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Full Length Article

Screening Mungbean Germplasm for Salt Tolerance using Growth Indices and Physiological Parameters

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Abstract

Soil salinity is a major abiotic stress that limits crop productivity. Mungbean is cultivated as a marginal crop in rice-based farming systems of Asia, suffering heavy yield loss due to soil salinity. Therefore, it is necessary to develop salt tolerant cultivars of mungbean for growing in saline areas. In this study, mungbean germplasm was evaluated for salt tolerance to introduce suitable varieties for cultivation in saline soils. Thirteen mungbean varieties (Mung-88, Ramzan, NM-92, NM-13-1, NM-2011, NM-121-25, Chakwal-97, NM-19-19, NM-20-21, NM-2006, NM-28, NM-51 and Var-6601) were grown under 0, 60, 80, 100 m*M* NaCl in laboratory conditions. Results indicated that germination stress tolerance index, shoot and root lengths stress tolerance index, fresh and dry weight stress tolerance indices, root to shoot lengths ratio, relative water contents and osmotic potential were decreased with increasing salinity levels while Na^+/K^+ ratio was increased by increasing salinity levels in all varieties. Positive and significant correlations found among germination stress tolerance index and fresh weight stress tolerance index and seedlings dry weight stress tolerance index. To evaluate the similarity index between mungbean varieties multivariate technique of cluster analysis (dendrogram) was used which grouped the data into three clusters. Salt tolerant varieties come in cluster-I (NM-92, Ramzan, Chakwal-97, NM-51 and Var-6601), medium tolerant in cluster-II (Mung-88, NM-121-25, NM-20-21, NM-13-1 and NM-2011) and salt sensitive in cluster-III (NM-28, NM-19-19 and NM-2006). In conclusion, mungbean variety NM-92 was highly salt tolerant while NM-28 was salt sensitive one. Germination stress tolerance index, shoot and root lengths indices, seedling dry and fresh weight indices, relative water contents and Na^+/K^+ ratio are the important growth indices and physiological parameters to be used as selection criteria to evaluate salt tolerance in mungbean varieties. © 2019 Friends Science Publishers

Keywords: Correlation analysis; NaCl; Tolerance indices; Mungbean varieties

Introduction

Among abiotic stresses, soil salinity is one of the most important constraints limiting the growth of plants, delaying the process of seed germination and ultimately affecting the crop yield. Salt stress exhibits diverse impact on plant biodiversity and crop productivity in arid and semi-arid regions of the world and considered as a main threat to food security (Farooq *et al.*, 2015). It has been estimated that more than 20% of irrigated land area is salt affected globally (Mickelbart *et al.*, 2015). Therefore, it is direly needed to use conventional and modern techniques to develop salt tolerant cultivars for obtaining high yield under saline soils (Hussain *et al.*, 2016).

The screening of germplasm is the basic requirement to select the salt-tolerant genotypes to get economic output from saline soils. The physiological indices could also be used as screening tool for evaluating the varieties at an early seeding stages rather than screening them at yielding stage due to simple selection criteria (Flowers and Yeo, 1995; Shahzad *et al.*, 2012; Hussain *et al*., 2013). Literature also provided evidences that the genotypic variations for stress tolerance can be analyzed in crop plants at germination and early growth stages by physiological indices (Zafar *et al*., 2015; Farooq *et al*., 2017; Tabasssum *et al*., 2017; Bajwa *et al*., 2018). The salt-tolerant potential at the seedling stage may determine the tolerance at vegetative as well as reproductive phase that have been effectively studied in *Triticum aestivum* (Ali and Gupta, 2012; Zafar *et al*., 2015; Tabasssum *et al*., 2017), *Sorghum bicolor* (Bafeel, 2014) and *Oryza sativa* (Hariadi *et al.*, 2015).

Mungbean (*Vigna radiata* L. Wilczek) is short duration crop and cultivated on marginal saline land due unavailability of fertile lands mostly occupied by staple food crops or crops having high income returns (Joshi *et al*., 2014). The quality of mungbean has been badly affected by

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salt stress causing severe yield and farmers' income losses (Saha *et al.*, 2010; Farooq *et al*., 2017). Therefore, selection of salt tolerant varieties is necessary for improvement of mungbean productivity under saline conditions.

Screening of mungbean germplasm may be helpful for introduction of tolerant varieties for economic cultivation of saline lands or useful in developing high yielding salt tolerant mungbean varieties. In this study, mungbean germplasm was evaluated for salt tolerance using some growth indices and physiological parameters as screening tools at early seedling stage. The main objective of this study was to identify the salt tolerant mungbean varieties which can be directly cultivated in salt-affected lands. Moreover, some growth indices and physiological parameters were also identified to be used as screening tools to evaluate mungbean germplasm for salinity tolerance.

