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TIME-DEPENDENT DETERMINATIVE BIOCHEMICAL TRAITS FOR SALT TOLERANCE MECHANISM IN MUNGBEAN (*Vigna radiata* (L.) R. WILCZEK)

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ABSTRACT

Mungbean is one of the commercially valuable pulse crops. Time-dependent biochemical modulations in the mungbean varieties PKV AKM 12-28 and VBN (Gg)3 exposed to 75, 100, and 125 mM NaCl were estimated, and the results were concluded through multivariate modeling. The cluster analysis gave two fairly distinct clusters that had similar biochemical responses. Results on the principal component analysis suggested that protein content (PC), total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, ABTS radical scavenging activity, proline content (PRC), total free amino acid (TFAA) content, and malondialdehyde (MDA) contents were dominant traits in the shoot as compared to the root. These can be taken as the primary indicators to assess the effect of salt stress on mungbean varieties. The discriminant analysis had identified TFC, MDA, and total sugar content (TSC) as discriminating variables between the roots and shoots. Further, MDA and TFC were identified as discriminating variables under different salt concentrations, and TSC was identified as a discriminating variable at different exposure durations. Discriminant partial least squares analysis further identified optimum biochemical modulations in the shoots of PKV AKM 12-28 and 75 mM NaCl. The salt treatment produced a strong biochemical modulation after 30 and 45 days, which helped plants survive under salt stress. The multivariate approaches efficiently interpreted time-dependent biochemical modulations in shoots and roots of mungbean varieties under salt stress.

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1 Introduction

Soil salinity as abiotic stress has affected over one billion hectares of the global arable land. Such landmasses are increasing annually by ~10% due to natural causes and human-made causes such as substandard agricultural practices like improper irrigation and the extensive use of chemicals (Shrivastava & Kumar 2015; Soltabayeva et al., 2021). According to one estimate, salinity-affected soil is equivalent to more than 6% of the global landmass (Ding et al., 2018). The soil salinity is usually caused by excess sodium and chloride ions (Fall et al., 2018). Salinity reduces the growth and development of crops due to ionic, osmotic, and oxidative stresses (Arzani & Ashraf 2016; Abid et al., 2020). Salinity is known to affect many essential cellular and metabolic processes adversely.

Mungbean, *Vigna radiata* (L.) R. Wilczek (Fabaceae) is an important dietary pulse crop. It is commonly cultivated worldwide and in several Indian states like Maharashtra, Rajasthan, Andhra Pradesh, Madhya Pradesh, and Orissa (Ram & Singh, 1993). In India, 3.45 million hectares were under mungbean cultivation during the twelfth plan (2012-2017), which gave 1.59 million tons of produce (Kumar & Pandey, 2018). It is a significant source of proteins, vitamins, antioxidants, and minerals (Nair et al., 2019). It is also used as green manure, fodder, and in pharmaceuticals and cosmetics industries (Tang et al., 2014). However, soil salinity affects its physiology and biochemistry culminating in retarding its growth, development, and production (Saha et al., 2010; Ghosh et al., 2011; Ghosh et al., 2015; Sehrawat et al., 2019).

The effects of NaCl stress on plant metabolism are generally studied by monitoring the variations or changes in the plant's biochemical responses (Ghosh et al., 2015; Sehrawat et al., 2015; Muchate et al., 2016; Kalaria, 2017; Shelke et al., 2017; Rahneshan et al., 2018). However, it becomes challenging to interpret and to draw conclusions from the complicated nature of biochemical responses and their interrelationships through the conventional approach. Moreover, the conventional approach can only provide quantitative data characteristics. However, it cannot determine conceptual descriptions and underlying reasons for dependencies among data attributes (Michalski & Kaufman, 1998). Cluster analysis (CA), principal component analysis (PCA), discriminant analysis (DA), discriminant partial least squares (DPLS), and Pearson's multiple correlation analysis (MCA) are statistical tools that are used to analyze and interpret complex datasets accurately (Simeonov et al., 2003; Singh et al., 2004; Sinha et al., 2009a; Sinha et al., 2009b; Shelke et al., 2017; Mundada et al., 2020). These methods can adequately analyze, interpret, and draw conclusions from complex interrelationships among attributes used in different environmental, biological, chemical, and ecotoxicological studies (Mujunen et al., 1998; Singh et al., 2004; Sinha et al., 2009a; Sinha et al., 2009b; Chunthaburee et al., 2015; Sarabi et al., 2016; Shelke et al., 2017; Mundada et al., 2020). In the present study, the effects of NaCl stress on the biochemical responses in mungbean varieties were investigated through multivariate techniques (CA, DA, PCA, DPLS, and MCA) to interpret the results and the complex relationships among many such attributes.

2 Materials and methods

2.1 Plant materials, growth, and salt treatment

Certified and healthy seeds of mungbean varieties PKU-AKM 12-28 and VBN (Gg)3 were procured from Pulses Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, and National Pulses Research Centre, Tamil Nadu Agricultural University, Tamil Nadu, respectively. Plants were grown in the Botanical garden of the Modern College of Arts, Science and Commerce, Shivajinagar, Pune- 5. The potting mixture was prepared from the sandy clay loam soil collected from the village Charholi in the district Pune (MS). Plants were grown in non-perforated plastic pots of 35 cm × 20 cm size. Each pot contained a 15 kg mixture of soil and farmyard manure in a 3:1 ratio.

Fifteen seeds of each variety were sown per pot. Thinning of plants was done after fifteen days of sowing to maintain five plants per pot. The salinity stress was given to these 15 day-old seedlings through Hoagland nutrient medium Hoagland & Arnon (1950) containing 0, 75, 100, and 125 mM NaCl (equivalent to 0.3, 7, 8, and 9 dsm^{-2} EC, respectively). Three hundred ml respective solution was added to each pot on every alternate day to maintain the potting mixture's desired EC until the experiments were concluded. Each treatment was replicated in three pots. Data was collected, /analyses were performed on two plants per pot after 15, 30, and 45 days of salt stress treatments.

2.2 Biochemical analysis

All the biochemical parameters were estimated spectrophotometrically on a microprocessor-based UV–Vis spectrophotometer (Bioera, India).

2.2.1 Protein content (PC)

Lowery et al.'s (1951) method with the Bovine serum albumin (BSA) as a standard protein was used to estimate proteins. The suspension of one gram tissue homogenized in 3 ml of 100 mM potassium phosphate buffer was centrifuged at 15000 rpm for 20 min at four ºC. The supernatant was used for protein estimation, and the intensity of blue color developed was measured at 550 nm.

2.2.2 Malondialdehyde (MDA) content

MDA content was estimated by Heath & Packer's (1968) method. One gram plant material was homogenized and mixed with 2 ml of

20 % trichloroacetic acid containing 0.5 % thiobarbituric acid. This mixture in 3 ml of 0.1 % trichloroacetic acid was centrifuged at 5000 rpm. Two ml of the supernatant was incubated for 30 minutes at 95 °C, and the absorbance was read at 532 nm. The absorbance was also recorded at 600 nm to subtract nonspecific absorption.

MDA content (nmol/g dry weight)=

$$\frac{[(A_{532} - A_{600}) \times \text{total volume(ml)} \times 1000]}{[\text{Extinction coefficient} \times \text{sample volume(ml)}] \times \text{Weight of plant tissue (g)}}$$

Where, extinction coefficient = 155

2.2.3 Extraction of total phenolics, flavonoids, and antioxidants

The extract was prepared by refluxing 40 mg of dried plant material in 5 ml of 80 % methanol for one hr. The extract was filtered through Whatman no. 1 filter paper fitted on the Buchner funnel, and the filtrate was evaporated to dryness. The residue was dissolved in 10 ml 80 % methanol.

2.2.3.1 Estimation of total phenolics (TPC)

Total phenolics were estimated by Swain & Hillis' (1959) method. Half ml of the extract was evaporated to dryness, and the residue was dissolved in 0.5 ml distilled water, to which 0.5 ml of Folin-Ciocalteu Phenol reagent was added. After 5 minutes, 1 ml of a saturated sodium carbonate solution was added to this mixture and incubated for one h at room temperature. The absorbance was read at 760 nm. Gallic acid was used as a standard phenol.

2.2.3.2 Estimation of total flavonoids (TFC)

Total flavonoids were estimated by the Balbaa et al. (1974) method. Half ml of extract was evaporated to dryness, and the residue was dissolved in 1 ml 0.1 M methanolic aluminium chloride. The yellow color developed was read at 420 nm. Rutin was used as a standard flavonoid.

