Mankar GD *et al.* (2021)
Notulae Scientia Biologicae
Volume 13, Issue 2, Article number 10936
DOI:10.15835/nsb13210936
Research Article

Morphological, physiological, and biochemical responses to NaCl-induced salt stress in mungbean (*Vigna radiata* L.) varieties

Ganesh D. MANKAR[1], Uttam R. WAYASE[1], Deepak B. SHELKE[2], Tukaram D. NIKAM[3], Rajkumar B. BARMUKH[1]*

[1]*Modern College of Arts, Science and Commerce (Autonomous), Post Graduate Research Centre, Department of Botany, Shivajinagar, Pune-5, Savitribai Phule Pune University, Pune-7, MS, India; gdmbotany@gmail.com; botanica5050@gmail.com; barmukhbotany@moderncollegepune.edu.in (*corresponding author)*
[2]*Department of Botany, Amruteshwar Arts, Commerce and Science College, Vinzar, Velha, Pune- 412213, MS, India dpk.shelke1@gmail.com*
[3]*Savitribai Phule Pune University, Department of Botany, Pune 411 007, MS, India; tdnikam@unipune.ac.in*

Abstract

Seventeen mungbean varieties [*Vigna radiata* (L.) R. Wilczek] were subjected to 100-400 mM salinity stress at the germination stage, and the indices of seed germination and early seedling growth were analysed. With the increasing salinity, seed germination and seedling growth attributes were affected in all varieties. Principal component analysis and hierarchical cluster analysis of varietal responses on the germination and seeding growth attributes at 400 mM NaCl separated seventeen varieties into four distinct clusters. Principal component analysis at lower salt stress levels indicated that the attributes of germination and early seedling growth are reliable to identify salt-tolerant mungbean varieties. In contrast, only germination attributes are reliable at higher salinity levels. Two salt-susceptible and salt-tolerant varieties were further assessed for NaCl-induced physiological and biochemical changes. Levels of proteins, secondary metabolites, osmolyte, and antioxidants were increased at lower salt concentrations but reduced at higher salt concentrations. Photosynthetic pigments decreased and membrane damage increased under salinity. Varieties that showed tolerance to salt stress can be used in salinity-affected agriculture fields after validating their salt tolerance in field experiments.

Keywords: antioxidants; germination; NaCl; osmolytes; photosynthetic pigments; secondary metabolites

Abbreviations: DW: dry weight; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FGP: final germination percentage; FW: fresh weight; GI: germination index; GRI: germination rate index; HCA: hierarchical cluster analysis; MCA: multiple correlation analysis; MDA: malondialdehyde content; PCA: principal component analysis; PC: proteins content; RL: root length; STI: salt tolerance index; SL: shoot length; SR: secondary roots; SV: seedling vigor; TGI: Timson germination index; %TWC: percent tissue water content; TPC: total phenolics content; TFC: total flavonoids content; PRC: total proline content; TSC: total sugars content; TFAA: total free amino acid content.

Received: 28 Mar 2021. Received in revised form: 04 Jun 2021. Accepted: 09 Jun 2021. Published online: 23 Jun 2021.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

Mungbean is an economically important pulse crop grown in many parts of the world. It is an important protein source rich in fibbers, amino acids, fatty acids, vitamins, and minerals. Salt stress is one of the major abiotic stress in mungbean production, which results in a decrease in growth and productivity (Nair *et al.*, 2019). Cations such as Na^+, Ca^{2+}, and Mg^{2+} and anions such as Cl^-, $SO4^{2-}$ and HCO^{3-} are the most common ions present in saline soil. Since the soils deteriorate due to Na^+ in particular and Na^+ and Cl^- both are toxic to plants, NaCl is considered the most critical salt (Yadav *et al.*, 2011). Excess NaCl in the surroundings and inside the cell impairs germination, morpho-physiological, photosynthetic, and biochemical processes and culminates in a threat to the plant's growth and survival (Sehrawat *et al.*, 2019).

Seed germination is an important agronomic and ecological process that determines the growth and productivity of higher plants. Screening of mungbean varieties at seed germination and early seedlings stage for stress tolerance is practical because plants are more susceptible to environmental stresses at the initial stages (Vibhuti *et al.*, 2015). Salinity adversely affects cellular and metabolic processes like photosynthesis, synthesis of proteins, nucleic acids, lipids, and carbohydrates, mechanisms detoxifying reactive oxygen species (ROS), and the levels of secondary metabolites (Parida and Das, 2005). Sustained seed germination, improved root and shoot growth, levels of photosynthetic pigments, and biochemical parameters confer salt tolerance during the initial phase of stand establishment. The crop varieties that are likely to have the mechanisms to avoid or tolerate salinity are precious and required for such harsh environments. Since mungbean varieties have performed differently under salinity stress (Nawaz *et al.*, 2020), a plant-based solution demands a thorough screening of tolerant crop varieties for better yield under a saline environment.

In light of this, the present investigation was undertaken with two objectives- i) identify mungbean varieties tolerant to salt stress during seed germination, and ii) analyse effects of NaCl salt stress on key physiological and biochemical parameters during seed germination and early seedling growth of salt-tolerant and salt-susceptible varieties.

Materials and Methods

Seed collection, growth, and treatment conditions

Healthy seeds of the following seventeen certified varieties of mungbean were procured: 'VBN(Gg)2' and 'VBN(Gg)3' (National Pulses Research Centre, Tamil Nadu Agricultural University, Vamban), 'IPM 401-3', 'IPM 302-2', 'IPM-2K-14-9', 'IPM 2-3', 'IPM 2-14', and 'IPM 99-125' (Indian Institute Of Pulse Research, Kanpur), 'PKU-AKM-4', 'PKU-AKM 12-28', 'PKU-AKM 8802', and 'PKV Greengold' (Pulses Research Unit, Akola), 'DGGV-2' (ICAR-Indian Institute for Pulses Research Regional Research Center, UAS Campus, Dharwad, Karnataka). The seeds of 'Utkarsha', 'Samrat', 'SML 668', and 'Swati 4' were collected from the local market.

Seeds were surface sterilized with 0.1% $HgCl_2$ for three min and subsequently washed five times with distilled water. Ten Seeds were set to germinate on a double-layered germination paper placed in sterile Petri plates (90 mm). The germination paper was moistened with 10 ml of 100 to 400 mM of NaCl solutions. The control was set by moistening the germination paper with 10 ml of distilled water. Petri plates were incubated in the dark at 25±2 °C for ten days. The experiment was performed in triplicate, and each replicate had ten seeds, and germination counts were taken on each day.

Germination attributes

After the germination run of ten days, seed germination was analysed by computing: final germination percentage (FGP), germination rate index (GRI), and germination index (GI) by following Kader (2005) and Timson germination index (TGI) as per Timson (1965).

Growth attributes

Five seedlings were randomly selected on the 11th day. Their growth was measured in terms of shoot length (SL), root length (RL), number of secondary roots (SR), fresh weight (FW), dry weight (DW), percent tissue water content (%TWC) as described by (Shelke *et al.*, 2017), and seedling vigour (SV) as per Abdul-Baki and Anderson (1973).

The multivariate analysis tools such as PCA and HCA are used to differentiate samples having different biological statuses, quality, and origin (Chunthaburee *et al.*, 2015). Therefore, PCA and HCA were used to identify stress-tolerant mungbean varieties. This analysis grouped seventeen varieties into clusters based on their responses under NaCl salt stress. STIs of attributes were subjected to PCA and HCA to evaluate the salt tolerance level in seventeen mungbean varieties. The salt tolerance indices (STIs) were calculated by dividing the value observed on an attribute under a given salinity level by the weight on the same attribute in control (Chunthaburee *et al.*, 2015).

Selection of varieties for photosynthetic and biochemical analysis

The most tolerant and susceptible varieties were selected for photosynthetic and biochemical analyses based on germination and growth attributes in all varieties. IC50 is the inhibitory concentration of salt at which the seed germination is inhibited by 50%. The IC50 treatment of salt stress in the most susceptible variety was used for further experiments along with one concentration below and above the IC50 concentration. Seeds were surface sterilized with 0.1% $HgCl_2$ for three min and subsequently washed five times with distilled water. Ten Seeds were set to germinate on a double-layered germination paper placed in sterile Petri plates (90 mm). The germination paper was moistened with 10 ml of 75, 100, and 125 mM NaCl solutions. The control was set by moistening the germination paper with 10 ml of distilled water. Seedlings were allowed to grow under controlled conditions (25±0.5 °C and 16:8 h Light: dark photoperiod) for up to ten days. After ten days, five seedlings were selected, and the shoots and roots of seedlings were harvested separately for further analysis. The experiment was performed in triplicate, and each replicate had ten seeds.

Photosynthetic pigments and biochemical analysis

The photosynthetic and biochemical parameters were estimated spectrophotometrically on a microprocessor-based UV-Vis spectrophotometer (Bioera, India).