Materials and Methods

Experiment Details

Thirteen mungbean varieties (Mung-88, RAMZAN, NM-92, NM-13-1, NM-2011, NM-121-25, Chakwal-97, NM-20-21, NM-19-19, NM-2006, NM-28, NM-51 and VAR-6601) were obtained from Plant Breeding and Genetics Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan and were tested under four different levels of salinity (0, 60, 80 and 100 m*M* NaCl). Seeds were surface-sterilized with 10% sodium hypochlorite for 3 min. and then washed three times with distilled water. Ten seeds of each variety were grown in plastic bowls filled with 1 kg washed river sand and saturated with test solutions of salinity (0, 60, 80 and 100 m*M* NaCl). These bowls were placed in a Growth Chamber (Sanyo-Gallenkamp, U.K.) running at 28 ± 2^{o}C with 10 h photoperiod with 80 μM S^{-1} m^{-2} light intensity. The seeds were considered germinated when radical length reached up to 5 mm. The experiment was laid in completely randomized design with three replications.

Physiological Indices

The germinated seeds were counted daily and promptness index (PI) was calculated using formula given. This index was used to calculate germination percentage (Ashraf *et al.*, 2008):

$$[\boldsymbol{PI} = nd1\ (1.00) + nd2\ (0.75) + nd3\ (0.50) + nd4\ (0.25)]$$

Here: $nd1, nd2, nd3$ and $nd4$ denoted the total number of seeds that germinated on first, second, third and fourth day, respectively.

A germination stress tolerance index (GSI) was calculated in terms of percentage as followed:

$$\left[\boldsymbol{GSI} = \left(\frac{PI\ of\ stressed\ seeds}{PI\ of\ control\ seeds}\right) \times 100\right]$$

After two weeks of the experiment, 5 plants from each replication of each treatment were harvested and their fresh weight, shoot and root lengths, osmotic potential (Ψ_s), root length/shoot length ratio (RL/SL) and relative water contents (RWC) were estimated. Plants were dried in an oven at 70^{o}C and their dry weights were recorded and dry weight was used for determination of Na^+/K^+ ratio.

Root and shoot length stress tolerance indices (RLSI, SLSI), fresh and dry weight indices (FWSI, DWSI) were calculated according to the following formulas:

$$\left[\boldsymbol{FWSI} = \left(\frac{Fresh\ weight\ of\ stressed\ plants\ (g)}{Fresh\ weight\ of\ control\ plant\ (g)}\right) \times 100\right]$$

$$\left[\boldsymbol{SLSI} = \left(\frac{Shoot\ length\ of\ stressed\ plants\ (cm)}{Shoot\ length\ of\ control\ plants\ (cm)}\right) \times 100\right]$$

$$\left[\boldsymbol{RLSI} = \left(\frac{Root\ length\ of\ stressed\ plants\ (cm)}{Root\ length\ of\ control\ plants\ (cm)}\right) \times 100\right]$$

$$\left[\boldsymbol{DWSI} = \left(\frac{Dry\ weight\ of\ stressed\ plants\ (g)}{Dry\ weight\ of\ control\ plants\ (g)}\right) \times 100\right]$$

Leaf relative water content (RWC) was measured by using the method of Weatherley (1950).

$$\left[\boldsymbol{RWC}\ (\%) = \left[\frac{(FW - DW)}{(TW - DW)}\right] \times 100\right]$$

Here fresh, dry and turgid weight represented by FW, DW and TW respectively.

Osmotic potential of leaf was measured by using osmometer (Wescor 5500, USA). The leaf sap was extracted to determine the osmolality. Sodium (Na^+) and potassium (K^+) contents were estimated from plant extract prepared from dried plant materials according to Wolf (1982) by flame photometer (Model PFP7, Jenway Ltd., UK).

Statistical Analysis

The collected data was statistically analyzed and subjected for analysis of variance (ANOVA) and subsequent comparison of means was performed by using the Ducan's test at 5% probability (Steel *et al*., 1997). Cluster and correlation analyses were performed using the MSTATC and Minitab 16.

Results

Seed germination was significantly reduced under salinity in all varieties of mungbean. The germination stress tolerance index (GSI) was (85%, 75 and 67%) under 60, 80 and 100 m*M* NaCl levels, respectively (Table 1). Maximum GSI was exhibited by variety MUNG-88 (98.8%, 98.3 and 96%) while it was minimum in Var-6601 (74.5%, 58.7, 42%) at 60, 80 and 100 m*M* NaCl, respectively. The overall varietal means and ranking indicated that MUNG-88 was at the top followed by NM-92 while Var-6601 was at bottom (13th position).