2.2.3.3 DPPH radical scavenging assay

A method by Blois (1958) was used to quantify antioxidants. A mixture of 200 µl extract and 1 ml DPPH (0.1 mM) was incubated in the dark for 30 min, after which its absorbance was recorded at 517 nm. The following formula was used to calculate the percentage radical scavenging potential.

$$\text{RSA (\%)} = (\text{Abs control} - \text{Abs sample} / \text{Abs control}) \times 100$$

2.2.3.4 ABTS radical scavenging assay

The method of Roberta et al. (1999) was followed for this assay. Two hundred µl extract was added to 1 ml of ABTS reagent (a mixture of equal volumes of 7 mM ABTS and 2.45 mM potassium persulphate incubated in the dark for 16 h at room temperature). After 10 min of incubation, the reaction mixture's absorbance was read at 734 nm. The following formula was used to calculate the percentage of radical scavenging activity

$$\text{RSA (\%)} = (A_{control} - A_{sample} / A_{control}) \times 100$$

2.2.4 Total proline content (PRC)

Proline content was estimated by Bates et al.'s (1973) method. Fifty mg of dry tissue sample was homogenized in 4 ml of 3 % sulfosalicylic acid, and the mixture was centrifuged at 3,000 rpm for 20 min, and the supernatant was collected. A mixture of 1 ml each of supernatant, ninhydrin, and glacial acetic acid was incubated in a boiling water bath for one h. The reaction was terminated by placing the test tubes in an ice bath. Four ml of toluene was added to the above mixture. The intensity of red color was read at 520 nm against toluene blank.

2.2.5 Total free amino acid content (TFAA)

Total amino acid content was estimated by Lee & Takahashi's (1966) method. One hundred mg tissue was homogenized in 3 ml of 80 % methanol. The homogenate refluxed for two h in a water bath was centrifuged, and the supernatant was collected. The residue was once again extracted with 3 ml of 80% methanol and centrifuged. The supernatants were pooled for further use. The mixture was evaporated in a water bath, and the residue was dissolved in 3.0 ml of 10% isopropyl alcohol. Two hundred µl of this sample was added to 3.8 ml of ninhydrin-citrate-glycerol reagent (a mixture of 1 ml of 1% ninhydrin in 0.5 M citrate buffer (pH 5.5), 2.4 ml glycerol, and 0.4 ml 0.5 M citrate buffer) and boiled for 12 min. The absorbance of the mixture was read at 570 nm. Glycine was used as a standard amino acid.

2.2.6 Total sugars content (TSC)

Total sugar content was estimated by Scott & Melvin's (1953) method. Twenty mg dry material was added in 1.25 ml 2.5 N HCl and incubated in a hot water bath for one h. Pinches of Na_2CO_3 were added to neutralize the acid, and the volume was adjusted to 25 ml with distilled water. To 1 ml of this solution, 4 ml of anthrone reagent was added and incubated in a hot water bath for 8-10 min. After cooling the contents to room temperature, the intensity of the dark green color developed was measured at 630 nm. D-glucose was used as standard sugar.

2.3 Statistical analyses

All the experiments were performed in triplicates and with a completely randomized block design (CRD). The data were presented as mean±SD. The root and shoot biochemical datasets for chemometric modeling consisted of nine variables subjected to

multivariate modeling. Multivariate modeling of these variables was performed through principal component analysis (PCA) and hierarchical cluster analysis (HCA) by using the PAST statistical package. The discriminant analysis (DA) was performed on the dataset by Statistica V 10.0 software by using the standard, forward stepwise and, backward stepwise modes. The dissimilarity-based partial least square analysis (DPLS) was performed in XL-Stat statistical software (Wunderlin et al., 2001; Singh et al., 2004; Sinha et al., 2009a; Sinha et al., 2009b). The correlations between the NaCl stressed plants' biochemical parameters at different NaCl concentrations and exposure durations were determined through Pearson's correlation method in SPPS statistical software version 20 (Chunthaburee et al., 2015; Shelke et al., 2017).

3 Results

Biochemical changes induced by NaCl concentrations and the exposure durations in the roots and shoots of PKU-AKM 12-28 and VBN (Gg)3 are presented in Tables 1 and 2. Cluster analysis (CA) was used to detect changes in the biochemical responses induced by NaCl stress. CA produced a dendrogram (Figure 1), where all the twelve combinations (four levels of NaCl concentrations and three exposure durations) for root and shoot tissues of PKU-AKM 12-28 and VBN (Gg)3 were grouped into two statistically significant clusters (susceptible and resistant). These were further divided into two subgroups (shoot and root) since the samples within these groups had similar characteristics concerning biochemical and physiological responses. Thus, the differences in the responses in root and shoots tissues of PKU-AKM 12-28 and VBN (Gg)3 under NaCl stress were observed. It also distinguished varieties based on the tolerance level.

The normalized dataset (combined roots and shoots) was subjected to the PCA analysis to evaluate (i) interactions between plant and NaCl stress, (ii) differential responses in root and shoot tissues subjected to different levels of NaCl stress, (iii) relationships among variables, and d. factors affecting these variables. The first three significant principal components (PCs) of PCA indicated 92.37% of the total variance. Figure 2 illustrates the loadings and scores of the first two PCs (PC1 vs. PC2). The first Two PCs represent 84.56% of the total variance and reflected the main groupings in the data set. The PC1 is determined mainly by PC, TPC, TFC, DPPH, ABTS, PRC, and TFAA with strong positive loadings, whereas in PC2, MDA alone showed high positive loading.

Differences between the responses in root and shoot tissues are presented in the plot's scores and were grouped into two distinct clusters. It may be noted that the shoot tissues differentiated prominently in terms of PC, TPC, TFC, DPPH, ABTS, PRC, and TFAA and MDA. It shows variations and differences in responses of root and shoot tissues of mungbean varieties on the dominance of the biochemical and physiological variables at all NaCl concentrations and exposure durations. The root and shoot tissues of the same variety or the same tissues of different varieties showed different response patterns. The shoot and root tissues of PKU-AKM 12-28 variety had high scores with PC1 compared to VBN (Gg)3, which indicates its high tolerance level to salinity stress.

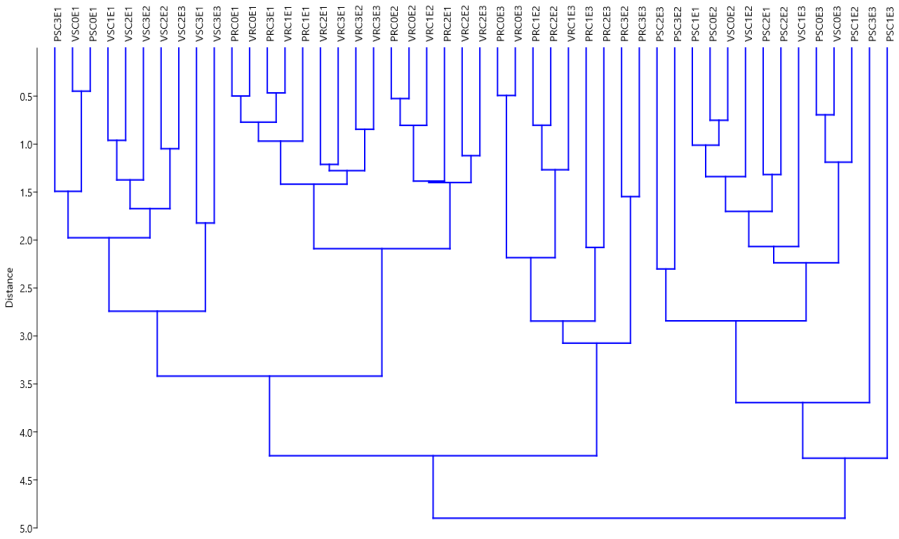

Figure 1 Dendrogram showing clustering of root and shoot tissue biochemical samples of the NaCl stressed plants of mungbean varieties

Table 1 Effects of NaCl stress on biochemical parameters in shoot tissue of *Vigna radiata* varieties PKU AKM 12-28 and VBN (Gg)3