Chlorophyll content was estimated by Arnon's (1949) method. The carotenoid content was estimated by Maclachlan and Zalik's (1963) method. The anthocyanins were estimated by Mancinelli's (1984) method. Protein content was estimated by the Lowry *et al.* (1951) method with bovine serum albumin (BSA) as a standard protein. Malondialdehyde (MDA) (nmol/g dry weight) was estimated by Heath and Packer's (1968)method. Total phenolics were estimated by Swain and Hillis's (1959)method, and gallic acid was used as a standard phenol. Total flavonoids were estimated by the Balbaa *et al.* (1974)method, and rutin was used as a standard flavonoid. The proline content was estimated by the Bates *et al.* (1973)method. Total amino acid content was estimated by Lee and Takahashi's (1966)method, and L-Serine was used as a standard amino acid. Total sugar content was estimated by Scott and Melvin's (1953) method, and D-glucose was used as a standard sugar. The radical scavenging activity (%RSA) was estimated using the Blois (1958) DPPH method.

Statistical analysis

All the experiments were performed with three replicates in a completely randomized block design (CRD). Each replicate had ten seeds. The data were analysed by one-way analysis of variance (ANOVA) using SPSS software version 20. Mean values of treatments were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$. The data were presented as a mean ± standard deviation. The PCA of salt tolerance indices (STIs) was performed using the PAST statistical package (Hammer *et al.*, 2001). Parameters that showed a higher contribution to PCA were subjected to HCA. Ward's complete linkage clustering method and squared Euclidean distance were used for HCA performed in SPSS software version 20.

Results and Discussion

Effect of salt stress on seed germination

The increasing salt concentration affected all the seed germination attributes (FGP, GI, GRI, and TGI) in all varieties. At 100 mM NaCl, FGP, GI, GRI, and TGI were reduced drastically in 'VBN(Gg)' up to 54%, 56%, 61%, and 56%, respectively. Relatively lesser reductions were observed in all germination parameters in 'IPM 401-3', 'IPM 302-2', 'IPM 99-125', 'PKU-AKM 12-28', 'PKV Greengold', 'DGGV-2', 'PKU-AKM 4', 'PKU-AKM 8802', and 'Samrat'. These varieties showed the highest FGP (100%). 'PKV Greengold' had the highest GRI, GI, and TGI (100%) among those varieties that germinated at 100 mM NaCl. At 200 mM NaCl, 'VBN (Gg)3' showed a significant reduction of 84.61%, 90.47%, 94.49%, 90.47% in FGP, GI, GRI, TGI, respectively. However, 'PKV Greengold', 'PKU-AKM 4', 'PKU-AKM 8802', 'IPM 302-2', and 'Swati-4' showed less reduction without any significant difference. At 300 mM, more than 50% reduction in germination was observed in 'IPM 2-3', 'DGGV-2', 'PKU-AKM 4', 'Swati 4', 'IPM 99-125', and 'VBN(Gg)2'. The highest germination (FGP) was observed in 'PKU-AKM 8802' (96.66%), followed by 'PKU-AKM 12-28' (76.66%). These two varieties showed the highest GI, GRI, and TGI. At 400 mM NaCl, only seed germination indices were calculated. 'VBN(Gg)2' and 'VBN(Gg)3' did not germinate at this highest salt-stress level. Less reduction in FGP was observed in 'PKU-AKM 8802' (46.6%), followed by 'PKU-AKM 12-28' (50%). GI and TGI were reduced by 70.66% in 'PKU-AKM 12-28' and 64.66% in 'PKU-AKM 12-28'. A higher reduction in GI and TGI was observed in all other varieties.

FGP, GI, GRI, and TGI are helpful to analyse the effects of stress on seed germination (Kader, 2005). Reduced seed germination resulted in weak growth and development in 'VBN(Gg)3' and 'VBN(Gg)2' and reflected a crucial determinant for salinity tolerance. Salinity affects water and nutrient uptake during seed germination by creating osmotic and ionic imbalances that reduce germination potential (Pandey and Penna, 2017). Podder *et al.* (2020) have reported a similarly reduced mungbean germination under salinity. Decreased seed germination with increased salt concentration was also reported in wheat (Bagwasi *et al.*, 2020) and rice (Öner *et al.*, 2020). Therefore, germination attributes are the most critical and valuable attributes that reflect time, rapidity, uniformity, and synchronization in seed germination under salt stress.

Effect of salt stress on seedling development

Salt stress causes a detrimental effect on root growth as roots are directly in contact with salt. Root growth directly correlates with other seedling growth attributes such as shoot length, secondary root, and biomass production. Early seedling growth parameters such as SL, RL, SR, SV, FW, DW, and %TWC are helpful attributes for screening salt-tolerant varieties since these attributes are also affected by salinity (Shelke *et al.*, 2017). Various early seedling growth parameters such as SL, RL, SR, SV, FW, DW, TWC% were affected by salt stress. At 100 mM NaCl, SL was reduced by more than 50% in all varieties screened, but a severe reduction was observed in 'IPM 401-3', 'IPM-2K-14-9', and 'Swati-4'. The RL was more reduced in 'PKU-AKM 4' than 'PKV Greengold'. Seedling vigor was dramatically reduced by more than 70% in 'VBN (Gg)3' and 'IPM-2K-14-9', and up to 34% reduction was observed in 'PKV Greengold' and 'IPM995'. SR was reduced by nearly 70% in I'PM 2-14' and 'Swati-4'. More than 50% reduction in FW was observed in almost all varieties. Moreover, a more significant reduction in FW and DW was noted in 'DGGV-2'. TWC was reduced by 2-7%.

At 200 mM NaCl, 84 to 94% reduction in SL was observed in all varieties. 'VBN (Gg)3', 'IPM 2-3', and 'IPM 2-14' showed more than 83% reduction in root length than other varieties. More than 80% reduction in SV was observed in all varieties; however, 'VBN (Gg)3' showed the highest reduction (98%). Secondary roots were not developed in 'VBN(Gg)2', 'VBN(Gg)3', 'IPM 401-3', 'IPM 2-3', 'IPM 2-14', 'PKV Greengold', 'PKU-AKM 4', and 'Samrat'. FW was reduced up to 84-96%. Among these varieties, 'DGGV2' had the least, and 'Swati-4' had a maximum reduction in FW. DW was reduced by 68-91%, with a greater reduction in 'Swati-4' among all varieties screened. TWC% was less reduced in 'DGGV2' than other varieties. A greater reduction in TWC% was observed in 'Swati-4'. At 300 mM NaCl, all the varieties screened showed SL and RL reduced by

up to 90%. There was not a significant difference in SL and RL in all the varieties. However, SV was less reduced in 'PKU-AKM 8802' and 'PKU-AKM 12-28' compared to other varieties. At 300 mM, FW was reduced to 95-97% in all varieties without significant differences in FW. The DW was reduced by 7-11% in all varieties. TWC% was reduced between 80-94% except in Utkarsha, which showed up to 66% reduction. At 400 mM NaCl, SL, RL, and SV were not measurable due to poor seedling growth, and only germination indices were the key parameters. At 400 mM NaCl, FW, DW, and TWC% could not be calculated.

These results corroborate those by (Rahneshan *et al.*, 2018) in *Pistacia vera*. The number of secondary roots (SR) differed in different varieties at low salt concentrations. SR was poorly developed in a few varieties with the increased salt concentration. In few varieties, SR was not developed at and above 300 mM NaCl salt stress. The number of secondary roots decreased with increased salinity in soybean varieties and was a deterministic feature under salinity (Shelke *et al.*, 2017). SV was reduced in all varieties at low salinity levels. However, it was reduced by more than 70% in all varieties at 200 mM NaCl. At higher salt concentrations, the differences in the reduction observed in all the varieties were negligible. The highest reduction was observed in 'VBN(Gg)3'. A similar decrease in SV was observed in rice varieties subjected to salt stress (Datir *et al.*, 2020). Seedling fresh weight, dry weight, and tissue water content reduced with increasing salt concentration. The reduction in these attributes affects the absorption of water and essential minerals. It also reduces root pressure, water, and mineral flow, and their transport from root to shoot, and thus the growth and development of plants (Liu *et al.*, 2020). Our results are in line with Rahman *et al.* (2016), who have reported a variety-dependent reduction in SL, RL, DW, FW, and SV with increased salinity. These, therefore, appear to be deterministic features in mungbean varieties as well. A similar result was reported in rice (Datir *et al.*, 2020) and soybean (Shelke *et al.*, 2017).