Salinity stress significantly decreased the shoot and root length (SLSI and RLSI) of all mungbean varieties. With the increase in salinity (60, 80 and 100 m*M* NaCl), the SLSI and RLSI were decreased significantly (66.3, 51.3 and 40%,

Table 1: Effect of various salinity levels on germination stress tolerance index, shoot length and root length stress tolerance index of different mungbean varieties

Mungbean varieties	Germination stress tolerance index (GSI%)					Shoot length stress tolerance index (SLSI%)					Root length stress tolerance index (RLSI%)				
	Salinity levels (m*M* NaCl)														
	60	80	100	Means	Ranking	60	80	100	Means	Ranking	60	80	100	Means	Ranking
Mung 88	98.8±6b-d	98.3±6b-d	96.8±1b-d	97.0±1a	1	73.6±0.8c	58.9±0.5c-e	46.7±1de	59.8±10cd	5	74.1±1bc	62.2±0.5a-c	51.6±0.3ab	62.7±13b-d	4
Ramzan	94.5±2a	88.5±3a	79.7±10a	87.6±7b	2	75.1±0.3a	66.5±0.3a	56.5±0.4ab	66.0±11ab	2	79.1±2bc	63.9±0b-d	50.3±0.7a-c	64.5±13a-c	3
NM-92	90.2±3ab	83.7±1ab	75.9±4ab	83.3±7bc	3	79.2±0.7a	64.9±0.2a	61.3±1a	68.5±12a	1	79.3±1a	68.8±1a	50.4±1a	66.2±14ab	2
NM-13-1	89.4±4a-c	83.5±3de	74.4±2b-d	82.4±7bc	4	49.6±0.3e	40.2±0.8e-g	17.1±1ef	35.6±12ij	12	67.1±0cd	49.4±0cd	35.9±1de	50.8±14f-i	9
NM- 2011	87.8±2b-d	82.6±1a-c	70.1±5bc	80.2±9cd	5	66.7±0.6de	50.3±0.4ef	37.5±0.3ef	51.5±13ef	8	69.0±0.7cd	51.6±1c-e	40.2±0.4b-d	53.6±14e-g	8
NM-121-25	87.1±1c-e	80.2±2e	69.3±10cd	78.9±8cd	6	68.6±0.4cd	52.3±0.6de	40.6±0.4ef	53.8±11ef	7	71.9±1ab	55.6±1ab	41.2±1ab	56.3±15d-f	7
Chakwal-97	84.4±1d-f	78.4±2b-d	67.5±10bc	76.8±8cde	7	76.3±0.1ab	62.0±0.5ab	51.9±0.2bc	63.4±14a-c	3	75.5±1c	58.8±2b-e	44.4±1b-d	59.6±15c-e	6
NM-19-19	84.0±2g	73.5±3f	66.7±3e	74.8±8def	8	61.8±0.3ef	48.8±0.4f-h	34.4±0.3f	48.3±14fg	9	83.5±2a	66.7±2a	57.1±2a	69.1±14a	1
NM-20-21	82.5±2ef	66.1±3c-e	64.1±1b-d	70.9±10efg	9	57.8±0.3e	38.9±0.5gh	27.0±0.4f	41.2±13hi	11	75.7±2cd	59.1±2b-e	47.1±2b-d	60.6±15c-e	5
NM-2006	80.4±1f	65.2±2de	63.0±2d	69.5±9fg	10	60.9±0.4e	44.1±0gh	27.3±1f	44.1±16gh	10	60.1±1cd	43.5±2c-e	26.7±1cd	43.4±17ij	12
NM-28	76.1±2g	63.4±3f	56.5±2e	65.3±9gh	11	45.5±0.4f	27.1±0.3h	18.3±0.3f	32.3±15j	13	54.3±2d	44.1±0.8e	24.5±1d	41.0±16 j	13
NM- 51	76.5±2a-c	61.5±10b	45.8±1b-d	61.3±15h	12	69.4±0.3bc	54.8±0.6cd	45.8±0.1c-e	56.6±12de	6	64.5±1c	44.0±0.3c	30.8±0.8cd	46.4±16h-j	11
Var-6601	74.5±2d-f	58.7±3de	42.9±0b-d	58.7±15h	13	70.9±0.7ab	61.2±0.4bc	52.8±0.3b-d	61.6±12b-d	4	70.4±1c	51.6±0.8c-e	21.4±0.9d	47.8±16g-i	10
Means	85.2±7A	75.7±11B	67.1±14C			66.3±10A	51.3±11B	40.0±14C			71.0±8A	55.0±8B	40.0±11C		

Means sharing different letters, within a row or column, are statistically different from each other at 5% probability level

respectively for SLSI) and 71%, 55 and 40% respectively for RLSI (Table 1). Under 60 m*M* NaCl, the maximum SLSI (79%) was exhibited by NM-92 closely followed by RAMZAN (75%) whereas, minimum SLSI was observed in variety NM-28 (45%). Under the highest salinity level (100 m*M* NaCl), variety NM-92 was successful in maintaining the highest SLSI (61%) whereas the lowest was in NM-13-1 (17%). The overall ranking nad varietal means showed that NM-92 ranked at first while NM-28 comes at the 13th position. Similarly, the minimum value for RLSI (54.3%) was exhibited by NM-28 at 60 m*M* NaCl level while in case of NM-20-21 it was maximum (83%). At 100 m*M* NaCl, the highest RLSI was observed in NM-20-21 (57%) and minimum was in Var-6601 (21.4%). Varietal means and ranking indicated that NM-19-19 and NM-92 were ranked as 1st and 2nd while NM-28 at the 13th position (Table 1).