variety	NaCl (mM)	Exposure duration (days)	Coding	Protein content (mg gm^{-1} FW)	MDA content (nmol gm^{-1} DW)	Total phenolic content (mg gm^{-1} DW)	Total flavonoid content (mggm^{-1} DW)	DPPH-RSA (Inhibition %)	ABTS-RSA (Inhibition %)	Proline content (µmol gm^{-1} DW)	Total sugar content (mg gm^{-1} DW)	Total free amino acid content (µmol gm^{-1} FW)
PKU AKM 12-28	0	15	PSC0E1	4.74 ± 0.40	170.32 ± 1.86	45.98 ± 1.55	5.72 ± 0.59	41.26 ± 2.13	42.44 ± 1.74	16.21 ± 1.08	91.63 ± 2.35	12.50 ± 0.73
		30	PSC0E2	5.59 ± 0.34	180.65 ± 4.59	51.03 ± 0.99	8.47 ± 0.64	58.54 ± 1.27	63.18 ± 1.21	24.20 ± 1.91	116.14 ± 2.65	13.80 ± 0.89
		45	PSC0E3	6.62 ± 0.36	189.76 ± 3.15	55.60 ± 0.96	10.14 ± 1.07	72.09 ± 1.16	79.65 ± 1.16	34.49 ± 2.21	157.21 ± 2.96	16.03 ± 0.07
	75	15	PSC1E1	5.57 ± 0.26	185.46 ± 5.57	59.84 ± 2.49	6.93 ± 0.43	5368 ± 2.02	57.56 ± 0.58	19.91 ± 1.41	117.49 ± 2.04	12.45 ± 0.89
		30	PSC1E2	6.08 ± 0.14	220.04 ± 2.93	66.67 ± 1.59	10.65 ± 0.33	64.85 ± 1.88	76.16 ± 1.74	29.00 ± 1.99	141.48 ± 2.91	14.99 ± 0.70
		45	PSC1E3	8.31 ± 0.90	250.32 ± 5.95	73.98 ± 1.56	13.40 ± 0.78	83.66 ± 0.78	90.31 ± 2.04	51.30 ± 1.28	202.95 ± 5.36	21.56 ± 1.60
	100	15	PSC2E1	6.98 ± 0.42	213.33 ± 6.87	51.25 ± 1.13	6.53 ± 0.31	46.40 ± 2.37	47.67 ± 2.10	22.06 ± 1.74	129.18 ± 2.95	15.26 ± 0.96
		30	PSC2E2	7.04 ± 0.13	244.82 ± 9.38	57.19 ± 1.19	8.88 ± 0.46	50.97 ± 0.92	55.04 ± 1.34	24.56 ± 1.44	164.62 ± 1.94	17.21 ± 0.49
		45	PSC2E3	7.53 ± 0.26	273.20 ± 7.75	65.27 ± 1.42	10.62 ± 1.03	61.53 ± 3.89	67.83 ± 1.46	21.17 ± 1.58	236.06 ± 2.61	18.54 ± 0.98
	125	15	PSC3E1	4.47 ± 0.23	236.22 ±12.17	38.82 ± 1.56	6.55 ± 0.40	38.07 ± 1.22	42.25 ± 1.68	24.14 ± 1.77	139.89 ± 1.37	10.42 ± 0.66
		30	PSC3E2	7.86 ± 0.28	259.44 ± 6.74	45.92 ± 1.45	8.02 ± 0.31	43.16 ± 2.49	49.61 ± 1.46	12.86 ± 1.35	201.84 ± 2.96	19.60 ± 0.60
		45	PSC3E3	5.51 ± 0.63	282.67 ± 9.92	37.27 ± 1.53	6.11 ± 0.43	31.88 ± 3.23	46.32 ± 2.62	9.16 ± 1.37	255.22 ± 3.68	12.48 ± 1.07
VBN (Gg)3	0	15	VSC0E1	4.54 ± 0.23	172.22 ± 9.12	44.17 ± 2.06	5.67 ± 0.60	38.96 ± 1.95	39.34 ± 2.93	19.83 ± 1.43	97.25 ± 2.78	11.30 ± 0.76
		30	VSC0E2	4.92 ± 0.25	172.56 ± 5.27	48.35 ± 1.04	7.77 ± 0.39	56.47 ± 1.70	53.49 ± 2.33	27.34 ± 0.95	123.03 ± 3.74	13.30 ± 0.85
		45	VSC0E3	5.88 ± 0.27	181.68 ± 7.74	58.19 ± 0.96	9.37 ± 1.01	75.04 ± 0.61	82.75 ± 3.20	30.83 ± 1.59	166.32 ± 3.06	14.45 ± 0.70
	75	15	VSC1E1	3.45 ± 0.31	215.91 ±14.98	44.08 ± 0.10	6.45 ± 0.59	38.15 ± 1.58	41.47 ± 2.62	3.41 ± 0.98	109.20 ± 2.50	8.31 ± 0.41
		30	VSC1E2	5.00 ± 0.13	246.02 ± 4.51	53.88 ± 1.50	9.75 ± 0.33	49.19 ± 0.92	64.73 ± 0.89	21.28 ± 1.45	130.63 ± 1.96	12.48 ± 0.77
		45	VSC1E3	5.65 ± 0.20	270.62 ± 6.05	49.29 ± 1.05	8.31 ± 0.44	57.61 ± 4.33	70.93 ± 2.66	9.56 ± 2.25	178.97 ± 3.41	14.34 ± 0.70
	100	15	VSC2E1	3.71 ± 0.34	246.19 ± 3.61	36.02 ± 1.60	5.05 ± 0.21	35.48 ± 2.26	37.79 ± 1.74	3.24 ± 1.00	87.74 ± 2.49	7.56 ± 1.41
		30	VSC2E2	4.56 ± 0.21	277.85 ± 6.45	45.97 ± 0.85	6.51 ± 0.31	42.60 ± 1.04	56.98 ± 1.16	4.23 ± 1.01	121.67 ± 1.76	11.22 ± 0.47
		45	VSC2E3	4.56 ± 0.19	300.39 ±10.73	42.32 ± 1.96	6.69 ± 1.06	36.69 ± 4.44	42.05 ± 3.20	5.02 ± 0.34	141.75 ± 2.61	11.10 ± 0.56
	125	15	VSC3E1	2.03 ± 0.17	262.54 ± 3.15	19.93 ± 1.39	3.63 ± 0.49	17.31 ± 2.43	27.71 ± 1.78	3.63 ± 0.22	62.65 ± 1.72	7.28 ± 0.46
		30	VSC3E2	3.53 ± 0.38	302.62 ± 6.37	29.31 ± 0.96	5.51 ± 0.42	36.41 ± 4.68	36.82 ± 4.12	1.46 ± 0.59	89.33 ± 2.07	10.55 ± 0.30
		45	VSC3E3	3.07 ± 0.28	336.00 ± 9.85	23.29 ± 1.24	4.51 ± 0.28	23.30 ± 0.97	27.33 ± 4.07	1.25 ± 0.14	126.20 ± 4.58	7.53 ± 0.58

Values represent mean±SD

Table 2 Effects of NaCl stress on physiological and biochemical parameters in root tissue of *Vigna radiata* varieties PKU AKM 12-28 and VBN (Gg)3