In summary, a stepwise increase in salinity caused a progressive reduction in seed germination and affected early seedling growth parameters in all mungbean varieties screened in the present investigation. These varieties showed varied responses to salinity stress from low to high salinity levels. For example, at 100 mM NaCl, the 'Samrat' variety was resistant, but it was salt-susceptible at a higher level of 400 mM NaCl. At 100 mM and 200 mM NaCl, germination parameters such as FGP, GI, TGI, GRI, and early seedling growth parameters such as SV, RL, SL, SR, FW, DW, and %TWC are vital parameters to assess salinity responses in different varieties and can be used alternatively. However, at higher salinity levels of 300 mM and 400 mM, only germination parameters such as FGP, GI, TGI, and GRI are critical to assess salt-tolerance response because of poor SL and RL in seedlings. Further, SL, RL, FW, DW, SV were reduced by more than 80% at and above 200 mM NaCl, and SR was absent at and above 300 mM NaCl in all varieties. 'VBN(Gg)3' was highly susceptible at all salinity levels, and it did not germinate at and above 300 mM NaCl. Likewise, 'VBN(Gg)2' also did not germinate at 400 mM NaCl. PKU-AKM 12-28 showed the highest tolerance at all salinity levels.

The results showed significant differences in germination indices across varieties subjected to 400 mM NaCl stress (Table 1). PCA and HCA analyses were performed using germination indices of seedlings of seventeen mungbean varieties exposed to 400 mM NaCl (Table 2) to discriminate them based on their tolerance levels. FGP, GRI, GI, and TGI are essential to understand time, rapidity, uniformity, rate, and synchronization of seed germination (Kader, 2005). The higher the FGP, the higher is the population size. Higher GI is shown by seeds having a greater germination percentage and also a high rate of germination. Thus, if seed germination is higher and faster, it results in a better GRI (Kader, 2005). The Timson germination index (TGI) is an important and widely used parameter to assess the seed germination rate (Timson, 1965). 'VBN(Gg)3' and 'VBN(Gg)2' did not germinate at this salt stress level, and hence their germination indices were zero, while 'PKU-AKM 12-28' and 'PKU-AKM 8802' showed the highest germination indices due to their salt-tolerant nature.

Table 1. Seed germination parameters in seventeen mungbean varieties under control and 400 mM NaCl stress

Variety	NaCl (mM)	FGP (%)	GI	GRI	TGI
'VBN(Gg)2'	0	86.67 ± 5.77	41.67 ± 3.06	7.83 ± 1.04	83.33 ± 6.11
	400	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
'VBN(Gg)3'	0	86.67 ± 5.77	42.00 ± 1.73	8.07 ± 0.12	84.00 ± 3.46
	400	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
'IPM 401-3'	0	100.00 ±0.00	43.00 ± 0.58	7.17 ± 0.00	86.00 ± 0.00
	400	33.33 ± 5.77	13.00 ± 1.73	1.56 ± 0.10	26.00 ± 3.46
'IPM 302-2'	0	100.00 ±0.00	50.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	33.33 ±15.28	11.33 ± 4.93	1.36 ± 0.63	22.67 ± 9.87
'IPM-2K-14-9'	0	90.00 ±10.00	42.33 ± 4.16	8.36 ± 0.55	84.67 ± 8.33
	400	33.33 ± 5.77	8.67 ± 2.08	1.00 ± 0.22	17.33 ± 4.16
'IPM 2-3'	0	100.00 ±0.00	47.67 ± 0.58	8.83 ± 0.29	95.33 ± 1.15
	400	23.33 ±11.55	4.67 ± 2.31	0.58 ± 0.29	9.33 ± 4.62
'IPM 2-14'	0	100.00 ±0.00	46.67 ± 2.52	9.08 ± 0.52	93.33 ± 5.03
	400	30.00 ±10.00	7.33 ± 0.58	0.95 ± 0.14	14.67 ± 1.15
'IPM 99-125'	0	100.00 ±0.00	44.67 ± 0.58	7.33 ± 0.29	89.33 ± 1.15
	400	33.33 ±15.28	14.00 ± 2.65	2.08 ± 0.14	28.00 ± 5.29
'PKU-AKM 12-28'	0	100.00 0.00	50.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	50.00 ±17.32	14.67 ± 4.04	1.75 ± 0.50	29.33 ± 8.08
'PKV Greengold'	0	100.00 ±0.00	50.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	13.33 ± 5.77	5.67 ± 2.89	0.94 ± 0.77	11.33 ± 5.77
'DGGV-2'	0	100.00 ±0.00	50.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	36.67 ± 5.77	15.33 ± 2.08	2.22 ± 0.19	30.67 ± 4.16
'PKU-AKM-4'	0	100.00 ±0.00	5]0.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	10.00 ± 0.00	4.33 ± 0.58	0.50 ± 0.00	8.67 ± 1.15
'PKU-AKM 8802'	0	100.00 ±0.00	50.00 ± 0.00	10.00 ±0.00	100.00 ± 0.00
	400	53.33 ±11.55	17.67 ± 5.77	2.03 ± 0.82	35.33 ± 11.55
'Utkarsha'	0	96.67 ± 5.77	39.00 ± 3.00	6.14 ± 0.76	78.00 ± 6.00
	400	30.00 ±10.00	6.67 ± 3.06	0.81 ± 0.34	13.33 ± 6.11
'SML 668'	0	100.00 ±0.00	48.00 ± 1.00	9.00 ± 0.50	96.00 ± 2.00
	400	20.00 ±10.00	4.67 ± 1.53	0.56 ± 0.21	9.33 ± 3.06
'Swati 4'	0	100.00 ±0.00	47.33 ± 0.58	8.67 ± 0.29	94.67 ± 1.15
	400	26.67 ± 5.77	5.33 ± 1.15	0.67 ± 0.14	10.67 ± 2.31
'Samrat'	0	100.00 ±0.00	49.67 ± 0.58	9.83 ± 0.29	99.33 ± 1.15
	400	6.67 ± 11.55	1.67 ± 2.89	0.23 ± 0.40	3.33 ± 5.77

FGP: final germination percentage, GI: germination index**,** GRI: germination rate index**,** TGI: Timson germination index. (Values represent mean± standard deviation)

The germination indices were converted to STIs (Table 2) subjected to PCA and HCA. The PCA (Figure 1) showed the discrimination of varieties. PC1 and PC2 described 95.42% and 3.82% of the variance. The eigenvalue of PC1 and PC2 constituted 3.82% and 0.15% of the variation, respectively. PC1 positively correlated with GI (0.99), TGI (0.99), GRI (0.97), and FGP (0.95). PC2 had a positive correlation with only FGP (0.31), and all other parameters showed a negative correlation. The 'PKU-AKM 8802', 'PKU-AKM 12-28', 'DGGV-2', 'IPM 99-125', and 'IPM 401-3' ranked positively with PC1 due to higher germination parameters, whereas 'VBN(Gg)3', 'VBN(Gg)2', and 'Samrat' ranked negatively where all the parameters were suppressed. The STIs of GI, TGI, GRI, and FGP ranged between 0.00 to 0.35, 0.00 to 0.35, 0.00 to 0.22, and

0.00 to 0.53, respectively. The 'VBN(Gg)2' failed to germinate at this NaCl concentration. The highest STIs for germination parameters were observed in 'PKU-AKM 12-28' and 'PKU-AKM 8802'.

Table 2. Stress tolerance indices (STIs) of seed germination parameters of seventeen mungbean varieties under 400 mM NaCl salt stress

Variety	FGP	GI	GRI	TGI
'VBN(Gg)2'	0	0	0	0
'VBN(Gg)3'	0	0	0	0
'IPM 401-3'	0.33	0.3	0.22	0.3
'IPM 302-2'	0.33	0.23	0.14	0.23
'IPM-2K-14-9'	0.37	0.2	0.12	0.2
'IPM 2-3'	0.23	0.1	0.07	0.1
'IPM 2-14'	0.3	0.16	0.1	0.16
'IPM 99-125'	0.4	0.31	0.28	0.31
'PKU-AKM 12-28'	0.5	0.29	0.18	0.29
'PKV Greengold'	0.13	0.11	0.09	0.11
'DGGV-2'	0.37	0.31	0.22	0.31
'PKU-AKM 4'	0.1	0.09	0.05	0.09
'PKU-AKM 8802'	0.53	0.35	0.2	0.35
'Utkarsha'	0.31	0.17	0.13	0.17
'SML 668'	0.2	0.1	0.06	0.1
'Swati 4'	0.27	0.11	0.08	0.11
'Samrat'	0.07	0.03	0.02	0.03

(FGP: final germination percentage, GI: germination index, GRI: germination rate index, TGI: Timson germination index)