Fresh weight stress tolerance index (FWSI) was considerably decreased (63%, 45 and 34% under 60, 80 and 100 m*M* NaCl, respectively). The highest FWSI was exhibited by NM-92 (85%), while minimum FWSI was measured in NM-13-1 (43%) at 60 m*M* NaCl stress. At 80 m*M* NaCl, maximum FWSI observed in RAMZAN (62%) while NM-28 was the poorest in performance (25%) for FWSI. The varietal means and ranking indicated that NM-92 and RAMZAN scored maximum points for FWSI and ranked as 1st and 2nd while the NM-28 was at 13th (Table 2).

Dry weight stress tolerance index (DWSI) of all mungbean varieties reduced significantly (70%, 58% and 37%) at 60, 80, 100 m*M* NaCl, respectively. At 60 m*M* NaCl level, maximum DWSI was recorded for NM-92 (89%) whereas the lowest (43%) was inNM-28. Under 100 m*M* NaCl salinity, the highest DWSI was estimated for NM-92 (70%) and it was the lowest in NM-28 (20%). The varietal means and overall ranking showed that NM-92 performed the best and was at first position, while NM-28 was the lowest for DWSI and ranked at 13th position (Table 2).

Osmotic potential (Ψs) was significantly decreased under increasing salinity levels in all mungbean varieties. The variety NM-92 exhibited (-0.85 MPa) Ψ_s at 0 m*M* NaCl and it decreased up to (-1.35 MPa) under 100 m*M* NaCl stress. Mungbean variety NM-28 showed (-0.99 MPa) under control conditions and it dropped up to (-1.77 MPa) at 100 m*M* NaCl stress (Table 3). The varietal means showed that variety NM-92 and Chakwal-97 exhibited less reduction in osmotic potential than variety NM-28.

Relative water contents (RWC) in all mungbean varieties were significantly influenced under different levels of salinity (Table 3). Maximum RWC (90, 78 and 59%) were observed in variety NM-92 under 0, 80 and 100 m*M* NaCl while minimum were recorded in Mung-88, Ramzan, NM-28 (79, 52 and 32%). The varietal means showed that variety NM-92 exhibited maximum RWC.

Salinity stress significantly affected the RL/SL ratio in all varieties of mungbean. At 0, 60, 80 and 100 m*M* NaCl level, the highest RL/SL ratio was recorded in variety NM-19-19 while the lowest ratio was observed in variety Chakwal-97 at 0 and 80 m*M* NaCl (1.16 and 1.08) while Ramzan exhibited lowest ratio at 60 m*M* NaCl (0.84) (Table 4). Based on varietal means, the highest RL/SL ratio was noted in variety NM-19-19 (Table 4).

The Na^+/K^+ ratio was significantly increased under salinity in all mungbean varieties (Table 4). At 0, 60, 80 and 100 m*M* NaCl stress, the highest ratio was observed in variety NM-28 (0.118, 0.32, 0.47, 0.79) while lowest was noted in NM-92 (0.427, 0.136, 0.233, 0.427). On basis of varietal means, the mungbean variety NM-28 showed highest Na^+/K^+ ratio.

The correlation data indicated that growth and physiological indices exhibited positive correlations among GSI and SLSI, DWSI and RWC, while negative and insignificant correlation existed among Na^+/K^+ and RL/SL ratio, RLSI and RWC. The correlation between FWSI and osmotic potential was negative but significant (Table 5).

In current study, cluster analysis dendrogram (Fig. 1) based on complete linkage correlation coefficient distance (CLCCD) which had split the mungbean varieties into three clusters. The cultivars with similar characters come in cluster 1-salt tolerant- varieties and second cluster contain -medium tolerant- while third comprised of-salt sensitive-. These varieties grouped together according to their salt tolerance

Table 2: Effect of various salinity levels on fresh weight stress tolerance index and dry weight stress tolerance index of different mungbean varieties