variety	NaCl (mM)	Exposure duration (days)	Coding	Protein content (mg gm^{-1} FW)	MDA content (nmol gm^{-1} DW)	Total phenolic content (mg gm^{-1} DW)	Total flavonoid content (mggm^{-1} DW)	DPPH-RSA (Inhibition %)	ABTS-RSA (Inhibition %)	Proline content (µmol gm^{-1} DW)	Total sugar content (mg gm^{-1} DW)	Total free amino acid content (µmol gm^{-1} FW)
PKU AKM 12-28	0	15	PRC0E1	1.95 ± 0.16	78.11 ± 5.20	17.92 ± 0.45	1.11 ± 0.128	16.63 ± 1.88	21.71 ± 2.62	4.62 ± 0.67	118.21 ± 2.06	4.25 ± 0.13
		30	PRC0E2	3.01 ± 0.13	80.86 ± 4.17	32.39 ± 2.05	1.21 ± 0.138	30.87 ± 2.25	43.80 ± 2.62	22.63 ± 1.75	135.49 ± 2.52	5.28 ± 0.09
		45	PRC0E3	3.84 ± 0.34	76.73 ± 7.30	46.01 ± 1.36	1.58 ± 0.060	76.70 ± 2.32	88.18 ± 1.46	35.49 ± 1.50	145.54 ± 1.39	5.75 ± 0.34
	75	15	PRC1E1	2.50 ± 0.27	77.76 ± 8.66	24.16 ± 2.07	1.49 ± 0.058	23.54 ± 1.16	29.84 ± 1.46	8.69 ± 0.98	131.58 ± 2.00	4.70 ± 0.14
		30	PRC1E2	3.29 ± 0.17	82.58 ± 1.79	54.02 ± 1.24	1.75 ± 0.078	56.39 ± 3.22	61.43 ± 3.20	25.07 ± 2.48	151.32 ± 3.04	5.39 ± 0.21
		45	PRC1E3	3.63 ± 0.17	86.37 ± 5.11	57.47 ± 2.03	2.08 ± 0.147	64.97 ± 1.62	72.87 ± 0.89	48.55 ± 2.11	213.01 ± 2.98	6.44 ± 0.21
	100	15	PRC2E1	3.01 ± 0.20	86.71 ± 2.73	32.65 ± 1.76	1.84 ± 0.080	32.04 ± 2.91	43.02 ± 1.74	4.48 ± 1.13	136.02 ± 2.62	5.31 ± 0.23
		30	PRC2E2	3.11 ± 0.10	98.75 ± 2.60	52.65 ± 1.10	1.84 ± 0.091	55.02 ± 1.15	57.17 ± 0.89	24.62 ± 1.03	184.58 ± 2.29	6.05 ± 0.18
		45	PRC2E3	2.17 ± 0.18	115.96 ± 2.19	46.91 ± 1.56	1.51 ± 0.012	52.39 ± 1.29	57.75 ± 1.46	66.59 ± 3.57	234.05 ± 3.26	4.49 ± 0.18
	125	15	PRC3E1	1.18 ± 0.11	98.41 ± 2.60	20.44 ± 1.41	0.95 ± 0.111	21.60 ± 2.03	24.03 ± 2.75	3.01 ± 0.48	132.15 ± 1.95	2.59 ± 0.22
		30	PRC3E2	2.77 ± 0.20	112.52 ± 5.75	36.88 ± 1.79	1.32 ± 0.102	41.59 ± 1.17	47.29 ± 3.78	34.72 ± 1.95	228.16 ± 3.62	4.92 ± 0.09
		45	PRC3E3	2.06 ± 0.13	132..82 ± 2.92	32.48 ± 1.54	1.13 ± 0.209	36.41 ± 4.79	40.70 ± 2.66	16.71 ± 0.90	254.23 ± 4.08	3.84 ± 0.16
VBN (Gg)3	0	15	VRC0E1	1.95 ± 0.21	76.04 ± 3.15	15.73 ± 1.52	1.00 ± 0.116	13.96 ± 4.31	28.88 ± 2.93	4.74 ± 1.49	108.29 ± 0.87	3.81 ± 0.50
		30	VRC0E2	2.55 ± 0.32	87.74 ± 6.77	35.46 ± 1.27	1.43 ± 0.079	33.17 ± 1.15	47.87 ± 1.46	25.61 ± 2.31	124.32 ± 3.24	5.17 ± 0.18
		45	VRC0E3	3.44 ± 0.31	81.20 ± 1.46	43.37 ± 1.32	1.74 ± 0.105	71.64 ± 1.94	84.30 ± 1.74	34.56 ± 0.88	153.90 ± 2.62	5.59 ± 0.26
	75	15	VRC1E1	1.79 ± 0.11	94.97 ± 1.03	19.72 ± 1.31	0.42 ± 0.030	20.63 ± 2.25	20.74 ± 2.93	2.40 ± 0.47	130.16 ± 2.22	3.81 ± 0.26
		30	VRC1E2	2.68 ± 0.21	105.29 ± 6.28	35.47 ± 1.03	0.82 ± 0.101	42.39 ± 1.58	45.54 ± 1.21	20.17 ± 1.42	120.42 ± 2.75	4.70 ± 0.26
		45	VRC1E3	2.97 ± 0.16	133.85 ± 4.38	38.71 ± 1.38	1.34 ± 0.071	51.78 ± 1.89	57.75 ± 4.36	24.20 ± 1.34	161.28 ± 1.70	4.93 ± 0.19
	100	15	VRC2E1	1.60 ± 0.16	102.19 ± 2.73	26.59 ± 0.92	0.77 ± 0.050	27.55 ± 4.13	32.36 ± 2.04	0.99 ± 0.10	92.87 ± 3.86	3.14 ± 0.20
		30	VRC2E2	2.02 ± 0.16	137.63 ± 3.63	28.68 ± 0.41	0.84 ± 0.025	32.89 ± 2.44	39.73 ± 0.34	17.48 ± 0.81	105.13 ± 3.63	4.14 ± 0.14
		45	VRC2E3	1.35 ± 0.12	154.84 ± 8.03	31.31 ± 1.27	0.86 ± 0.120	39.04 ± 1.29	44.19 ± 1.16	7.38 ± 0.67	134.33 ± 3.11	3.87 ± 0.12
	125	15	VRC3E1	0.90 ± 0.11	127.31 ± 4.65	16.61 ± 0.81	0.53 ± 0.056	19.90 ± 1.75	22.29 ± 2.62	0.56 ± 0.11	71.64 ± 2.62	1.78 ± 0.09
		30	VRC3E2	1.67 ± 0.09	152.09 ± 5.30	23.15 ± 1.03	0.81 ± 0.075	25.77 ± 1.52	31.59 ± 3.78	11.56 ± 1.23	97.47 ± 2.60	3.32 ± 0.22
		45	VRC3E3	1.08 ± 0.11	185.46 ± 1.46	19.29 ± 1.26	0.66 ± 0.053	21.84 ± 5.04	26.55 ± 2.42	7.29 ± 1.11	112.95 ± 2.66	2.26 ± 0.24

Values represent mean±SD

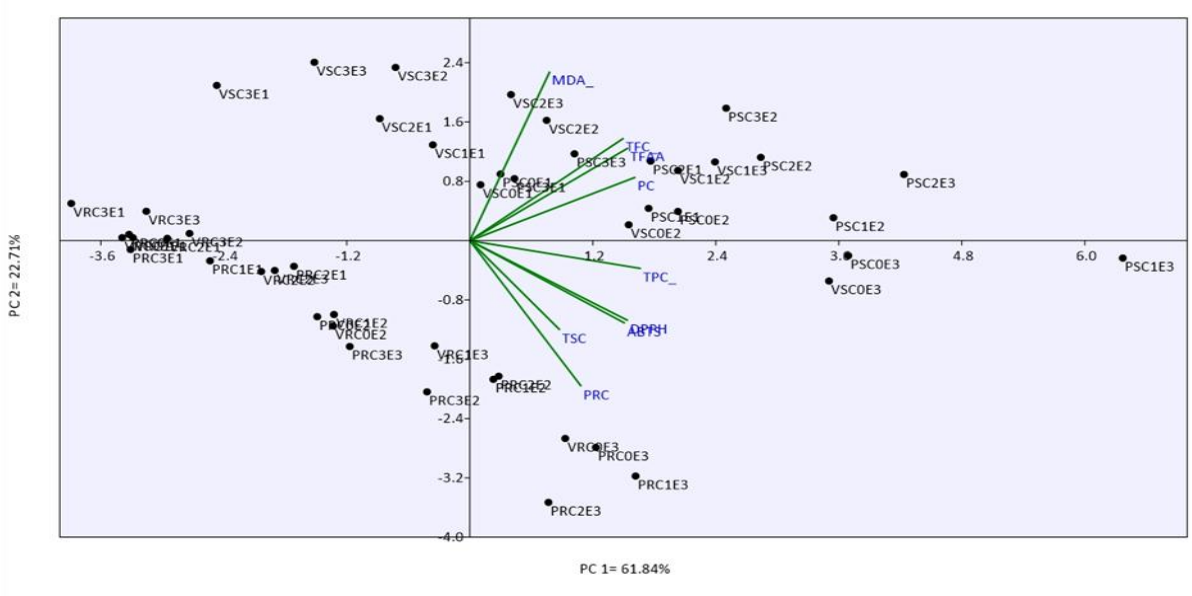

Figure 2 PCA scores and loadings of the first two PCs obtained from the shoot and root biochemical dataset of mungbean varieties

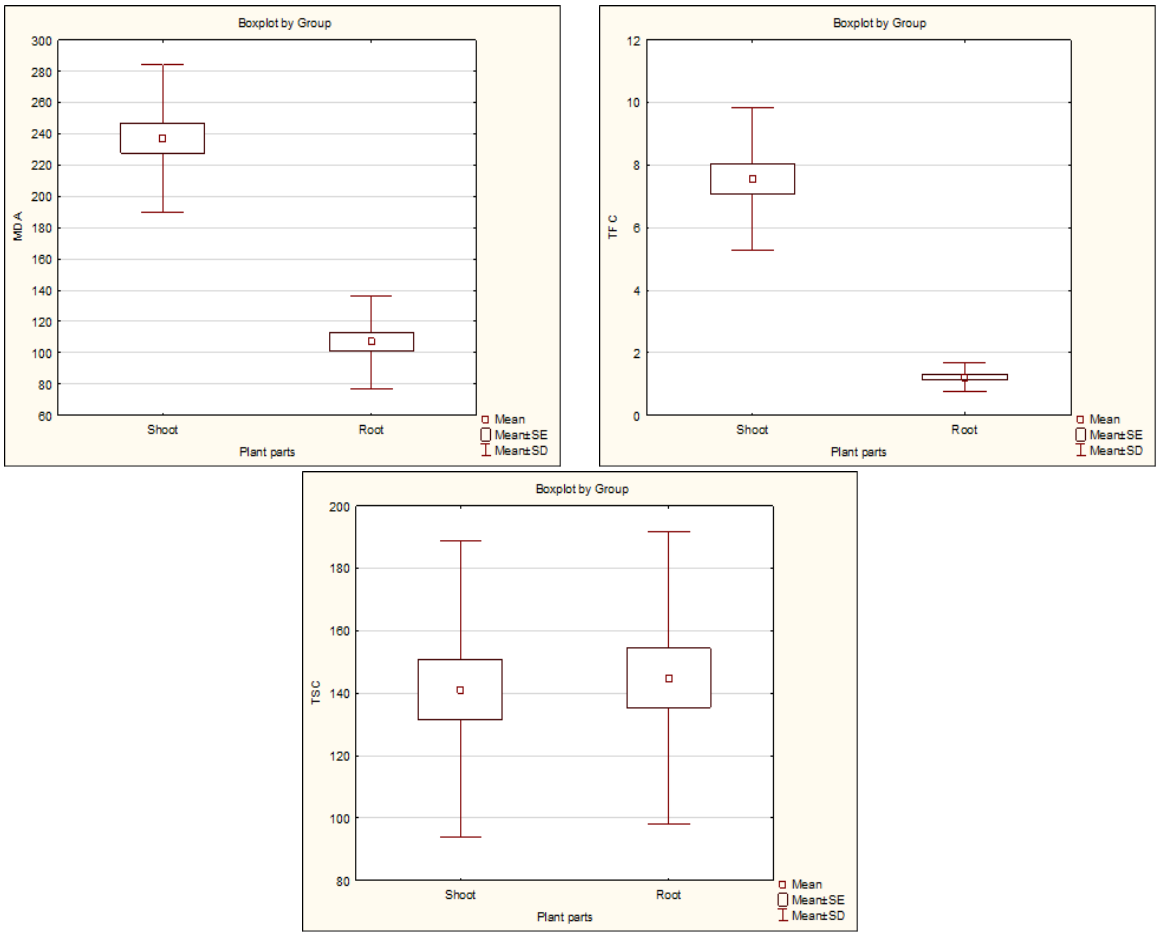

Figure 3 Box whisker plots-Variation in root and shoot biochemical samples a) MDA b) TFC c) TSC