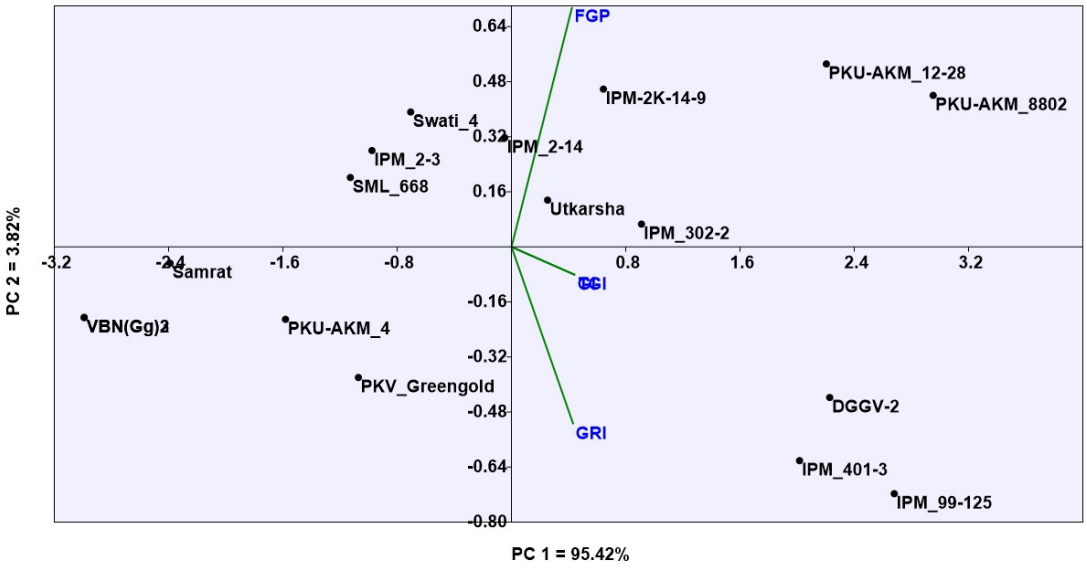


Figure 1. Biplot of principal components 1 and 2 of the PCA obtained from germination data on seventeen mungbean varieties exposed to 400 mM NaCl

FGP: final germination percentage, GI: germination index, GRI: germination rate index, TGI: Timson germination index.

PCA results were further confirmed by cluster analysis, which was performed on parameters responsible for most of the variation accounted for by PC1 and PC2. At 400 mM NaCl, SL, RL, and SV were not measurable due to poor seedling growth. Only germination indices were the key parameters to separate all varieties into four clusters at the highest salinity level (400 mM NaCl) (Fig 2). The 'VBN(Gg)2', 'VBN(Gg)3', and 'Samrat' from cluster I represent a highly susceptible group of varieties with low germination indices. 'IPM 2-14', 'Utkarsha', 'IPM 302-2', and 'IPM-2K-14-9' separated in cluster II represented moderately susceptible varieties. In contrast, 'IPM 2-3', 'SML 668', 'Swati 4', 'PKV Greengold', and 'PKU-AKM-4' separated in cluster III are moderately tolerant varieties. This separation is because of better germination indices in cluster II than cluster III. The 'PKU-AKM 12-28', 'PKU-AKM 8802', 'IPM 401-3', 'DGGV-2', and 'IPM 99-125' varieties separated in cluster IV represent salt-tolerant varieties with higher germination compared to all other clusters.

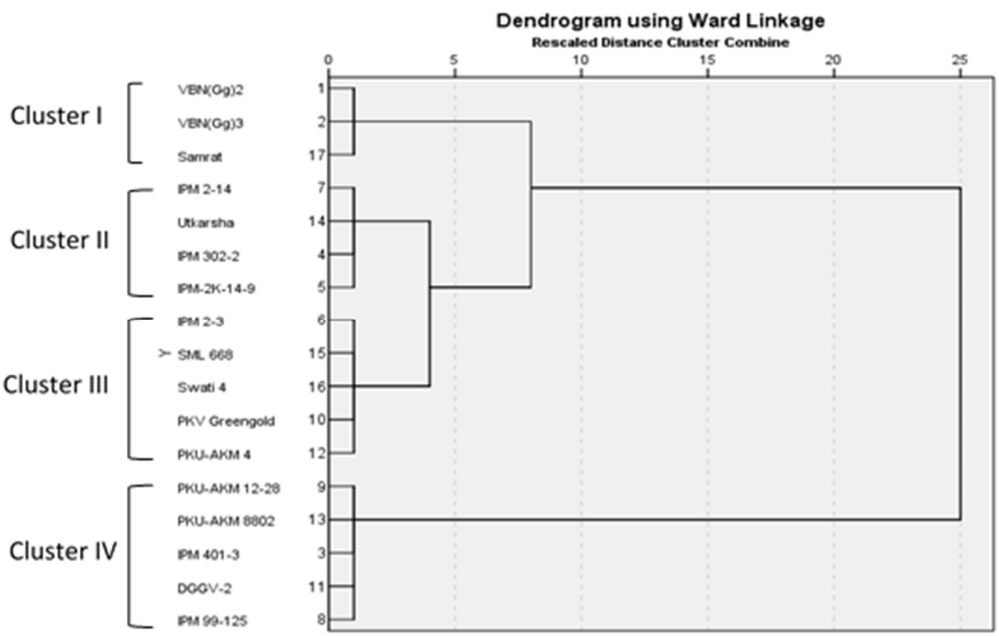


Figure 2. Hierarchical cluster analysis based on germination parameters on seventeen mungbean varieties subjected to 400 mM NaCl stress

Effect of salt stress on photosynthetic and biochemical attributes at early seedling growth in mungbean varieties

Two salt susceptible varieties ('VBN (Gg)3' and 'VBN (Gg)2') and two salt-tolerant varieties ('PKU-AKM 12-28' and 'PKU-AKM 8802') were selected to explore the salt-responsive photosynthetic and biochemical attributes at early seedling growth. These varieties were subjected to 75, 100, and 125 mM NaCl stress, and various photosynthetic and biochemical parameters in shoot and root biomass were measured.

Effect of salt stress on photosynthetic pigments

Salinity alters photosynthetic, biochemical, physiological, and metabolic processes, the extent of which varies with the level of stress and ultimately limits crop productivity (Shahid *et al.*, 2020). Plant growth is severely affected due to alteration in photosynthesis. Photosynthesis is hampered under salinity stress due to a reduction in chlorophyll contents or synthesis (Shin *et al.*, 2020).

In the present study, amounts of photosynthetic pigments, carotenoids, and anthocyanins were significantly reduced under salt stress (Figure 3A-3E). Damage to the photosynthetic pigments increased with the increasing salt concentration (Figure 3A-3E) and the 125 mM NaCl stress was the most damaging. These results corroborate those by Datir *et al.* (2020) in wheat and Regni *et al.* (2019) in olive. Low chlorophyll

content observed in mungbean plants under salinity may be associated with increased oxidative stress (Regni *et al.,* 2019) and the activation of chlorophyll degradation by the chlorophyllase enzyme (Datir *et al.*, 2020).

Salinity also affected carotenoid contents in mungbean varieties. Carotenoid levels were relatively less affected in 'PKU-AKM 12-28' at 75 and 100 mM NaCl concentration (Figure 3D). At the highest salinity stress level, the maximum reduction in carotenoids was observed in 'VBN(Gg)3'. Similar results were reported by Romanenko *et al.* (2017) in the alga *Acutodesmus dimorphus*. The enhanced carotenoid content improved salt tolerance in VBN(Gg)2. This result confirms carotenoid's potential role as antioxidants to detoxify ROS effects in plants during salinity stress (Verma and Mishra, 2005).

Literature is meager on anthocyanin levels in the vegetative tissue under salt stress. Anthocyanin content was marginally high in 'PKU-AKM 12-28' at 125 mM NaCl, whereas the other three varieties showed a 30-35% decrease in anthocyanin content (Figure 3E). High anthocyanin content was shown to induce an active protective response in *Oryza sativa* under salinity stress (Chutipaijit *et al.*, 2009). Eryilmaz (2006) had observed that chlorophyll content decreases, whereas anthocyanin content is elevated in different parts of seedlings under salinity. In sorghum, Jeon *et al.* (2020) have reported an increased anthocyanin production in salt-tolerant genotype 'Nampungchal' and reduced anthocyanin levels in the salt-susceptible 'Sodamchal' genotype. The mechanism of anthocyanin biosynthesis under salt stress is poorly understood and needs to be explored in detail (Eryilmaz, 2006).

