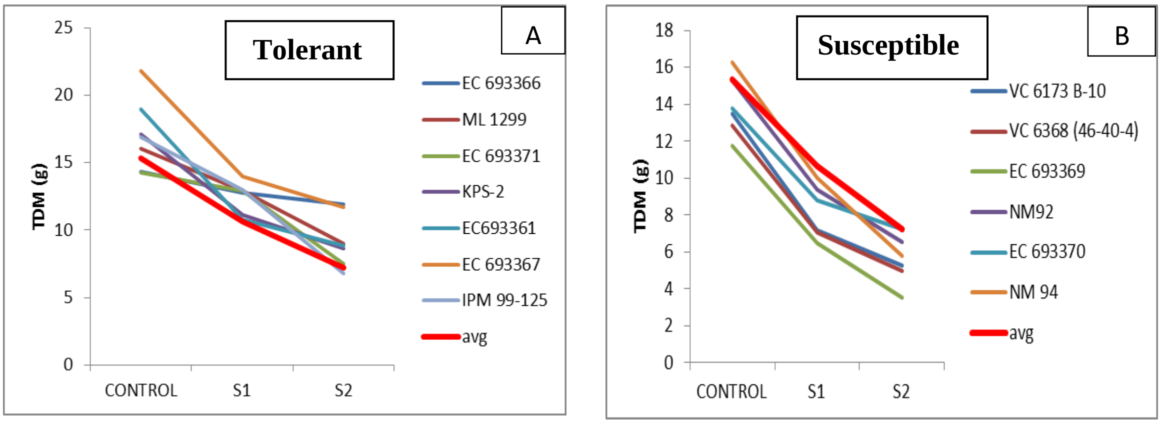


**Figure 6: Screening of mungbean lines based on average value for TDM in relation to pooled average (A) Tolerant lines sits above the average line (B) Susceptible lines sits below the average line.**

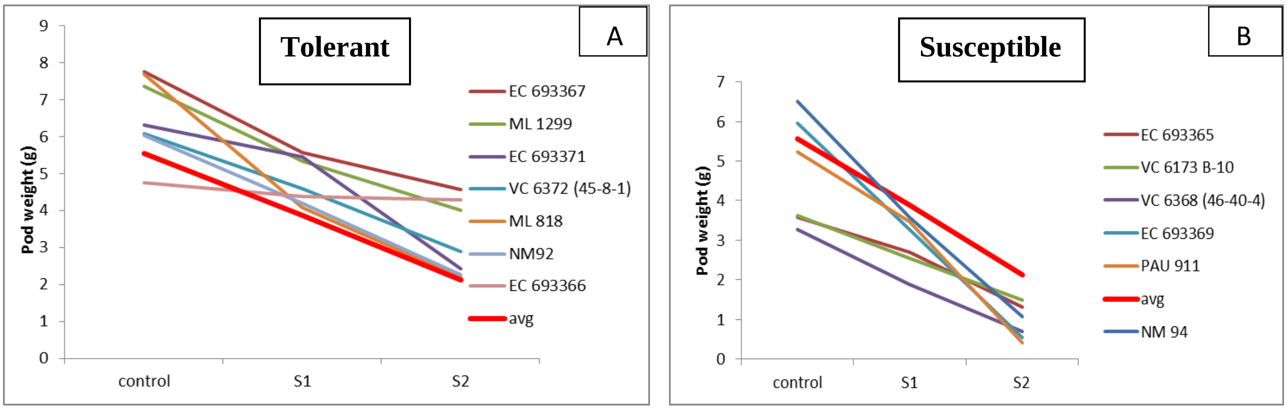


**Figure 7: Screening of mungbean lines based on average value of pod weight in comparison to pooled average (A) Tolerant lines fall above the average line (B) Susceptible lines fall below the average line.**