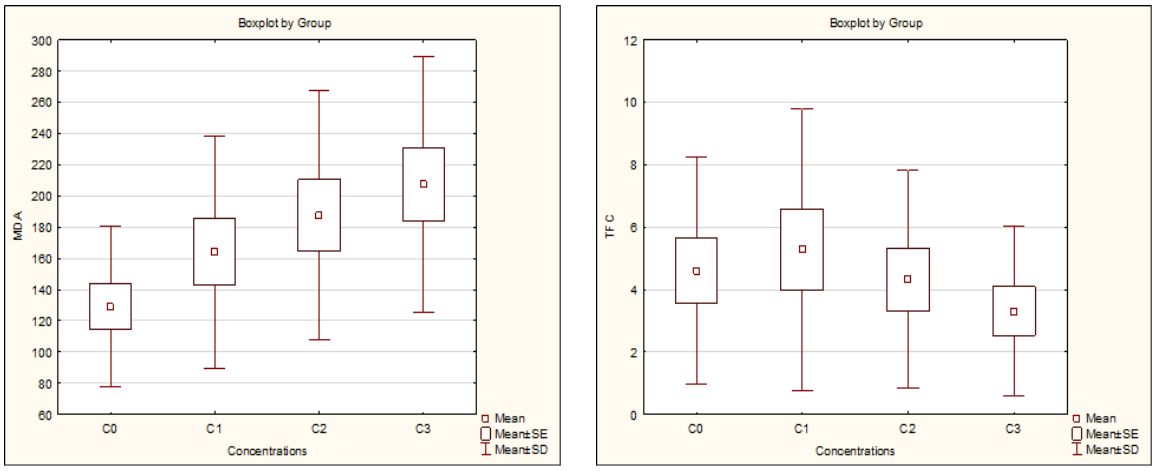

Figure 4 Box whisker plots-Variation induced by NaCl concentrations on biochemical samples a) MDA b) TFC

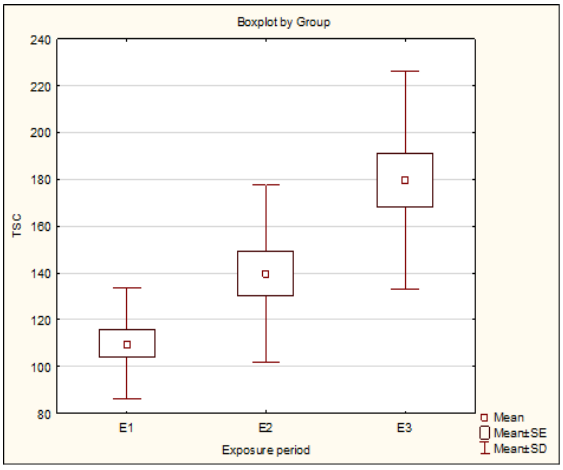

Figure 5 Box whisker plots-Variation induced by NaCl exposure duration in TSC

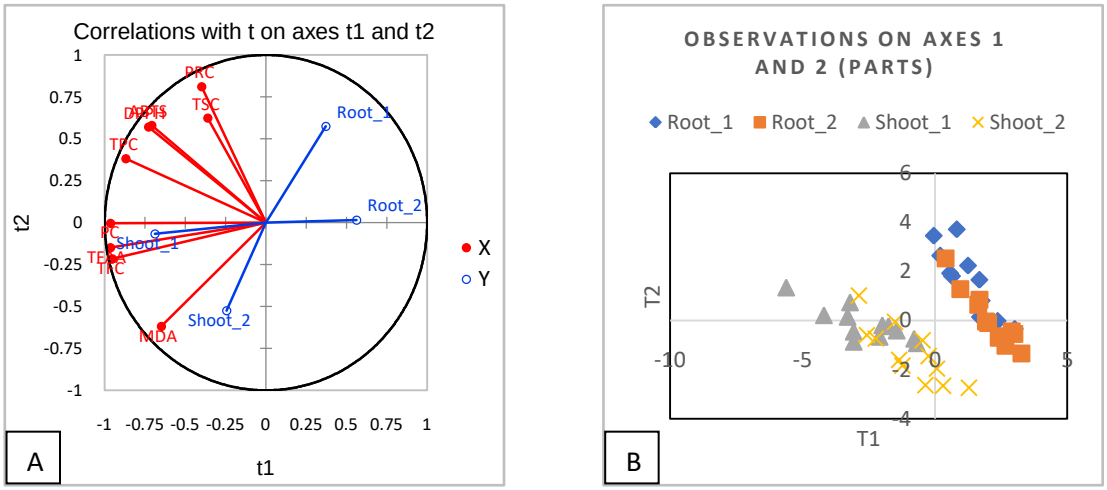

Figure 6 DPLS a) loadings and b) scores of the first two components obtained for the combined root and shoot data set (Where, Root_1: Root of PKU-AKM 12-28; Shoot_1: Shoot of PKU-AKM 12-28; Root_2: Root of VBN(Gg)3; Shoot_2: Shoot of VBN(Gg)3)

Table 3 a) Classification functions and b) Classification matrix for discriminant analysis of variation between the root–shoot biochemical samples of the mungbean

a)

	Linear Discriminant Functions for Groups Coefficients[a]	
	Shoot	Root
Standard DA mode		
PC	3.9285	0.7262
MDA	0.2779	0.1041
TPC	0.5569	0.4586
TFC	1.0462	-2.6927
DPPH	0.0017	-0.0002
ABTS	-0.1135	0.1292
PRC	-0.1019	-0.1783
TSC	-0.0931	0.0238
TFAA	0.2407	0.1149
Constant	-52.9722	-16.1920
Forward DA mode		
TFC	1.6812	-2.1738
MDA	0.2663	0.1021
TSC	-0.0839	0.0220
PC	4.9181	1.6464
ABTS	0.0524	0.2196
TFAA	0.2320	0.1003
DPPH	0.0028	0.0007
Constant	-49.4559	-13.4974
Backward DA mode		
MDA	0.1942	0.05870
TFC	4.1359	-0.09097
TSC	-0.0276	0.06046
Constant	-37.3836	-8.15575

[a]Discriminant function coefficient for shoot and root

b)

Monitoring groups	Correct assignations %	Groups assigned by DA	
		Shoot	Root
Standard DA mode			
Shoot	100.0000	24	0
Root	100.0000	0	24
Total	100.0000	24	24
Forward DA mode			
Shoot	100.0000	24	0
Root	100.0000	0	24
Total	100.0000	24	24
Backward DA mode			
Shoot	100.0000	24	0
Root	100.0000	0	24
Total	100.0000	24	24

Table 4 a) Classification functions and b) classification matrix for discriminant analysis of variation between NaCl concentrations and biochemical samples of the mungbean varieties under salinity

a)

	Linear Discriminant Functions for Groups			
	Coefficients[a]			
	C0	C1	C2	C3
Standard DA mode				
PC	-2.6069	-2.4868	-2.7521	-2.7702
MDA	0.0853	0.1172	0.1500	0.1700
TPC	0.7307	1.1376	1.1772	0.7568
TFC	-3.9099	-4.1047	-5.2000	-4.8061
DPPH	-0.2536	-0.2759	-0.2375	-0.0705
ABTS	0.2991	0.1840	0.1018	-0.0001
PRC	-0.2070	-0.3020	-0.2950	-0.2032
TSC	0.0317	0.0563	0.0675	0.0959
TFAA	1.4351	0.6108	1.0152	1.0674
Constant	-16.1155	-24.3347	-28.5462	-26.5231
Forward DA mode				
TPC	0.5963	0.9971	1.0462	0.6854
MDA	0.0902	0.1221	0.1550	0.1737
TFC	-3.4560	-3.6579	-4.7333	-4.4051
TSC	0.0343	0.0597	0.0694	0.0931
ABTS	0.0934	-0.0364	-0.0939	-0.0776
TFAA	0.3196	-0.4594	-0.1570	-0.0825
PRC	-0.2028	-0.3006	-0.2884	-0.1840
Constant	-15.4720	-23.6481	-27.9147	-26.1477
Backward DA mode				
MDA	0.03423	0.05008	0.07686	0.10176
TFC	-0.23513	-0.45302	-0.97984	-1.47946
Constant	-3.05324	-4.29882	-6.47752	-9.48695