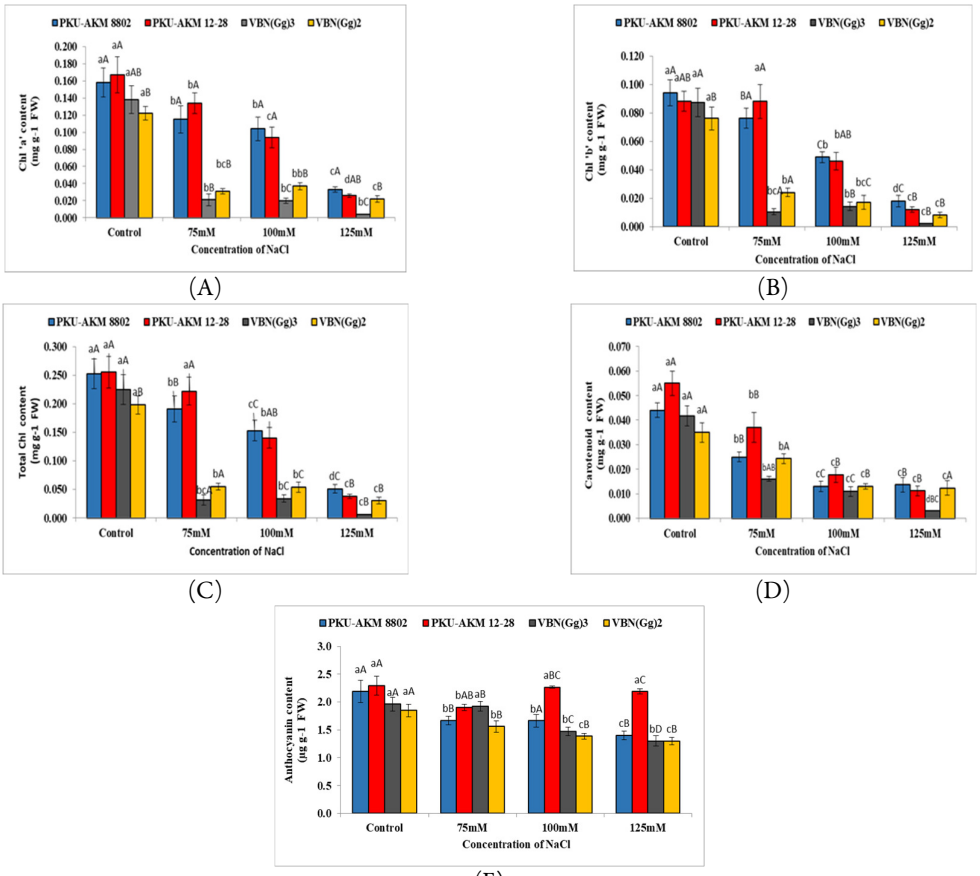
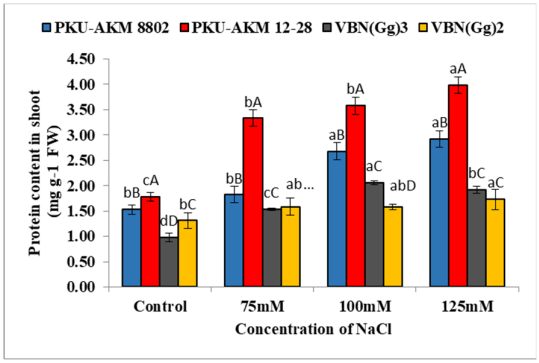


Figure 3. Effect of salt stress on plant pigments of 10-day old seedlings of *Vigna radiata* varieties
A) Chlorophyll 'a'; B) Chlorophyll 'b'; C) Total chlorophyll; D) Carotene and E) Anthocyanin content. Each value represents the mean of three replicates, and the vertical bar indicates the standard deviation. Small letters denote significant difference between treatments, and capital letters denote significant difference between varieties at a 0.05% significance level as per Duncan's Multiple Range Test (DMRT)

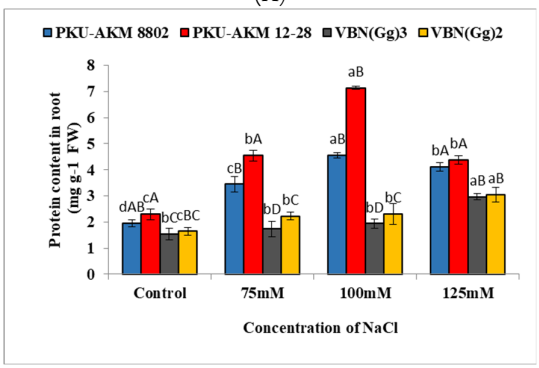
Effect of salt stress on protein content

The biochemical attributes were indeed influenced by increasing salt concentration in the selected four varieties. Protein content was increased upon exposure to salt stress in all varieties. Shoot protein content increased significantly under salt stress in 'PKU-AKM 12-28' (1.01-fold and 1.23-fold), followed by 'PKU-AKM 8802' (0.75 and 0.91-fold) at 100 and 125 mM NaCl concentration, respectively (Figure 4A). In 'VBN (Gg)2' and 'VBN (Gg)3', protein content increased relatively less compared to control at the highest salt concentration. In 'VBN (Gg)3', protein content increased at 100 mM NaCl but decreased at 125 mM NaCl.

Root protein was increased dramatically in 'PKU AKM 12-28' (2.1-fold) and 'PKU-AKM 8802' (1.32-fold) at 100 mM NaCl compared to 'VBN (Gg)2' (41%) and 'VBN (Gg)3' (0.26-fold) (Figure 4B). Protein content was high at all NaCl concentrations in 'PKU AKM 12-28' and 'PKU-AKM 8802'. The higher protein content in susceptible varieties could be due to enhanced detoxification pathways (Alharby *et al.*, 2019). Under salinity, plants significantly increase the levels of proteins such as photosynthetic pathway proteins, enzymes involved in scavenging ROS and osmolyte biosynthesis, late embryogenesis abundant proteins (LEA proteins), and membrane proteins, and carbohydrate and energy metabolism proteins (Arif *et al.*, 2020).



(A)



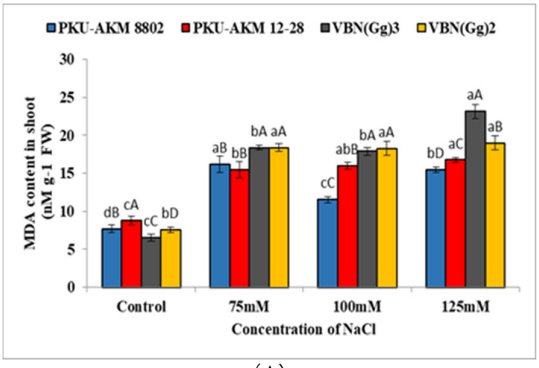
(B)

Figure 4. Effect of salt stress on the protein content of 10-day old seedlings of *Vigna radiata* varieties
A) Shoot protein content and B) Root protein content. Each value represents the mean of three replicates, and the vertical bar indicates the standard deviation. Small letters denote significant difference between treatments, and capital letters denote significant difference between varieties at a 0.05% significance level according to Duncan's Multiple Range Test (DMRT)

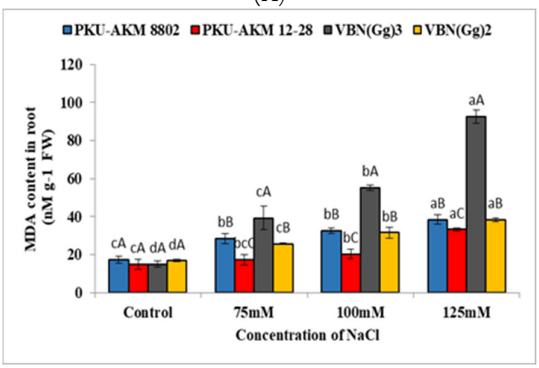
Effect of salt stress on lipid peroxidation

MDA, an output of oxidative stress and membrane damage, was used to measure the intensity of membrane damage in shoots and roots exposed to salinity stress (Campo *et al.*, 2014). Untreated normal seedlings have lower MDA content due to relatively less membrane damage caused by reactive oxygen species (ROS) generated as a by-product of plant aerobic metabolism. Various environmental stresses result in excessive ROS production, causing progressive oxidation of membranes (Sharma *et al.*, 2012), and therefore,

elevated MDA levels. Increased salt concentrations elevated the MDA content in mungbean, and it can be used as a vital biomarker to discriminate crop varieties. Membrane damage increased with increasing salt concentration from 75-125 mM NaCl. In the shoot, MDA content was significantly increased in 'VBN (Gg)2' by 1.43-fold and by 1.81-fold in 'VBN (Gg)3' as compared to 'PKU-AKM 12-28' (0.7-fold) and 'PKU-AKM 8802' (1.1-fold) at 75 mM NaCl. Further, it increased in 'VBN (Gg)3' by 2.53-fold than in 'PKU-AKM 12-28' at 125 mM NaCl (Figure 5A). In the root, the highest MDA content was found in 'VBN (Gg)3', which was increased by 5.14-fold compared to other varieties, which showed a nearly 1.2-1.3-fold increase in MDA at 125 mM NaCl (Figure 5B). Such an increase in MDA content with increasing salt concentrations was also observed more in the sensitive wheat genotype than the tolerant ones (Datir *et al.*, 2020).