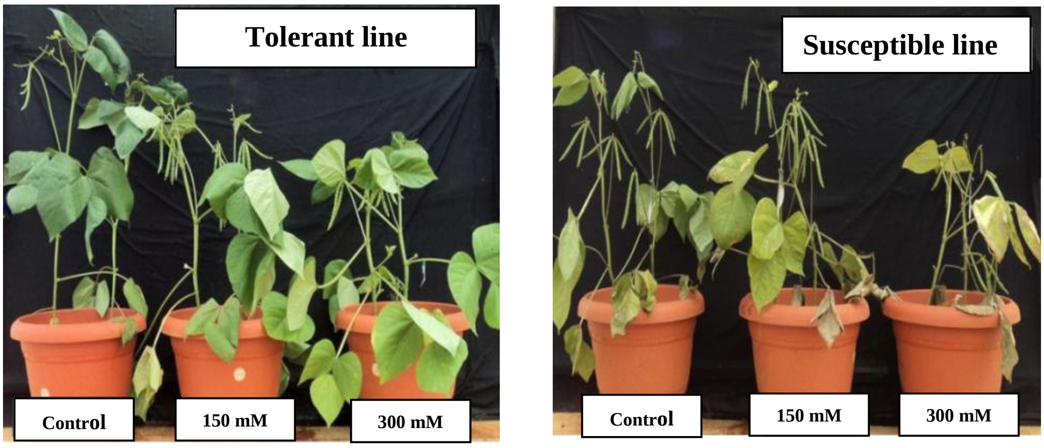


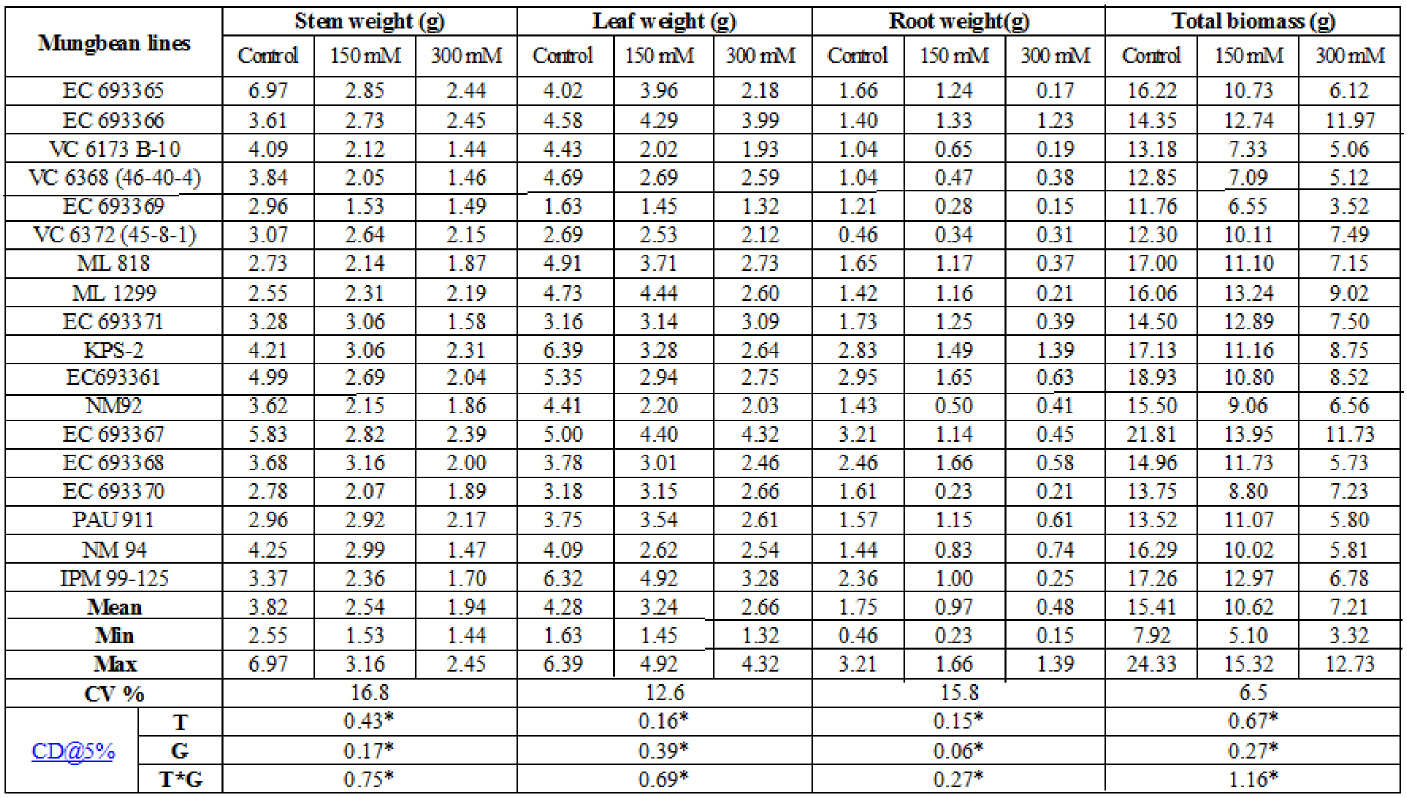
**Figure 8: Tolerant and susceptible mungbean lines at control, 150 and 300 mM of salinity stress (whole plant level).**