Mungbean varieties	Fresh weight stress tolerance index (FWSI %)					Dry weight stress tolerance index (DWSI %)				
	Salinity treatments (m*M* NaCl)									
	60	80	100	Means	Ranking	60	80	100	Means	Ranking
Mung 88	66.5 ± 0.3a-c	43.4 ± 1.0ab	35.7 ± 0.4a-d	48.5 ± 16de	7	84.5 ± 0.1ab	61.1 ± 0.1a-d	28.6 ± 0.04b-d	58.0 ± 10def	6
Ramzan	77.6 ± 0.2ab	62.1 ± 0.0a	46.6 ± 1.0a-c	62.1 ± 15ab	2	79.8 ± 0.2ab	68.1 ± 0.2ab	42.5 ± 0.04a	63.5 ± 12cd	4
NM-92	85.0 ± 0.3a	59.6 ± 1.0a	55.1 ± 1.0ab	66.6 ± 15 a	1	89.0 ± 0.1ab	73.2 ± 0.1a-c	70.0 ± 0.03a	77.4 ± 17 a	1
NM-13-1	71.3 ± 0.01f	50.8 ± 0.2d	39.6 ± 0de	53.9 ± 14cd	5	49.0 ± 0.2ab	38.4 ± 0.06a-d	26.3 ± 0.04b-d	37.9 ± 19j	12
NM- 2011	44.4 ± 0.2ef	36.5 ± 0.3cd	24.6 ± 0.04a	35.2 ± 14 hi	11	63.7 ± 0.1ab	51.2 ± 0.03b-d	30.4 ± 0.02bc	48.4 ± 20gh	9
NM- 121-25	68.5 ± 1a-c	44.8 ± 0.2ab	37.9 ± 0.1a-e	50.4 ± 14de	6	74.6 ± 0.2ab	58.2 ± 0.1a-d	34.6 ± 0.02b	55.8 ± 20ef	7
Chakwal-97	57.2 ± 0.2a-d	47.5 ± 0.1bc	33.6 ± 0.1a-e	46.1 ± 16ef	8	82.6 ± 0.1ab	76.7 ± 0.05d	56.9 ± 0.02cd	72.1 ± 20ab	2
NM-19-19	73.5 ± 0.1c-e	55.8 ± 0.2bc	40.7 ± 0.1a-d	56.7 ± 15bc	4	67.7 ± 0.02b	56.5 ± 0.05cd	31.5 ± 0.03b-d	51.9 ± 18fg	8
NM-20-21	54.6 ± 0.1b-e	34.4 ± 0.2cd	27.2 ± 0.2b-e	38.7 ± 15gh	10	62.9 ± 0.04ab	47.1 ± 0.06b-d	24.5 ± 0.02b	44.9 ± 16hi	10
NM-2006	58.9 ± 0.2f	38.9 ± 0.2d	28.5 ± 0.3e	42.1 ± 16fg	9	54.4 ± 0.1b	42.0 ± 0.03b-d	26.1 ± 0.03b-d	40.8 ± 15ij	11
NM-28	43.1 ± 0.2ef	31.0 ± 0.3cd	22.4 ± 0.2de	32.2 ± 16 i	12	42.6 ± 0.02b	39.5 ± 0.05b-d	20.4 ± 0.02d	34.1 ± 14j	13
NM- 51	45.5 ± 0.2a-c	24.6 ± 0.2a	19.4 ± 0.6a	29.8 ± 13i	13	77.3 ± 0.05b	64.8 ± 0.02b-d	37.8 ± 0.03b-d	60.0 ± 11de	5
Var-6601	79.9 ± 0.3d-f	58.7 ± 0.3cd	40.6 ± 0.2a-e	59.7 ± 13bc	3	81.8 ± 0.1a	72.7 ± 0.1a	47.9 ± 0.02a	67.5 ± 11bc	3
Means	63.5± 14A	45.2 ± 11B	34.8 ± 10C			70 ± 14A	57.7 ± 13B	36.7 ± 14C		

Means sharing different letters within a row or column are statistically different from each other at 5% probability level

Table 3: Effect of various salinity levels on osmotic potential and relative water contents of different mungbean varieties