(A)



(B)

Figure 5. Effect of salt stress on lipid peroxidation (in terms of MDA content) in 10-day old seedlings of *Vigna radiata* varieties

A) Shoot MDA content and B) Root MDA content. Each value represents the mean of three replicates, and the vertical bar indicates the standard deviation. Small letters denote significant difference between treatments, and capital letters denote significant difference between varieties at a 0.05% significance level according to Duncan's Multiple Range Test (DMRT)

Effect of salt stress on phenolics, flavonoids, and antioxidants

Natural antioxidants such as phenolic and flavonoid compounds play an essential role under stress. These metabolites have various biological functions in plants, the significant being protection from ROS generated under various environmental stresses such as salt stress (Khare *et al.*, 2020). The elevated levels of phenolics and flavonoids observed in the present investigation support the observations by (Bistgani *et al.*, 2019). These secondary metabolites act as antioxidants, mitigate oxidative stress, and scavenge the reactive oxygen species (ROS)(Selmar and Kleinwächter, 2013). Phenolic content in shoot and root was increased in 'PKU AKM 12-28' and 'PKU-AKM 8802' compared to 'VBN (Gg)2' and 'VBN (Gg)3' at 125 mM NaCl (Figure 6A). It was increased in the root of 'PKU AKM 12-28' and 'PKU-AKM 8802' by 24% and decreased in 'VBN (Gg)2' and 'VBN (Gg)3' by 38% and 63%, respectively, at 125 mM NaCl. However, 'VBN (Gg)3'

showed increased phenolic content at 100 mM NaCl in the root (Figure 6B). In the shoot, flavonoid content increased in 'PKU AKM 12-28' by 14% and 'PKU-AKM 8802' by 33% but decreased in 'VBN (Gg)2' by 53% and 'VBN (Gg)3' by 35% at 75 mM NaCl. However, it was decreased in all varieties at 125 mM NaCl by 41-52% (Figure 6C). In the root, flavonoid content increased in 'PKU-AKM 12-28' by 96% and decreased in 'VBN (Gg)2' by 57% at 100 mM NaCl. At 125 mM NaCl, it was significantly reduced in 'VBN (Gg)2' and 'VBN (Gg)3' compared to 'PKU-AKM 12-28' and 'PKU-AKM 8802' (Figure 6D). Such an increase in these secondary metabolites in salt-tolerant varieties was also reported by Chutipaijit *et al.* (2009) in salt-tolerant rice varieties than salt-sensitive ones.

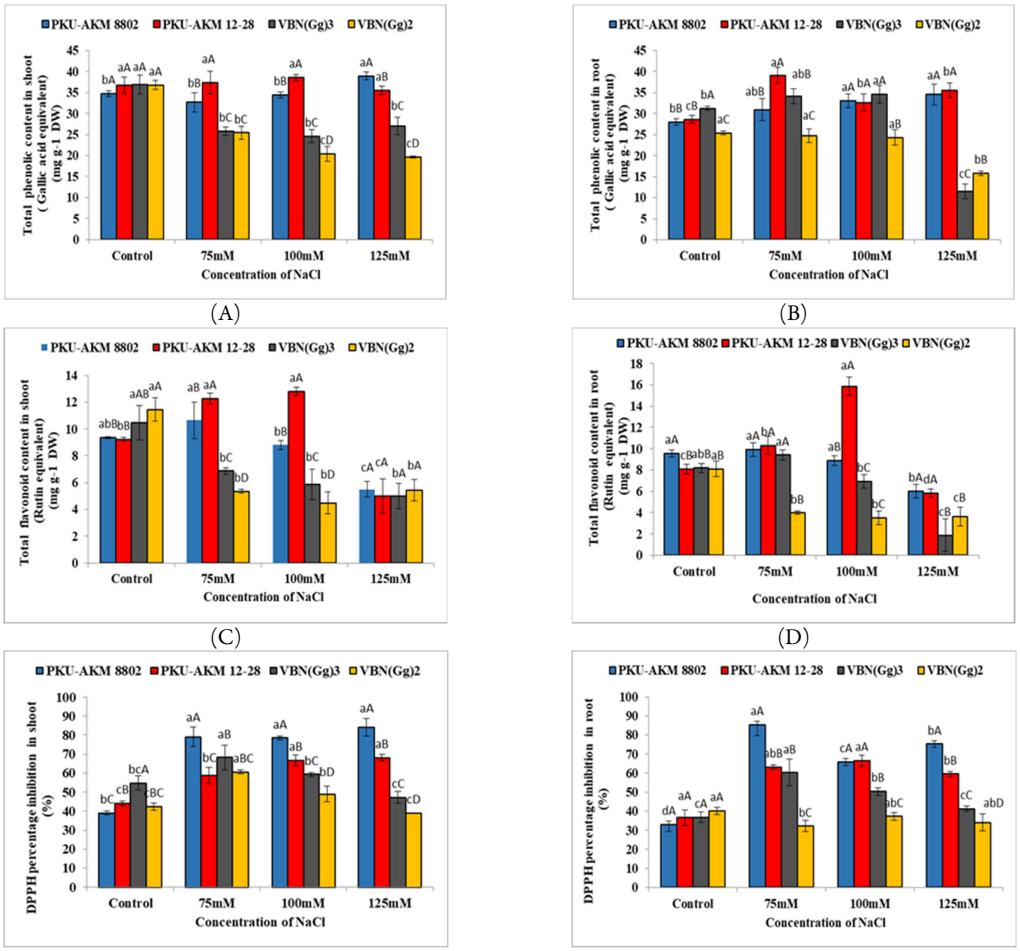


Figure 6. Effect of salt stress on phenol and flavonoid content and antioxidants of 10-day old seedlings of *Vigna radiata* varieties

A) Shoot phenolic content; B) Root phenolic content; C) Shoot flavonoids content; D) Root flavonoids content; E) Shoot DPPH radical scavenging activity and F) Root DPPH radical scavenging activity. Each value represents the mean of three replicates, and the vertical bar indicates the standard deviation. Small letters denote significant difference between treatments, and capital letters denote significant difference between varieties at a 0.05% significance level according to Duncan's Multiple Range Test (DMRT)

Where phenolic and flavonoid content correlates with antioxidant nature, DPPH assay is a nonenzymatic antioxidant activity which is a direct and sensitive method to investigate savaging of ROS by antioxidants (Golkar *et al.*, 2020). In the present study, DPPH radical scavenging activity significantly increased in both shoot and root as the salt concentration increased from 75-125 mM NaCl in 'PKU-AKM

12-28' and 'PKU-AKM 8802' (Figure 6E-6F). However, it was initially increased at 75 and 100 mM NaCl and later decreased at 125 mM NaCl in 'VBN (Gg)2' and 'VBN (Gg)3'. However, DPPH radical scavenging was higher in 'PKU-AKM 8802' than 'VBN (Gg)2' at all salt concentrations. In this regard, the increase in DPPH activity under salt stress corroborates with studies in chickpea (Kaur *et al.*, 2014).

Effect of salt stress on osmolyte accumulation

High accumulation of different osmolytes like proline, amino acids, and total sugars was observed in the mungbean varieties exposed to NaCl stress (Figure 8). In the shoot, proline content was significantly higher in 'PKU-AKM 12-28' and 'PKU-AKM 8802' than 'VBN (Gg)2' and 'VBN (Gg)3'. At 125 mM NaCl, the highest increase in proline content in the shoot was found in 'PKU-AKM 12-28' (by 5.48-fold) and in 'VBN (Gg)3' (by 1.25-fold) (Figure 7A). At 125 mM NaCl, proline content was significantly increased by 3.26-fold in the roots of 'PKU-AKM 12-28', whereas in the roots of 'VBN (Gg)2' and 'VBN (Gg)3', it increased by 0.4-fold and 0.27-fold respectively (Figure 7B). Proline content was significantly increased in root than shoot. Proline also acts as an antioxidant by stabilizing the membranes, scavenging free radicals, stabilizing proteins and protein complexes, and maintaining the osmotic balance (Muchate *et al.*, 2016).