**Table 2. Range value of biometric parameters of selected 18 mungbean lines (at whole plant level) under different salinity stress levels**

| Plant Traits | Treatment | Range value | CD *P*≤0.05 |
|---|---|---|---|
| Plant height (cm) | Control | 23.3-124.0 | 2.64* |
| | 150 mM | 18-118 | |
| | 300 mM | 16-85.7 | |
| Number of leaves | Control | 14-37 | 5.15* |
| | 150 mM | 12.3-31 | |
| | 300 mM | 11.0-28.6 | |
| Root length (cm) | Control | 31.6-70.9 | 2.3* |
| | 150 mM | 19.5-48.67 | |
| | 300 mM | 13.4-39.7 | |
| Root volume | Control | 1.4-17.5 | 1.4* |
| | 150 mM | 0.6-15.4 | |
| | 300 mM | 0.3-3.5 | |
| Relative water content (RWC, %) | Control | 61.7-88.2 | 0.79* |
| | 150 mM | 1.2-4.2 | |
| | 300 mM | 0.3-2.6 | |

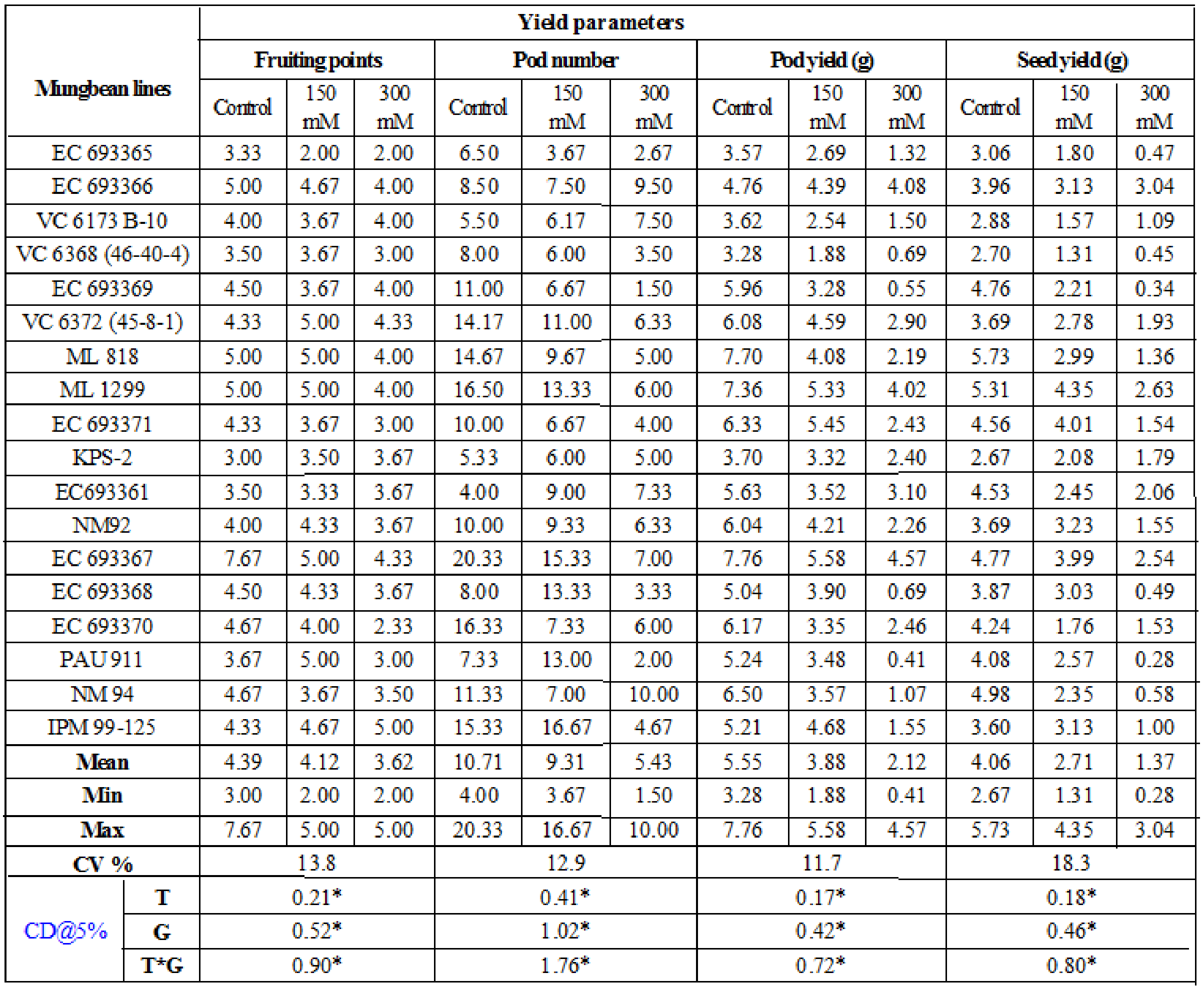
**Note:** Biometric parameters (plant height, number of leaves, root length, root volume and relative water content) were gradually decreased with increase in salinity concentration from 150 and 300 mM NaCl in all mungbean lines compared to respective non-treated control (values are significant at P≤0.05).