Mungbean varieties	Osmotic potential (Ψs; -MPa)					Relative water contents (RWC; %)				
	Salinity treatments (NaCl in m*M*)									
	0 m*M*	60 m*M*	80 m*M*	100 m*M*	Means	0 m*M*	60 m*M*	80 m*M*	100 m*M*	Means
Mung 88	0.87 ± 0.2a	0.98 ± 0.3d-f	1.27 ± 0.2a	1.33 ± 0.2a	1.11 ± 0.2de	79.00 + 20a	42.00 ± 10e	66.00 ± 6b	33.00 ± 3b	55.00 ± 21c
Ramzan	0.86 ± 0.1a	1.03 ± 0.03c-f	1.41 ± 0.2a	1.60 ± 0.2b	1.22 ± 0.3a-d	82.00 ± 15a	68.00 ± 10a-d	52.00 ± 2c	48.00 ± 6ab	62.00 ± 15b
NM-92	0.85 ± 0.2a	0.93 ± 0.03f	1.12 ± 0.1a	1.35 ± 0.2b	1.06 ± 0.2e	90.00 ± 5a	68.00 ± 4a-d	78.00 ± 10a	59.00 ± 10a	73.00 ± 13a
NM-13-1	0.93 ± 0.2a	0.94 ± 0.04ef	1.42 ± 0.1a	1.79 ± 0.3b	1.27 ± 0.4ab	88.00 ± 10a	58.00 ± 8d	69.00 ± 10ab	38.00 ± 8a	63.00 ± 20b
NM- 2011	0.88 ± 0.1a	1.05 ± 0.04c-e	1.37 ± 0.2a	1.42 ± 0.4a	1.18 ± 0.2b-e	81.00 ± 14a	68.00 ± 10a	65.00 ± 5b	40.00 ± 10b	63.00 ± 17b
NM- 121-25	0.84 ± 0.1a	1.21 ± 0.1ab	1.44 ± 0.2a	1.63 ± 0.3a	1.28 ± 0.3ab	86.00 ± 7a	72.00 ± 2a-c	61.00 ± 1bc	44.00 ± 4ab	65.00 ± 17b
Chakwal-97	0.87 ± 0.1b	0.97 ± 0.02d-f	1.18 ± 0.1a	1.28 ± 0.3a	1.07 ± 0.1e	85.00 ± 10a	74.00 ± 4ab	61.00 ± 4bc	45.00 ± 5ab	66.00 ± 17b
NM-19-19	0.95 ± 0.02b	1.13 ± 0.1a-c	1.40 ± 0.2a	1.62 ± 0.3a	1.27 ± 0.2ab	89.00 ± 4a	62.00 ± 7cd	77.00 ± 2a	36.00 ± 6b	66.00 ± 22b
NM-20-21	0.90 ± 0.3a	1.11 ± 0.1bc	1.33 ± 0.2b	1.51 ± 0.2a	1.21 ± 0.2b-d	88.00 ± 10a	78.00 ± 3a	62.00 ± 3b	42.00 ± 2b	67.00±20ab
NM-2006	0.91 ± 0.02a	0.97 ± 0.02d-f	1.31 ± 0.1a	1.65 ± 0.2a	1.21 ± 0.3b-d	86.00 ± 10a	72.00 ± 1a-c	63.00 ± 10b	36.00 ± 3b	64.00 ± 21b
NM-28	0.99 ± 0.03a	1.23 ± 0.1a	1.39 ± 0.1b	1.77 ± 0.3a	1.34 ± 0.3a	90.00 ± 10a	76.00 ± 1a	52.00 ± 4c	32.00 ± 10b	62.00 ± 25b
NM- 51	0.91 ± 0.04a	1.06 ± 0.06cd	1.24 ± 0.2a	1.36 ± 0.2a	1.14 ± 0.1c-e	87.00 ± 10a	71.00 ± 10a-c	63.00 ± 3b	41.00 ± 20b	66.00 ± 19b
Var-6601	0.91 ± 0.9a	1.10 ± 0.1bc	1.42 ± 0.2a	1.51 ± 0.4a	1.23 ± 0.2a-c	80.00 ± 20a	63.00 ± 3b-d	60.00 ± 5bc	46.00 ±20ab	62.00 ± 13b
Means	0.80 ± 0.04A	1.00 ± 0.1B	1.31 ± 0.1C	1.50 ± 0.1D		85.00 ± 30A	71.00 ± 90B	59.00 ± 70C	41.00 ± 70D	

Means sharing different letters, within a row or column, are statistically different from each other at 5% probability level

Table 4: Effect of various salinity levels on root length: shoot length ratio and Na^+: K^+ ratio of different mungbean varieties