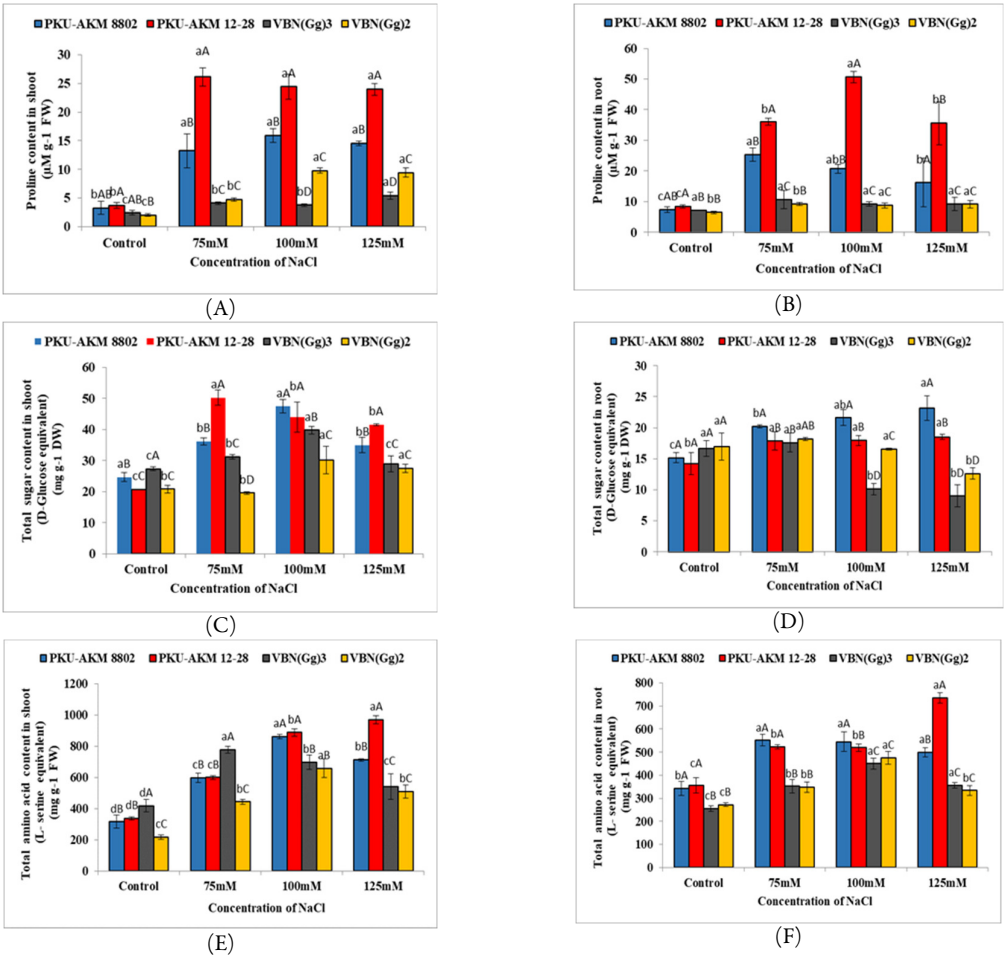


Figure 7. Effect of salt stress on osmolyte accumulation of 10-day old seedlings of *Vigna radiata* varieties A) Shoot proline content; B) Root proline content; C) Shoot total sugar content; D) Root total sugar content; E) Shoot amino acid content and F) Root amino acid content. Each value represents the mean of three replicates, and the vertical bar indicates the standard deviation. Small letters denote significant difference between treatments, and capital letters denote significant difference between varieties at a 0.05% significance level according to Duncan's Multiple Range Test (DMRT)

At 100 and 125 mM NaCl, 'VBN (Gg)2' and 'VBN (Gg)3' showed a remarkable decrease in sugar content than 'PKU-AKM 12-28' and 'PKU-AKM 8802' (Figure 7C-7D). The levels of sugars increase under salinity, and their role in osmotic adjustment has been validated (Marzec *et al.*, 2013; Ali *et al.*, 2018). At 75, 100, and 125 mM NaCl, amino acid content was significantly increased in the shoot and root of 'PKU-AKM 12-28' and 'PKU-AKM 8802' than 'VBN (Gg)2' and 'VBN (Gg)3' (Figure 7E-7F). Shahid *et al.* (2013) and Verma *et al.* (2018) have also reported increased amino acid content under salinity.

Conclusions

The present study concludes that the NaCl-induced salinity stress significantly affects the germination of seeds and early seedling growth in mungbean. Among the seventeen mungbean varieties, 'PKU-AKM 12-28' and 'PKU-AKM 8802' were the least affected by NaCl stress, whereas 'VBN(Gg)2' and 'VBN(Gg)3' were the most affected. These findings suggest that seed germination and growth attributes of mungbean seedlings can be used as traits for a rapid assortment of salt stress-tolerant varieties. Comparatively higher contents of photosynthetic pigments, proteins, secondary metabolite, osmolytes, antioxidants, and lower MDA content in the seedlings of 'PKU-AKM 12-28' and 'PKU-AKM 8802' suggest the salt-tolerant nature of these varieties. However, since this assessment of salt stress tolerance in the seventeen mungbean varieties is based on seeds germinated in Petri plates, field-based experiments are needed for validating the results. Further, studies on proteomics and genomics in these mungbean varieties would also be appropriate to know and validate genes and proteins conferring salt tolerance.

Authors' Contributions

GDM: Performed the experiments, collected and analysed the data, URW: Analysed data and prepared draft manuscript, DBS: Analysed data and prepared draft manuscript, TDN: Designed the experiments, and RBB: Designed the experiments, analysed the data, and finalized the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Dr. Neeta M. Patil, Head, Department of Botany, and Principal Dr. R. S. Zunjarrao, for providing the necessary infrastructure required to conduct the research. The authors gratefully acknowledge all institutes and companies for providing seeds of mungbean varieties. This work was supported by the Council of Scientific and Industrial Research (CSIR), New Delhi, MS, India, through the CSIR-JRF fellowship to Mr. Ganesh Mankar (grant number- 08/658(0001)/2017-EMR-I).

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abdul-Baki AA, Anderson JD (1973). Vigor determination in soybean seed by multiple criteria 1. Crop Science 13(6):630-633. *https://doi.org/10.2135/cropsci1973.0011183X001300060013x*

Alharby HF, Al-Zahrani HS, Hakeem KR, Iqbal M (2019). Identification of physiological and biochemical markers for salt (NaCl) stress in the seedlings of mungbean [*Vigna radiata* (L.) Wilczek] genotypes. Saudi Journal of Biological Sciences 26(5):1053-1060. *https://doi.org/10.1016/j.sjbs.2018.08.006*

Ali S, Khan N, Nouroz F, Erum S, Nasim W, Shahid MA (2018). *In vitro* effects of GA3 on morphogenesis of CIP potato explants and acclimatization of plantlets in field. In Vitro Cellular and Developmental Biology - Plant 54(1):104-111. *https://doi.org/10.1007/s11627-017-9874-x*

Arif Y, Singh P, Siddiqui H, Bajguz A, Hayat S (2020). Salinity induced physiological and biochemical changes in plants: An omic approach towards salt stress tolerance. Plant Physiology and Biochemistry 156(7):64-77. *https://doi.org/10.1016/j.plaphy.2020.08.042*

Arnon DI (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiology 24(1):1-15. *https://doi.org/10.1104/pp.24.1.1*

Bagwasi G, Agenbag GA, Swanepoel PA (2020). Effect of salinity on the germination of wheat and barley in South Africa. Crop, Forage & Turfgrass Management 6(1):e20069. *https://doi.org/10.1002/cft2.20069.*

Balbaa SI, Zaki AY, El Shamy AM (1974). Total flavonoid and rutin content of the different organs of *Sophora japonica* L. Journal of AOAC International 57(3):752-755. *https://doi.org/10.1093/jaoac/57.3.752*

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39(1):205-207. *https://doi.org/10.1007/BF00018060*

Bistgani ZE, Hashemi M, DaCosta M, Craker L, Maggi F, Morshedloo MR (2019). Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. Industrial Crops and Products 135:311-320. *https://doi.org/10.1016/j.indcrop.2019.04.055*

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. Nature 181:1199-1200. *https://doi.org/10.1038/1811199a0*

Campo S, Baldrich P, Messeguer J, Lalanne E, Coca M, Segundo BS (2014). Overexpression of a calcium-dependent protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiology 165(2):688-704. *https://doi.org/10.1104/pp.113.230268*

Chunthaburee S, Dongsansuk A, Sanitchon J, Pattanagul W, Theerakulpisut P (2015). Physiological and biochemical parameters for evaluation and clustering of rice cultivars differing in salt tolerance at seedling stage. Saudi Journal of Biological Sciences 23(4):467-477. *https://doi.org/10.1016/j.sjbs.2015.05.013*

Chutipaijit S, Cha-Um S, Sompornpailin K (2009). Differential accumulations of proline and flavonoids in indica rice varieties against salinity. Pakistan Journal of Botany 41(5):2497-2506. *http://www.pakbs.org/pjbot/PDFs/41(5)/PJB41(5)2497.pdf*

Datir S, Singh N, Joshi I (2020). Effect of NaCl-induced salinity stress on growth, osmolytes and enzyme activities in wheat genotypes. Bulletin of Environmental Contamination and Toxicology 104(3):351-357. *https://doi.org/10.1007/s00128-020-02795-z*

Eryılmaz F (2006). The relationships between salt stress and anthocyanin content in higher plants. Biotechnology & Biotechnological Equipment 20(1): 47-52. *https://doi.org/10.1080/13102818.2006.10817303*