**Table 3. Genotypic variation in growth traits and total biomass of selected 18 mungbean lines**



| Mungbean lines | | Stem weight (g) | | | Leaf weight (g) | | | Root weight(g) | | | Total biomass (g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM |
| EC 693365 | | 6.97 | 2.85 | 2.44 | 4.02 | 3.96 | 2.18 | 1.66 | 1.24 | 0.17 | 16.22 | 10.73 | 6.12 |
| EC 693366 | | 3.61 | 2.73 | 2.45 | 4.58 | 4.29 | 3.99 | 1.40 | 1.33 | 1.23 | 14.35 | 12.74 | 11.97 |
| VC 6173 B-10 | | 4.09 | 2.12 | 1.44 | 4.43 | 2.02 | 1.93 | 1.04 | 0.65 | 0.19 | 13.18 | 7.33 | 5.06 |
| VC 6368 (46-40-4) | | 3.84 | 2.05 | 1.46 | 4.69 | 2.69 | 2.59 | 1.04 | 0.47 | 0.38 | 12.85 | 7.09 | 5.12 |
| EC 693369 | | 2.96 | 1.53 | 1.49 | 1.63 | 1.45 | 1.32 | 1.21 | 0.28 | 0.15 | 11.76 | 6.55 | 3.52 |
| VC 6372 (45-8-1) | | 3.07 | 2.64 | 2.15 | 2.69 | 2.53 | 2.12 | 0.46 | 0.34 | 0.31 | 12.30 | 10.11 | 7.49 |
| ML 818 | | 2.73 | 2.14 | 1.87 | 4.91 | 3.71 | 2.73 | 1.65 | 1.17 | 0.37 | 17.00 | 11.10 | 7.15 |
| ML 1299 | | 2.55 | 2.31 | 2.19 | 4.73 | 4.44 | 2.60 | 1.42 | 1.16 | 0.21 | 16.06 | 13.24 | 9.02 |
| EC 693371 | | 3.28 | 3.06 | 1.58 | 3.16 | 3.14 | 3.09 | 1.73 | 1.25 | 0.39 | 14.50 | 12.89 | 7.50 |
| KPS-2 | | 4.21 | 3.06 | 2.31 | 6.39 | 3.28 | 2.64 | 2.83 | 1.49 | 1.39 | 17.13 | 11.16 | 8.75 |
| EC693361 | | 4.99 | 2.69 | 2.04 | 5.35 | 2.94 | 2.75 | 2.95 | 1.65 | 0.63 | 18.93 | 10.80 | 8.52 |
| NM92 | | 3.62 | 2.15 | 1.86 | 4.41 | 2.20 | 2.03 | 1.43 | 0.50 | 0.41 | 15.50 | 9.06 | 6.56 |
| EC 693367 | | 5.83 | 2.82 | 2.39 | 5.00 | 4.40 | 4.32 | 3.21 | 1.14 | 0.45 | 21.81 | 13.95 | 11.73 |
| EC 693368 | | 3.68 | 3.16 | 2.00 | 3.78 | 3.01 | 2.46 | 2.46 | 1.66 | 0.58 | 14.96 | 11.73 | 5.73 |
| EC 693370 | | 2.78 | 2.07 | 1.89 | 3.18 | 3.15 | 2.66 | 1.61 | 0.23 | 0.21 | 13.75 | 8.80 | 7.23 |
| PAU 911 | | 2.96 | 2.92 | 2.17 | 3.75 | 3.54 | 2.61 | 1.57 | 1.15 | 0.61 | 13.52 | 11.07 | 5.80 |
| NM 94 | | 4.25 | 2.99 | 1.47 | 4.09 | 2.62 | 2.54 | 1.44 | 0.83 | 0.74 | 16.29 | 10.02 | 5.81 |
| IPM 99-125 | | 3.37 | 2.36 | 1.70 | 6.32 | 4.92 | 3.28 | 2.36 | 1.00 | 0.25 | 17.26 | 12.97 | 6.78 |
| **Mean** | | 3.82 | 2.54 | 1.94 | 4.28 | 3.24 | 2.66 | 1.75 | 0.97 | 0.48 | 15.41 | 10.62 | 7.21 |
| **Min** | | 2.55 | 1.53 | 1.44 | 1.63 | 1.45 | 1.32 | 0.46 | 0.23 | 0.15 | 7.92 | 5.10 | 3.32 |
| **Max** | | 6.97 | 3.16 | 2.45 | 6.39 | 4.92 | 4.32 | 3.21 | 1.66 | 1.39 | 24.33 | 15.32 | 12.73 |
| **CV %** | | 16.8 | | | 12.6 | | | 15.8 | | | 6.5 | | |
| CD@5% | **T** | 0.43* | | | 0.16* | | | 0.15* | | | 0.67* | | |
| | **G** | 0.17* | | | 0.39* | | | 0.06* | | | 0.27* | | |
| | **T*G** | 0.75* | | | 0.69* | | | 0.27* | | | 1.16* | | |