Mungbean varities	Root length/ Shoot length ratio (RL/SL)					Sodium potassium ratio (Na^+/K^+)				
	Salinity treatments (NaCl in m*M*)									
	0 m*M*	60 m*M*	80 m*M*	100 m*M*	Means	0 m*M*	60 m*M*	80 m*M*	100 m*M*	Mean
Mung 88	1.620±0.06b-e	1.590 ± 0.1b	1.720 ± 0.06b-d	1.800 ± 0.2bc	1.680 ± 0.09a	0.048±0.007ef	0.142 ± 0.02b	0.235 ± 0.1c	0.438 ± 0.1d	0.215±0.16a
Ramzan	1.200 ± 0.2fg	0.840 ± 0.1b	1.140 ± 0.1cd	1.050 ± 0.03ef	1.050 ± 0.15b	0.116 ± 0.20a	0.298 ± 0.1a	0.451 ± 0.2a	0.781 ± 0.2a	0.411±0.28b
NM-92	1.490 ± 0.3c-f	1.490 ± 0.2b	1.570 ± 1b-d	1.270 ± 0.1de	1.450 ± 0.12bc	0.045 ± 0.002f	0.136 ± 0.02b	0.233 ± 0.1c	0.427 ± 0.1d	0.210±0.16a
NM-13-1	1.180 ± 0.07fg	1.620 ± 0.2b	1.340 ± 1b-d	1.260 ± 0.2de	1.350 ± 0.19b-d	0.096±0.003bc	0.146 ± 0.02b	0.259 ± 0.1bc	0.622 ± 0.01bc	0.280±0.23a
NM- 2011	1.430 ± 0.2d-g	1.550 ± 0.3b	1.430 ± 0.1b-d	1.410 ± 0.1c-e	1.450 ± 0.06b-d	0.062±0.002de	0.254 ± 0.1a	0.386 ± 0.1ab	0.698±0.001ab	0.350±0.26b
NM-121-25	1.890 ± 0.1b	2.000 ±0.1ab	2.000 ± 1b	2.000 ± 1b	1.970 ± 0.05c-e	0.085 ± 0.004c	0.280 ± 0.1a	0.434 ± 0.01a	0.703±0.001ab	0.375±0.26b
Chakwal-97	1.160 ± 0.1b-e	1.150 ±0.04b	1.080 ± 0.02d	1.000 ± 0ef	1.090 ± 0.07d-f	0.055 ± 0.1d-f	0.148 ± 0.01b	0.249 ± 0.01c	0.446 ± 0.01d	0.224±0.16a
NM-19-19	2.420 ± 0.3a	3.220 ± 1a	3.220 ± 0.2a	3.390 ± 0.2a	3.060 ± 0.43d-f	0.117 ± 0.01a	0.319 ± 0.01a	0.459 ± 0.01a	0.791 ± 0.1a	0.421±0.28b
NM-20-21	1.360 ± 0.3e-g	1.760 ± 1ab	1.960 ± 0.02b-c	1.910 ±0.08bc	1.740 ± 0.27e-g	0.091±0.002bc	0.264 ± 0.1a	0.414 ± 0.01a	0.732 ± 0.01ab	0.375±0.27b
NM-2006	1.770 ± 0.2bc	1.800 ± 0.3b	1.760 ± 0.04b-d	1.750±0.05b-d	1.770 ± 0.021fg	0.069 ± 0.01d	0.166 ± 0.03b	0.251 ±0.01bc	0.449 ± 0.01d	0.233±0.16a
NM-28	1.680 ± 0.2b-d	1.800 ± 0.2b	1.840 ± 0.1b-d	1.210 ± 0.1e	1.630 ± 0.28g	0.118 ± 0.01a	0.321 ± 0.1a	0.476 ± 0.1a	0.799 ± 0a	0.428±0.28b
NM- 51	1.430±0.03d-g	1.320 ± 0.1b	1.160 ± 0.1b-d	0.950 ± 0.05ef	1.210 ± 0.20g	0.103±0.002ab	0.288 ± 0.1a	0.443 ± 0.01a	0.735 ± 0.01ab	0.392±0.26b
Var-6601	1.310±0.07e-g	1.160 ± 0.1b	1.110 ± 0.1d	0.640 ± 0.04f	1.050 ± 0.28g	0.056 ±0.02d-f	0.154±0.004b	0.236 ± 0.1c	0.489 ± 0.1cd	0.233±0.18a
Means	1.530 ± 0.30A	1.630±0.51B	1.640 ± 0.50C	1.510 ± 0.60D		0.080 ± 0.03A	0.221 ± 0.08B	0.341 ± 0.1C	0.620 ± 0.1D	

Means sharing different letters, within a row or column, are statistically different from each other at 5% probability level

potential such as Cluster 1: NM-92, Ramzan, Chakwal-97, NM-51, Var-6601; Cluster 2: Mung-88, NM-121-25, NM-20-21, NM-13-1, NM-2011; Cluster 3: NM-28, NM-19-19 and NM-2006.

Discussion

Results of this study disclosed that growth indices and physiological parameters *i.e.*, GSI, SLSI, RLSI, FWSI, DWSI, RL/SL ratio, RWC and osmotic potential were significantly decreased in all varieties of mungbean while Na^+/K^+ ratio increased under saline conditions (Table 1-5). At increasing levels of salinity (60, 80 and 100 m*M* NaCl), seed germination stress tolerance index decreased significantly in all mungbean varieties (Table 1). These findings are in agreement with Islam and Karim (2010), who reported that increased levels of salinity drastically reduced the GSI and germination percentage in rice genotypes. Salt

Table 5: Correlation among different growth indices and physiological parameters used for screening mungbean germplasm

Variables	GSI	DWSI	FWSI	SLSI	RLSI	RWC	Ψ_s (-MPa)	RL/SL
DWSI	0.56*							
FWSI	0.39	0.33*						
SLSI	0.77**	0.65*	0.65**					
RLSI	-0.07	0.18	0.59*	0.23				
RWC	0.03*	0.02	0.26	0.36	0.222			
Ψ_s(-MPa)	-0.58*	-0.06	-0.50**	-0.66	-0.311	-0.35		
RL/SL	0.49	-0.04	-0.01	0.31	0.180	0.27	-0.74**	
Na^+/K^+	-0.40	-0.34	0.11	-0.39	0.005	0.01	0.55*	-0.58*

**= Significant ($p < 0.01$); GSI, DWSI, FWSI, SLSI, RLSI, RWC, Ψs, RL/SL and Na^+/K^+ ratio

Here GSI = Germination stress tolerance index; FWSI = Fresh weight stress tolerance index; DWSI = Dry weight stress tolerance index; SLSI = Shoot length stress tolerance index; RLSI = Root length stress tolerance index; RWC = Relative water contents