Golkar P, Bakhshi G, Vahabi MR (2020). Phytochemical, biochemical, and growth changes in response to salinity in callus cultures of *Nigella sativa* L. In Vitro Cellular and Developmental Biology - Plant 56(2):247-258. *https://doi.org/10.1007/s11627-020-10058-z*

Hammer Ø, Harper DA, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1):9.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. Archives of Biochemistry and Biophysics 125(1):189-198. *https://doi.org/10.1016/0003-9861(68)90654-1*

Jeon D, Lee S, Choi S, Seo S, Kim C (2020). Effect of salt stress on the anthocyanin content and associated genes in *sorghum bicolor* L. Korean Journal of Agricultural Science 47(1):105-117. *https://doi.org/10.7744/kjoas.20200003*

Kader MA (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales 138:65-75. *https://royalsoc.org.au/images/pdf/journal/138_Kader.pdf*

Kaur N, Kumar A, Kaur K, Gupta AK, Singh I (2014). DPPH radical scavenging activity and contents of H_2O_2, malondialdehyde and proline in determining salinity tolerance in chickpea seedlings. Indian Journal of Geo-Marine Sciences 51(5):407-415. *http://hdl.handle.net/123456789/29890*

Khare S, Singh NB, Singh A, Hussain I, Niharika K, Yadav V, ... Amist N (2020). Plant secondary metabolites synthesis and their regulations under biotic and abiotic constraints. Journal of Plant Biology 63(3):203-216. *https://doi.org/10.1007/s12374-020-09245-7*

Lee YP, Takahashi T (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. Analytical Biochemistry 14(1):71-77. *https://doi.org/10.1016/0003-2697(66)90057-1*

Liu C, Zhao X, Yan J, Yuan Z, & Gu M (2020). Effects of Salt stress on growth, photosynthesis, and mineral nutrients of 18 pomegranate (*Punica granatum*) cultivars. Agronomy 10(1):1-17. *https://doi.org/10.3390/agronomy10010027*

Lowry OH, Rosebrough NJ, Farr AL, Randall R J (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193:265-275.

Maclachlan S, Zalik S (1963). Plastid structure, chlorophyll concentration, and free amino acid composition of a chlorophyll mutant of barley. Canadian Journal of Botany 41(7):1053-1062. *https://doi.org/10.1139/b63-088*

Mancinelli AL (1984). Photoregulation of anthocyanin synthesis. Plant Physiology 75(2):447-453. *https://doi.org/10.1104/pp.75.2.447*

Marzec M, Muszynska A, Gruszka D (2013). The role of strigolactones in nutrient-stress responses in plants. International Journal of Molecular Sciences 14(5):9286-9304. *https://doi.org/10.3390/ijms14059286*

Muchate NS, Nikalje GC, Rajurkar NS, Suprasanna P, Nikam TD (2016). Physiological responses of the halophyte *Sesuvium portulacastrum* to salt stress and their relevance for saline soil bio-reclamation. Flora: Morphology, Distribution, Functional Ecology of Plants 224(7):96-105. *https://doi.org/10.1016/j.flora.2016.07.009*

Nair RM, Pandey AK, War AR, Hanumantharao B, Shwe T, Alam AKMM, ... Schafleitner R (2019). Biotic and abiotic constraints in mungbean production-progress in genetic improvement. Frontiers in Plant Science 10:1340. *https://doi.org/10.3389/fpls.2019.01340*

Nawaz M, Khan A, Ashraf MY (2020). Screening mungbean germplasm for salt tolerance using growth indices and physiological parameters 22(3):401-406. *https://doi.org/10.17957/IJAB/15.1078*

Öner F, Soysal AÖS (2020). Determination of germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties under stress conditions. Notulae Scientia Biologicae 12(3): 693–701. *https://doi.org/10.15835/nsb12310766*

Pandey M, Penna S (2017). Time course of physiological, biochemical, and gene expression changes under short-term salt stress in *Brassica juncea* L. Crop Journal 5(3):219-230. *https://doi.org/10.1016/j.cj.2016.08.002*

Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: A review. Ecotoxicology and Environmental Safety 60:324-349. *https://doi.org/10.1016/j.ecoenv.2004.06.010*

Podder S, Ray J, Das D, Sarker BC (2020). Effect of salinity (NaCl) on germination and seedling growth of mungbean (*Vigna radiata* L.). Journal of Bioscience and Agriculture Research 24(2):2012-2019. *https://doi.org/10.18801/jbar.240220.246*

Rahman MM, Habib MA, Sikdar MSI, Md S, Islam MS (2016). Evaluation of mungbean genotypes for salt tolerance at seedling stage and alleviation of saline stress by gypsum. Fundamental and Applied Agriculture 1(1):39-43. *http://www.f2ffoundation.org/faa/wp-content/uploads/sites/2/2016/05/FAA-2016-008_Tech-Editor.pdf*

Rahneshan Z, Nasibi F, Moghadam AA (2018). Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two *pistachio* rootstocks. Journal of Plant Interactions 13(1):73-82. *https://doi.org/10.1080/17429145.2018.1424355*

Regni L, Del Pino AM, Mousavi S, Palmerini CA, Baldoni L, Mariotti R, ... Proietti P (2019). Behavior of four olive cultivars during salt stress. Frontiers in Plant Science 10:867. *https://doi.org/10.3389/fpls.2019.00867*

Romanenko EA, Romanenko PA, Babenko LM, Kosakovskaya IV (2017). Salt stress effects on growth and photosynthetic pigments' content in algoculture of *Acutodesmus dimorphus* (Chlorophyta). International Journal on Algae 19(3):271-282. *https://doi.org/10.1615/InterJAlgae.v19.i3.70*

Scott TA, Melvin EH (1953). Determination of dextran with anthrone. Analytical Chemistry 25(11):1656-1661. *https://doi.org/10.1021/ac60083a023*

Sehrawat N, Yadav M, Sharma AK, Kumar V, Bhat KV (2019). Salt stress and mungbean [*Vigna radiata* (L.) Wilczek]: effects, physiological perspective and management practices for alleviating salinity. Archives of Agronomy and Soil Science 65(9):1287-1301. *https://doi.org/10.1080/03650340.2018.1562548*

Selmar D, Kleinwächter M (2013). Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. Industrial Crops and Products 42(1):558-566. *https://doi.org/10.1016/j.indcrop.2012.06.020*

Shahid MA, Ashraf MY, Pervez MA, Ahmad R, Balal RM, Garcia-Sanchez F (2013). Impact of salt stress on concentrations of Na+, Cl-and organic solutes concentration in pea cultivars. Pakistan Journal of Botany 45(3):755-761. *https://www.pakbs.org/pjbot/PDFs/45(3)/06.pdf*

Shahid MA, Sarkhosh A, Khan N, Balal RM, Ali S, Rossi L, ... Garcia-Sanchez F (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. Agronomy 10(7):938. *https://doi.org/10.3390/agronomy10070938*

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 17037. *https://doi.org/10.1155/2012/217037*

Shelke DB, Pandey M, Nikalje GC, Zaware BN, Suprasanna P, Nikam TD (2017). Salt responsive physiological, photosynthetic and biochemical attributes at early seedling stage for screening soybean genotypes. Plant Physiology and Biochemistry 118:519-528. *https://doi.org/10.1016/j.plaphy.2017.07.013*

Shin YK, Bhandari SR, Jo JS, Song JW, Cho MC, Yang EY, Lee JG (2020). Response to salt stress in lettuce: Changes in chlorophyll fluorescence parameters, phytochemical contents, and antioxidant activities. Agronomy 10(11):1627. *https://doi.org/10.3390/agronomy10111627*

Swain T, Hillis WE (1959). The phenolic constituents of Prunus domestica. I.-The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture 10(1):63-68. *https://doi.org/10.1002/jsfa.2740100110*

Timson J (1965). New method of recording germination data. Nature 207(4993):216-217. *https://doi.org/10.1038/207216a0*

Verma SS, Verma RS, Verma SK, Yadav AL, Verma AK (2018). Impact of salt stress on plant establishment, chlorophyll and total free amino acid content of ber (*Zizyphus mauritiana* Lamk.) cultivars. Journal of Pharmacognosy and Phytochemistry 7(2):556-559. *https://www.phytojournal.com/archives/2018/vol7issue2/PartH/7-1-362-203.pdf*

Verma S, Mishra SN (2005). Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. Journal of Plant Physiology 162(6):669-677. *https://doi.org/10.1016/j.jplph.2004.08.008*

Vibhuti SC, Bargali K, Bargali SS (2015). Seed germination and seedling growth parameters of rice (*Oryza sativa*) varieties as affected by salt and water stress. Indian Journal of Agricultural Sciences 85(1):102-108.

Yadav S, Irfan M, Ahmad A, Hayat S (2011). Causes of salinity and plant manifestations to salt stress: a review. Journal of Environmental Biology 32(5):667.