**Table-4. Genotypic variation in yield traits and pod/seed yield of selected 18 mungbean lines**



| Mungbean lines | | Yield parameters | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Fruiting points | | | Pod number | | | Pod yield (g) | | | Seed yield (g) | | |
| | | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM | Control | 150 mM | 300 mM |
| EC 693365 | | 3.33 | 2.00 | 2.00 | 6.50 | 3.67 | 2.67 | 3.57 | 2.69 | 1.32 | 3.06 | 1.80 | 0.47 |
| EC 693366 | | 5.00 | 4.67 | 4.00 | 8.50 | 7.50 | 9.50 | 4.76 | 4.39 | 4.08 | 3.96 | 3.13 | 3.04 |
| VC 6173 B-10 | | 4.00 | 3.67 | 4.00 | 5.50 | 6.17 | 7.50 | 3.62 | 2.54 | 1.50 | 2.88 | 1.57 | 1.09 |
| VC 6368 (46-40-4) | | 3.50 | 3.67 | 3.00 | 8.00 | 6.00 | 3.50 | 3.28 | 1.88 | 0.69 | 2.70 | 1.31 | 0.45 |
| EC 693369 | | 4.50 | 3.67 | 4.00 | 11.00 | 6.67 | 1.50 | 5.96 | 3.28 | 0.55 | 4.76 | 2.21 | 0.34 |
| VC 6372 (45-8-1) | | 4.33 | 5.00 | 4.33 | 14.17 | 11.00 | 6.33 | 6.08 | 4.59 | 2.90 | 3.69 | 2.78 | 1.93 |
| ML 818 | | 5.00 | 5.00 | 4.00 | 14.67 | 9.67 | 5.00 | 7.70 | 4.08 | 2.19 | 5.73 | 2.99 | 1.36 |
| ML 1299 | | 5.00 | 5.00 | 4.00 | 16.50 | 13.33 | 6.00 | 7.36 | 5.33 | 4.02 | 5.31 | 4.35 | 2.63 |
| EC 693371 | | 4.33 | 3.67 | 3.00 | 10.00 | 6.67 | 4.00 | 6.33 | 5.45 | 2.43 | 4.56 | 4.01 | 1.54 |
| KPS-2 | | 3.00 | 3.50 | 3.67 | 5.33 | 6.00 | 5.00 | 3.70 | 3.32 | 2.40 | 2.67 | 2.08 | 1.79 |
| EC693361 | | 3.50 | 3.33 | 3.67 | 4.00 | 9.00 | 7.33 | 5.63 | 3.52 | 3.10 | 4.53 | 2.45 | 2.06 |
| NM92 | | 4.00 | 4.33 | 3.67 | 10.00 | 9.33 | 6.33 | 6.04 | 4.21 | 2.26 | 3.69 | 3.23 | 1.55 |
| EC 693367 | | 7.67 | 5.00 | 4.33 | 20.33 | 15.33 | 7.00 | 7.76 | 5.58 | 4.57 | 4.77 | 3.99 | 2.54 |
| EC 693368 | | 4.50 | 4.33 | 3.67 | 8.00 | 13.33 | 3.33 | 5.04 | 3.90 | 0.69 | 3.87 | 3.03 | 0.49 |
| EC 693370 | | 4.67 | 4.00 | 2.33 | 16.33 | 7.33 | 6.00 | 6.17 | 3.35 | 2.46 | 4.24 | 1.76 | 1.53 |
| PAU 911 | | 3.67 | 5.00 | 3.00 | 7.33 | 13.00 | 2.00 | 5.24 | 3.48 | 0.41 | 4.08 | 2.57 | 0.28 |
| NM 94 | | 4.67 | 3.67 | 3.50 | 11.33 | 7.00 | 10.00 | 6.50 | 3.57 | 1.07 | 4.98 | 2.35 | 0.58 |
| IPM 99-125 | | 4.33 | 4.67 | 5.00 | 15.33 | 16.67 | 4.67 | 5.21 | 4.68 | 1.55 | 3.60 | 3.13 | 1.00 |
| **Mean** | | 4.39 | 4.12 | 3.62 | 10.71 | 9.31 | 5.43 | 5.55 | 3.88 | 2.12 | 4.06 | 2.71 | 1.37 |
| **Min** | | 3.00 | 2.00 | 2.00 | 4.00 | 3.67 | 1.50 | 3.28 | 1.88 | 0.41 | 2.67 | 1.31 | 0.28 |
| **Max** | | 7.67 | 5.00 | 5.00 | 20.33 | 16.67 | 10.00 | 7.76 | 5.58 | 4.57 | 5.73 | 4.35 | 3.04 |
| **CV %** | | 13.8 | | | 12.9 | | | 11.7 | | | 18.3 | | |
| CD@5% | **T** | 0.21* | | | 0.41* | | | 0.17* | | | 0.18* | | |
| | **G** | 0.52* | | | 1.02* | | | 0.42* | | | 0.46* | | |
| | **T*G** | 0.90* | | | 1.76* | | | 0.72* | | | 0.80* | | |