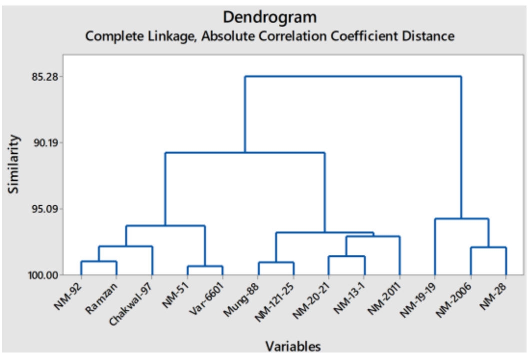

Fig. 1: Dendogram from cluster analysis for salt tolerance in different mungbean varieties based on growth indices and physiological parameters: a screening tool. Clusters detail; Cluster 1: NM-92, Ramzan, Chakwal-97, NM-51, Var -6601; Cluster 2: Mung-88, NM-121-25, NM-20-21, NM-13-1, NM-2011; Cluster 3: NM-19-19, NM-2006, NM-28

induced inhibition in seed germination could be attributed to osmotic stress and specific ion toxicity (Huang and Redmann, 2013). However, the reduction in seed germination is also due to accretion of sodium ions and chloride ions which prevents the sufficient absorption of water under salt stress. Salt stress markedly declined the shoot and root length in all seedlings of mungbean (Table 1). The shoot growth decreased under salinity due to loss of turgidity in the meristematic tissues resulting from the repressed water movement from the root zone (Alam *et al*., 2004). The root growth also disrupted due to specific ion effect which changed the root morphology and anatomy. According to some previous reports, the reduction in root length under salinity is an adaptive mechanism for plants to avoid and decrease absorption of salts (Hasegawa *et al.*, 2000). However, the variations among varieties regarding shoot length may be due to some genetic changes (Krishnamurthy *et al.*, 2007). The results of current experiment are in accordance with Alherby *et al.* (2018), who reported a decline in root length and shoot length of mungbean seedlings under salinity stress. Our findings regarding biomass reduction are similar with results of Hapsari and Trustinah (2018), who suggested that plant biomass decreased significantly in mungbean varieties under increasing levels of salt stress. However, the biomass reduction is due to interrupted biochemical and physiological mechanisms (Craine, 2005) as well as due to production of fewer leaves which reduces the photosynthetic area and dry matter accumulation (Puvanitha and Mahendran, 2017).

The osmotic potential (Ψs) of mungbean varieties also decreased under saline media which is in accordance with previous reports in *Raphinus sativus* (Noreen *et al.*, 2012), *Helianthus annuus* (Akram *et al.*, 2012) and *Pisum sativum* (Noreen *et al.*, 2010). According to Sanchez-Blanco *et al.* (1991), *Lycopersicon esculentum* and *Pennellii* plants also exhibited low Ψs under salinity stress, due to loss of water. Relative water contents show the plant water status; therefore, any reduction in RWC directly reflect the plant water deficit environment. The higher soluble salt concentration may induce the disturbance and imbalance in plant water relation and slows down the uptake of minerals and water from soil. Such disturbances may result in ionic toxicity and osmotic stress (Jiang *et al*., 2014). Our results regarding RWC confirmed the findings of Chutipaijit *et al.* (2009) and Amirjani (2010) who also suggested that salt tolerant cultivars exhibited higher RWC than that of salt susceptible varieties under saline conditions. The Na^+/K^+ ratio was significantly influenced under salinity in all mungbean varieties (Table 4). Higher Na^+/K^+ may damage the cell membrane resulting in replacement of calcium ions with sodium and leakage of potassium. Shabala and Cuin (2008), reported that Na^+/K^+ ratio considerably increased under salt stress and estimation of Na^+/K^+ ratio is an important indicator for plant response to stress.

Correlation analysis exhibited important associations among growth indices and physiological parameters. The correlation analysis of present study indicated a very positive and significant correlation among GSI and SLSI, FWSI and DWSI while non-significant correlation found between Na^+/K^+ and RL/SL ratio and RLSI (Table 5). The negative and significant relationship existed between GSI and osmotic potential. These results are similar with Kausar *et al.* (2012), found a positive correlation between physiological indices and stress tolerance in *Brassica* and *Sorghum* and concluded that physiological indices reflect the stress tolerance in plants. The reports by Zafar *et al.* (2015), on evaluation of *Triticum aestivum* varieties also revealed positive correlation among physiological indices. The data clearly showed that varities with higher ranking (RWC, Ψs, RL/SL ratio, GSI, RLSI, SLSI, FWSI and DWSI) are salt tolerant varieties of mungbean (*Vigna radiata L.* Wilczek). However, mungbean variety NM-92 scored maximum points and ranked at first position therefore, placed in cluster 1 in dendrogram (Fig. 1) and regarded as salinity tolerant variety. While variety NM-28 was the lowest in scores (below average) and come in cluster 3 (Fig. 1) therefore, categorized as sensitive one. NM-92 can be grown in saline soil having less than 80 m*M* NaCl salinity and may be used in breeding program to develop high yielding salt tolerant mungbean varieties.

Conclusion

Results disclosed the germination, shoot and root lengths, seedlings fresh and dry weight indices and root/shoot ratio, RWC, Na^+/K^+ ratio are the important growth indices and physiological parameters to be used as selection criteria to select mungbean germplasm for salinity tolerance at early seedling growth. Moreover, the mungbean variety NM-92 is the most salt tolerant variety which can be used to grow in saline conditions.

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