[a]Discriminant function coefficient for different concentrations of NaCl

b)

Monitoring groups	Correct assignations %	Groups assigned by DA			
		C0	C1	C2	C3
Standard DA mode					
C0	91.6667	11	1	0	0
C1	50.0000	2	6	3	1
C2	75.0000	0	2	9	1
C3	100.0000	0	0	0	12
Total	79.1667	13	9	12	14
Forward DA mode					
C0	83.3333	10	2	0	0
C1	50.0000	2	6	3	1
C2	75.0000	0	2	9	1
C3	100.0000	0	0	0	12
Total	77.0833	12	10	12	14
Backward DA mode					
C0	83.33334	10	2	0	0
C1	16.66667	5	2	5	0
C2	33.33333	2	2	4	4
C3	50.00000	0	1	5	6
Total	45.83333	17	7	14	10

Table 5 a) Classification functions and b) classification matrix for discriminant analysis of variation between NaCl exposure duration and biochemical samples of the mungbean varieties under salinity

a)

	Linear Discriminant Functions for Groups Coefficients[a]		
	E1	E2	E3
Standard DA mode			
PC	-6.6606	-9.7591	-11.0212
MDA	0.1627	0.2068	0.2558
TPC	0.1025	0.0418	-0.3390
TFC	-4.8394	-6.1462	-6.2375
DPPH	0.0960	0.1013	0.4313
ABTS	0.7033	0.9848	1.1109
PRC	-0.1350	-0.1026	-0.1049
TSC	0.1374	0.1757	0.2517
TFAA	2.6701	3.9021	3.8457
Constant	-26.6541	-42.9207	-64.1950
Forward DA mode			
TSC	0.1505	0.1946	0.2714
ABTS	0.4540	0.6400	0.7656
TPC	-0.1978	-0.3131	-0.6998
MDA	0.1090	0.1399	0.1869
PC	-4.4178	-5.7326	-7.2157
DPPH	0.1748	0.2485	0.5769
Constant	-18.1337	-30.3862	-51.1537
Backward DA mode			
TSC	0.07841	0.09983	0.1283
Constant	-5.40130	-8.07316	-12.6247

[a]Discriminant function coefficient for different Exposure time to NaCl

b)

Monitoring groups	Correct assignations %	Groups assigned by DA		
		E1	E2	E3
Standard DA mode				
E1	87.50000	14	2	0
E2	81.25000	1	13	2
E3	87.50000	0	2	14
Total	85.41666	15	17	16
Forward DA mode				
E1	81.25000	13	3	0
E2	68.75000	3	11	2
E3	87.50000	0	2	14
Total	79.16666	16	16	16
Backward DA mode				
E1	62.50000	10	6	0
E2	25.00000	8	4	4
E3	56.25000	1	6	9
Total	47.91667	19	16	13

Discriminant analysis (DA) was used to investigate further NaCl-stress-induced variations in biochemical parameters in the root and shoot tissues. The entire data set was divided into two groups (shoot and root), and linear DA was performed. Tables 3a and 3b shows the DFs and CMs generated from DA. The standard, forward, and backward stepwise DA modes constructed DFs, including all 9, 7, and 3 parameters, respectively, and depicted the corresponding CMs assigning 100% cases correctly. Forward stepwise DA showed that TFC, MDA, TSC, PC, ABTS, TFAA, and DPPH were followed by three variables - TFC, MDA, TSC in the backward stepwise DA with the same number of correct assignations by the DA mode. Thus, the DA results suggest that TFC, MDA, TSC are the most significant parameters to distinguish between two plant tissues (roots and shoots) exposed to NaCl stress. It further suggests that these parameters account for most of the expected variations in the biochemical parameters. The TFC, MDA, and TSC play a crucial role in the classification of the two clusters. Both CA and DA identified significant differences in root and shoot responses concerning biochemical changes in the mungbean varieties exposed to NaCl stress. DA identified the presence of significant differences between the root and shoot responses expressed in terms of discriminating variables (TFC, MDA, and TSC). As identified by DA, box and whisker plots of selected parameters showing shoot and root responses are given in Figure 3a-3c. TFC and MDA showed variations in root and shoot tissues under salinity. However, TSC did not change much in root and shoot.

Table 6 Pearson's correlation in the physiological and biochemical parameters in the roots and shoots of the NaCl exposed plants of *Vigna radiata*

	SPC	SMDA	STPC	STFC	SDPPH	SABTS	SPRC	STSC	STFAA	RPC	RSMDA	RTPC	RTFC	RDPPH	RABTS	RPRC	RTSC	RTFAA
SPC	1																	
SMDA	-.140	1																
STPC	.813**	-.423*	1															
STFC	.833**	-.194	.897**	1														
SDPPH	.028	-.240	.212	-.044	1													
SABTS	.739**	-.305	.873**	.925**	.056	1												
SPRC	.766**	-.536**	.797**	.814**	.053	.776**	1											
STSC	.686**	.249	.470*	.584**	-.100	.512*	.347	1										
STFAA	.810**	.035	.640**	.665**	.004	.606**	.493*	.610**	1									
RPC	.758**	-.452*	.810**	.809**	.050	.873**	.761**	.419*	.610**	1								
RSMDA	-.388	.925**	-.614**	-.415*	-.217	-.461*	-.696**	.070	-.225	-.580**	1							
RTPC	.797**	-.022	.783**	.895**	-.137	.834**	.679**	.644**	.639**	.799**	-.251	1						
RTFC	.847**	-.353	.806**	.737**	.143	.745**	.813**	.517**	.633**	.835**	-.515**	.803**	1					
RDPPH	.669**	-.032	.666**	.824**	-.160	.858**	.619**	.607**	.605**	.785**	-.200	.906**	.698**	1				
RABTS	.677**	-.151	.680**	.813**	-.153	.873**	.677**	.560**	.560**	.833**	-.277	.877**	.743**	.977**	1			
RPRC	.747**	.014	.686**	.831**	-.119	.760**	.580**	.724**	.617**	.615**	-.149	.800**	.627**	.765**	.765**	1		
RTSC	.740**	.144	.503*	.574**	-.054	.459*	.371	.958**	.667**	.440*	-.056	.626**	.552**	.523**	.476*	.698**	1	
RTFAA	.808**	-.444*	.874**	.821**	.067	.831**	.752**	.441*	.597**	.949**	-.569**	.809**	.852**	.725**	.770**	.626**	.488*	1

**. Correlation is significant at p= 0.01 (2-tailed); *. Correlation is significant at p= 0.05 (2-tailed).

Where, SPC: proteins content in shoot , SMDA: malondialdehyde content in shoot, STPC: total phenolics content in shoot, STFC: total flavonoids content in shoot, SDPPH: 2,2-Diphenyl-1-picrylhydrazyl-Radicle scavenging activity in shoot, SABTS: (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))- Radicle scavenging activity in shoot: SPRC: total proline content in shoot, STSC: total sugars content in shoot, STFAA: total free amino acid content in shoot, RPC: proteins content in root , RMDA: malondialdehyde content in root, RTPC: total phenolics content in root, RTFC: total flavonoids content in root, RDPPH: 2,2-Diphenyl-1-picrylhydrazyl-Radicle scavenging activity in root, RABTS: (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))- Radicle scavenging activity in root: RPRC: total proline content in root, RTSC: total sugars content in root, RTFAA: total free amino acid content in root,