## DISCUSSION

In plants, the most critical stage during seedling growth is the seed germination that determines effective crop establishment and production. Increasing salinity stress levels during mungbean seed germination significantly reduced the germination and seedling performance. Salinity adversely affects the plant growth at all stages, particularly at seedling and reproductive stages, which dramatically reducing the yield [17]. In the present study, it was observed that a significant reduction in shoot and root lengths in mungbean seedlings caused by salt stress was ameliorated by providing an induction treatment (with small dose of NaCl) prior to exposure to the lethal concentration. The percent survival was relatively more and reduction in recovery growth was appreciable (Figs.2 and 3) in pre-treated plants over nontreated plants, which indicates that pre-treatment has an assimilative effect in acclimation process as reported earlier in soybean [18] and rice [19].

Many earlier reports have shown the acclimation effect of treatment with low concentration of NaCl on improved tolerance leading to high growth rate in plants when exposed to a sublethal dose of salt treatment. The salt acclimated plants were shown to have high osmotic adjustment increased activity of certain ROS scavenging enzymes and induced expression of certain stress- related genes. [14] reported that pre-treatment with a sub-lethal dose of NaCl was able to overcome adverse effects of stress imposed by NaCl. Mungbean plants could acclimatize to lethal levels of salinity through pre-treatment with sub-lethal doses, which resulted in increased growth and photosynthesis in seedlings and modified the activities of antioxidant enzymes. Further, pre-treatment could enhance the antioxidant metabolism and thus partially ameliorating the negative effects of salinity mediated injury, enhanced ROS scavenging mechanism, these changes leading to expression of stress proteins which support better seedling growth.

Salinity has its own effects on the plant growth and development right from the germination till seed yield. In the present study, all recorded parameters decreased compared to control plants as NaCl concentration increased. The reduction of plant growth under salinity was due to the effect of salinity on the different vital activities of plants, such as declined enzymatic activity, metabolism, cell division and photosynthesis [20], and also caused a decrease on the assimilation of $CO_2$ through the effect on the opening of stomata and the sufficiency of photosynthesis process [21].Growth inhibition under salt stress may be due to the diversion of energy from growth to maintenance respiration [22]. Increasing salinity concentration levels leads to an increase on the absorbance of some essential elements that activated the action of some enzymes, which were essential for the protein synthesis [23].

The result explains that yield and yield attributes significantly decreased with higher salinity levels. Reduced yield in mungbean under salt stress may attributes to more flowers shedding, reduced photosynthetic efficiency to fill the developing seeds, which may lead to reduced number of seeds/pod or plant and dry matter yield of individual seed and shattering of the pods [24]. But in a few cases, like tolerant lines NM92, EC 693357, EC 693358, EC 693366, EC 693367, VC 6372 (45-8-1), ML 818, ML 1299, EC 693371, no matter what the stress was, the plants put forth appreciable pod yield, might attribute to better pollen fertility and translocation of photosynthates towards the pod and seed components. Whereas in susceptible lines, salinity stress resulted in shrivelled seeds [25] and impaired pod-setting leading to reduced pod number and seed yield [26].