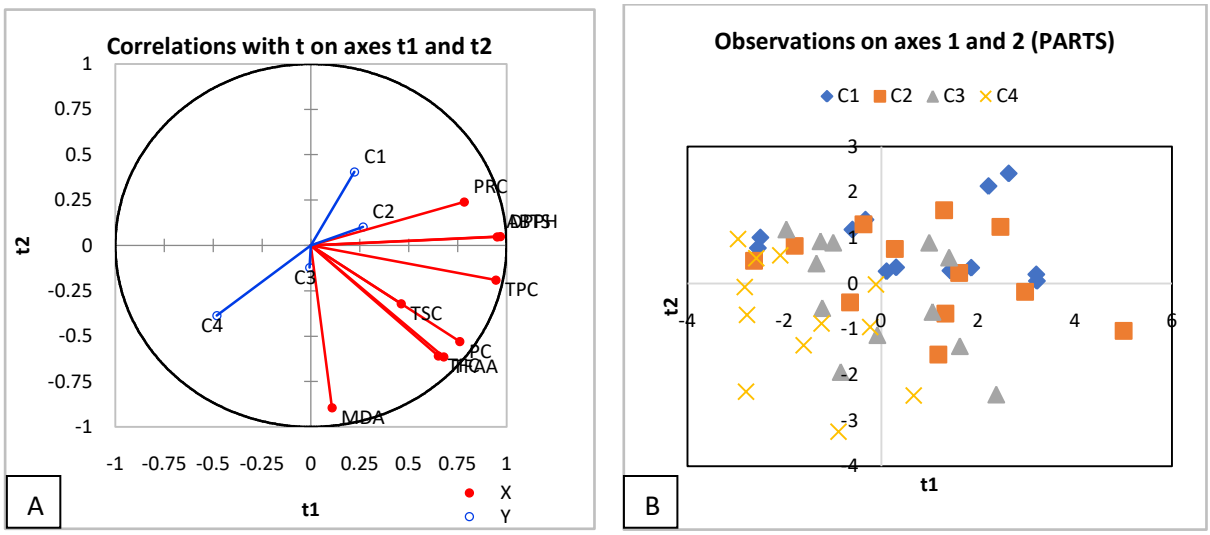

Figure 7 DPLS a) loadings and b) scores of the first two components obtained for concentrations of NaCl (Where, C1- 0 mM NaCl; C2- 75 mM NaCl; C3- 100 mM NaCl; C4- 125 mM NaCl)

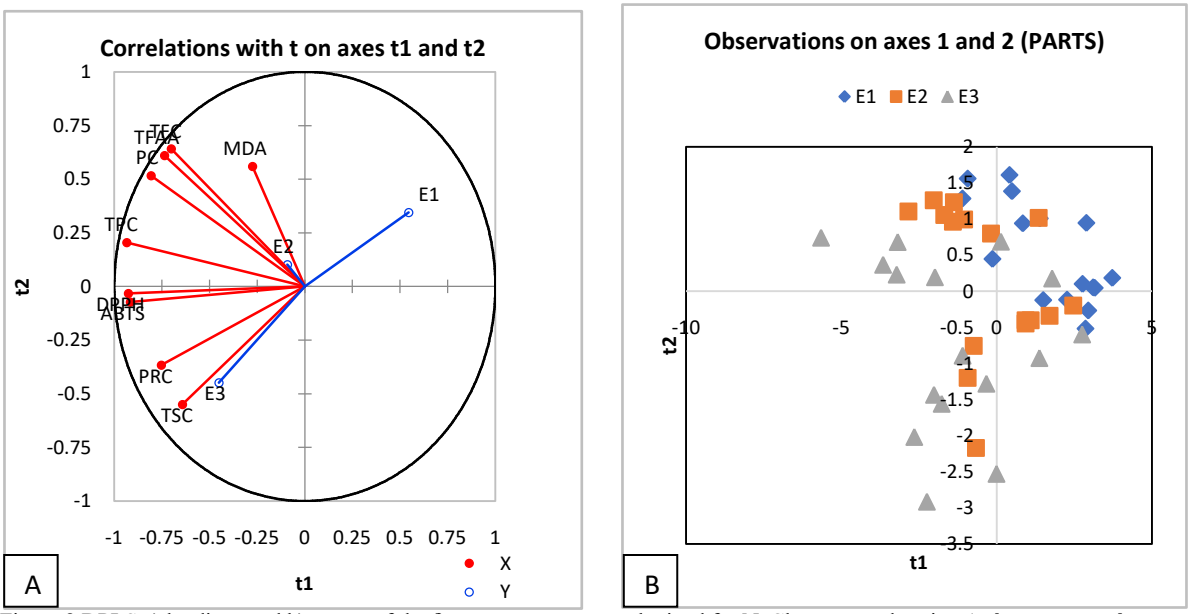

Figure 8 DPLS a) loadings and b) scores of the first two components obtained for NaCl exposure duration (Where, E1- 15 days; E2- 30 days; E3- 45 days of exposure period after salt treatment)

The effect of salt stress on the mungbean varieties was studied through DA performed on measured variables. The category variables (Y) were the four NaCl concentrations to which mungbean varieties were exposed. Table 4a shows the DFs and CMs obtained from modes of DA, viz., standard, forward stepwise, and backward stepwise. These DA modes constructed DFs, including all nine and two parameters, respectively, and rendered the corresponding CMs (Table 4b), assigning 79.17%, 77.08%, and 45.83% cases correctly. This result on DA suggests that MDA and TFC were significant parameters to differentiate the four sets of the plant responses corresponding to four NaCl concentrations. As identified by DA, box and whisker plots of selected parameters showing different NaCl concentration responses are given in Figure 4a-b. TFC and MDA showed variations in biochemical changes under salinity at different NaCl concentrations.

The effect of salt stress exposure duration in the mungbean varieties was also studied through DA performed on measured

variables. The category variables (Y) were the three exposure duration (E1, E2, and E3). The DA in standard and forward stepwise modes constructed DFs that included all 9 and 7 and 1 parameters for E1, E2, and E3, respectively (Table 5a), and produced corresponding CMs (Table 5b) assigning 85.41%, 79.16, and 47.91% cases correctly. Thus, the DA results revealed that TSC (Figure 5) is the most critical parameter to discriminate between the three sets of exposure durations.

Differences in responses of the NaCl-stressed mungbean varieties' root and shoot tissues were also studied through DPLS. The Score and loadings plots of the first two components (Figure 6a and 6b) illustrate the distribution pattern of response variables in the two sample groups. The PC, MDA, TFC, TPC, and TFAA dominated in the shoot compared to root in both verities. DPPH and ABTS activity are more dominated in shoot than the root of PKU-AKM 12-28 and root and shoot of VBN (Gg)3. PRC and TSC are more dominated in shoot and root of PKU-AKM 12-28. PC, TFC, TPC, DPPH, ABTS, and TFA were dominant in root and shoot of PKU-AKM 12-28 compared to VBN (Gg)3. However, MDA was more dominated in root and shoot of VBN (Gg)3 compared to (PKU-AKM 12-28).

The effect of NaCl concentrations on mungbean varieties was also studied through DPLS performed on measured variables. The score and loadings plot of the first two components are presented in Figure 7a and 7b. At higher NaCl concentration (C4=125 mM), PC, TPC, TFC, DPPH, ABTS, PRC, and TFAA showed more decline, and the MDA was increased more as compared to that observed at all other concentrations. At 75 mM (C2), PC, TPC, TFC, DPPH, ABTS, TSC, TFAA dominated more as compared to C1 (control), C3 (100 mM), and C4. Further, the effect of exposure durations on mungbean varieties' responses was also studied through DPLS performed on measured variables. The score and loadings plots of the first two components are presented in Figure 8a and 8b. These results suggest that at low exposure duration E1 (15 days), biochemical parameters are least affected. With increased exposure duration, parameters were affected more prominently at E2 (30 days) and E3 (45 days). The parameters were greatly influenced at E3 compared to E2.

It was observed that MDA variations were negatively correlated with all other parameters except PRC and TSC under salt stress. Variations in shoot MDA was positively correlated with root MDA ($r=0.92^{**}$). High ($r=0.90^{**}$) positive correlation was observed in variations among shoot TPC, TFC, DPPH, and ABTS. Moreover, nearly 70 to 80% positive correlation was observed among root and shoot TPC, TFC, DPPH, and ABTS. Variation in shoot PRC was positively correlated with root TFC ($r=0.81^{**}$). Variation in shoot and root TSC was positively correlated ($r=0.96^{**}$) with each other. Furthermore, PC in the root was positively correlated with TFAA in the root ($r=0.94^{**}$). TPC in the root was positively correlated with DDPH in the root ($r=0.90^{**}$). Finally, DPPH and ABTS activities in the root showed ($r=0.97^{**}$) a positive correlation with each other (Table 6).

4 Discussion

Salt stress affects the growth, development, and production of crops through osmotic and ionic stress (Liang et al., 2017; Zelm et al., 2020). In India, Mungbean is an economically important and significant dietary pulse crop cultivated, which is also susceptible to salt stress (Ghosh et al., 2015; Sehrawat et al., 2019). In the last decades, its production is reduced due to its susceptibility to different environmental stresses at different stages of its life cycle (Sehrawat et al., 2015). Soil salinity is one of the major stresses that has severely reduced its growth and global yield. Salt stress equivalent to 50 mM NaCl can cause a more than 60% reduction in the yield (Abd-Alla et al., 1998). Salinity alters biochemical processes such as protein synthesis (Alharby et al., 2019). lipid formation in plasma membranes (Datir et al., 2020), levels of secondary metabolites like phenolics and flavonoids (Isah, 2019), and antioxidant defense mechanism to scavenge reactive oxygen species (Taïbi et al., 2016) and synthesis of osmoprotectants like proline, amino acids, and sugars (Gupta & Huang 2014; Yang et al., 2020). Therefore, the current study examined PC, MDA, TPC, TFC, DPPH, ABTS, PRC, TSC, TFAA under 0, 75, 100 and 125 mM NaCl stress. Varieties of crops like soybean (Shelke et al., 2017), rice (Chunthaburee et al., 2015), and watermelon (Sarabi et al., 2016) showed different biochemical responses under salinity stress. Hence, in the present investigation, we compared the effects of salt stress in two mungbean varieties PKV AKM 12-28 and VBN (Gg)3.