## CONCLUSION

Salt tolerance is a polygenic trait, genotype and developmental stage specific. Lack of dependable technique and suitable parameter for screening constraint in developing salt tolerance in mungbean. Low productivity of mungbean highlights the need of its genetic improvement to maintain its productivity in salt-affected soils. The development of salt tolerant cultivars is the most promising and efficient gateway to reduce the lethal effects of soil salinity. An insufficient precipitation results in extensive reliance on irrigation and a considerable proportion of underground water in most of these areas is of poor quality [27]. Mungbean is an important pulse crop, but owing to poor quality of water/soil, the productivity of this crop is not optimal under such conditions.

From the results of the present investigation, it can be concluded that salinity affects early growth of mungbean seedlings. Pre-treatment with sub lethal dose of NaCl ameliorates the injurious effects of NaCl to some extent by increasing the growth, activities of antioxidant enzymes and accumulation of osmolytes for osmotic adjustments. Therefore plants can acclimatize to lethal level of salinity and can improve its production ability under saline conditions. Confirmatory field experiments of identified salinity tolerant lines have been planned to explore the field level tolerance.

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**Appendix-1: Mungbean lines used in the study**

| S.No. | Mungbean line ID | World Vegetable Center Code |
|---|---|---|
| 1 | EC693356 (VC 6153B-20P) | AVMU0402 |
| 2 | EC693357 (VC 6465-8-5-2) | AVMU1202 |
| 3 | EC693358 (VC 6469-12-3-4A) | AVMU1001 |
| 4 | EC693360 (VC 6486-10-51) | AVMU1002 |
| 5 | KPS-1 (VC 1973A) | AVMU8501 |
| 6 | KPS-2 (VC 2778A) | AVMU8601 |
| 7 | EC693361 (VC 6489-9-1) | AVMU1006 |
| 8 | NM 92 (VC 6370-92) | AVMU9701 |
| 9 | EC693362 (VC 6492-59A) | AVMU0801 |
| 10 | VC 3960-88 | AVMU8902 |
| 11 | VC 6153B-20G | AVMU0401 |
| 12 | EC 693363 (VC 6493-44-1) | AVMU1007 |
| 13 | EC 693364 (VC 6506-127) | AVMU1201 |
| 14 | EC 693365 (VC 6510-151-1) | AVMU1003 |
| 15 | EC 693366 (VC 6512-6A) | |
| 16 | VC 6173 B-10 | |
| 17 | EC 693367 (PDMA 54) | |
| 18 | VC 6368 (46-40-1) | |
| 19 | VC 6368 (46-40-4) | AVMU0201 |
| 20 | EC 693368 (PUSA 9074) | |
| 21 | VC 6369 (53-97) | |
| 22 | EC 693369 (TV 03980A-G) | |
| 23 | VC 6372 (45-8-1) | |
| 24 | EC 693370 (TV 03717B-G) | |
| 25 | ML 818 | |
| 26 | ML 1299 | |
| 27 | ML 1628 | |
| 28 | ML 1666 | |
| 29 | PAU 911 | |
| 30 | NM 94 (VC 6371-94) | AVMU0001 |
| 31 | IPM 02-14 | |
| 32 | PDM 139 | |
| 33 | IPM 205-7 | |
| 34 | IPM 02-17 | |
| 35 | EC 693371 (TV 01493A-G) | |
| 36 | EC 693372 (VO 1352B-G) | |
| 37 | EC 693374 (VO 6381A-G) | |
| 38 | EC 693376 (TV 03719A-G) | |
| 39 | IPM 99-125 | |
| 40 | IPM 02-3 | |

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