HCA is an unsupervised pattern identification method that exposes the underlying behavior or intrinsic structure of datasets without any *a priori* assumption about the dataset to classify or separate objects of the system into different clusters or categories based on its similarity or nearness (Singh et al., 2004; Sinha et al., 2009a; Sinha et al., 2009b; Shelke et al., 2017; Pongprayoon et al., 2019; Dehnavi et al., 2020). It is the most common approach in which clusters are formed sequentially by pairing most similar objects and forming higher clusters. The similarity between the two samples is given by Euclidean distance, and this 'distance' is calculated based on the 'difference' between the analytical values of the two samples (Otto, 1998). HCA was performed to uncover similarities or dissimilarities in the root and shoot responses based on the biochemical and physiological changes under salinity.

The CA results suggest a diverse response to salinity stress at the variety level, as evident from the separate clusters of PKU AKM 12-28 and VBN (Gg)3. These findings agree with previous studies in Sorghum (Dehnavi et al., 2020) and rice (Chunthaburee et al., 2015). Thus, at the same stress level, shoot and root tissues may

show entirely different responses. The plants take up NaCl through their root system and translocate it to the shoot. Therefore, roots play a crucial role in the salt tolerance of plants since they are the first point of contact that controls the uptake and translocation of salts and nutrients. Despite the direct exposure of the roots to the saline environment, their growth is less affected due to salt than that of the shoots (Munns & Tester, 2002). The NaCl concentration gradient along the plant axis may induce different biochemical and physiological responses in root and shoot tissues.

In the PCA analysis of a combined dataset of root and shoot, a close association of PC, TPC, TFC, DPPH, ABTS, PRC, and TFAA was observed in PC1, which is indicative of enhanced synthesis of proteins, amino acids, osmoprotectants such as proline, antioxidant compounds and secondary metabolites to counteract NaCl stress. The protein content is considered one of the critical indicators under stress in plants since it increases under salinity due to enhanced activity of detoxification pathways (Alharby et al., 2019). Protein content in plants increased under salinity due to an increase in the proteins involved in photosynthetic pathways, osmolyte synthesis pathways, ROS scavenging mechanisms, carbohydrate, and energy metabolism (Arif et al., 2020). The phenolics are one of the main groups of secondary metabolites. They function to protect plants against UV light, defense against pathogens, and pigmentation to attract pollinators and protect from ROS. Phenolics accumulate in plants under various environmental stresses, such as salt stress (Isah, 2019; Khare et al., 2020). Chutipaijit et al. (2009) have reported increased flavonoid content under salt stress in salt-tolerant rice cultivar compared to the salt-sensitive one. Antioxidant defense mechanism plays a vital role under salinity. It protects plants from oxidative damage of biomolecules like DNA (Kaur et al., 2014). Our observation of an increase in phenolics and flavonoids under salinity is supported by Valifard et al. (2014) and Bistgani et al. (2019). The proline is also increased under salinity for scavenging ROS, maintaining membrane integrity, osmotic adjustment, and stabilizing protein complexes (Muchate et al., 2016; Abid et al., 2020). These results are in line with those reported by Shahid et al. (2013) and Verma et al. (2018), who have demonstrated an increase in the amino acid contents in various pea and ber cultivars subjected to salt stress, respectively. The elevated levels of amino acids reduced the damages caused by salinity stress (Ashraf & Harris 2004). The PC2 is positively correlated with MDA, which supports earlier work of Sairam et al. (2002), Ashraf and Ali (2008), and Datir et al. (2020), which showed increased plasma membrane lipid peroxidation under salinity. The score plot of the first two principal components (PC1 and PC2) of the combined dataset (shoot and root) reflect the pattern of variations and differences in root and shoot tissue in terms of biochemical parameters over the entire salinity stress used in the experiments. In our study, the shoot's MDA content was elevated more in VBN (Gg)3 than PKU AKM 12-28 at 30 and 45 days of exposure to NaCl. The salinity elevates MDA levels due to the excessive generation of free radicals, which disrupts cellular functioning, affects lipid metabolism, cell membrane properties, and ion transport (Nigam & Schewe 2000; Alzahib et al., 2021). Further, Bor et al. (2003), Chaparzadeh et al. (2004), Shi et al. (2007), Datir et al. (2020), and Alzahib et al. (2021) have also observed salinity elevated MDA levels in beet, marigold, cucumber, wheat, and tomato respectively. At lower salinity levels, the PKU AKM 12-28 showed higher protein content, which tended to decline with increasing salinity. On the contrary, the protein content in VBN (Gg)3 decreased at every salinity level. These results are supported by the observations of Gomathi et al. (2013) and Mohammad et al. (2019). These studies reported increased protein content under salinity in rice *and Tagetes minuta*, respectively, due to differential accumulation of proteins and enhanced expression of polypeptides.

The accumulation of the phenolics and flavonoids was induced at a lower salinity level (75 mM). However, their levels declined at moderate and high salinity levels (100 and 125 mM). Salinity-induced oxidative damage may occur through generating excess reactive oxygen species (ROS) that can attack DNA, proteins, lipids, and carbohydrates. The ROS may occur in non-radical forms ($^1O_2$ and H_2O_2) as well as free radical forms (OH•, $O_2^{\bullet -}$, RO•, and HO_2•) (Gill & Tuteja, 2010). Plants produce antioxidant phenolic compounds such as phenolics and flavonoids to eliminate these ROS (Navarro et al., 2006; Petridis et al., 2012; Isah, 2019). However, the accumulation of phenolics under salinity stress may vary in different varieties of the same plant, as Hichem et al. (2009) showed in maize and Ghosh et al. (2011) in rice. The present investigation corroborates these observations since the phenolic compounds were induced more in PKU AKM 12-28 compared to VBN (Gg)3. The mungbean showed a relative tolerance to 75 mM NaCl stress by increasing phenolic and flavonoid levels. With the increase in salinity level, the imbalance between ROS generation and antioxidants synthesis reduced the efficiency to scavenge ROS. The flavonoids can function as antioxidants under environmental stresses, including salinity (Babu et al., 2003; Tattini et al., 2006; Agati et al., 2012; Babaei et al., 2020). We observed that the change in antioxidant potential was almost similar to the changes in TPC. It indicates a close relationship between phenolics compounds levels and the antioxidant potential (Huang et al., 2006; Ben Taârit et al., 2012; Khare et al., 2020). The results of the present investigation are in line with those in buckwheat sprout (Lim et al., 2012), maize (Hichem et al., 2009), and few Chinese medicinal plants (Wong et al., 2006). A significant correlation was reported between the phenolic content and antioxidant capacity in these plants as well.

The DA results indicate TFC, MDA, and TSC to be the most significant parameters to discriminate between two different plant

tissues (shoot and root) under the same salt stress. These account for most of the variations in biochemical changes studied in the present investigation. MDA and TFC are significant parameters to differentiate four sets of plant responses corresponding to four concentrations of NaCl. These results also reveal the TSC as the most critical parameter to discriminate among the three levels of stress exposure durations.

DPLS models the relationship between the independent variable (X) and dependent variable (Y) simultaneously to identify latent variables (LVs) in X that will predict the latent variables in Y. To verify variables and directions in multivariate space, discriminant partial-least square (DPLS) analysis is used. It enables the determination of variables and directions in multivariate space, which discriminate against the known classes in the calibration set. The DPLS was applied to study the differences in the shoot and root tissues' responses under salt stress in mungbean plants. The DPLS grouped shoot and root tissues separately in different quadrants of loading plots, indicating dominant parameters in both groups. The PC, MDA, TFC, TPC, and TFAA were dominant in the shoot compared to the root. Variations in the dominance of parameters in shoot and root tissues at the variety level were also observed. DPLS analysis-inferred differences between root and shoot tissues' responses under salt stress were as per expected lines. The PC, TPC, TFC, DPPH, ABTS, TSC, TFAA are relatively more dominant at a lower salt concentration (75 mM) than moderate (100 mM) and higher (125 mM) salt concentrations. All the parameters are affected by salt stress given for longer durations. Multiple correlation analysis showed that the variation in most of the biochemical and physiological parameters in shoot and root positively correlated with each other except MDA content, which correlated negatively. The application of a multivariate modeling technique to analyze the effects of salt stress on biochemical attributes in the root and shoot tissues of two varieties of mungbean demonstrated the grouping of variables and their interrelationship between shoot and root tissues and identified significant variables responsible for differential behavior.

5 Conclusion

Multivariate modeling (CA, DA, PCA, DPLS, and MCA) was performed to investigate the effects of NaCl stress and subsequent biochemical changes measured in the mungbean varieties PKU-AKM 12-28 and VBN(Gg)3. This technique provided information on the differential pattern for changes in biochemical parameters in the root and shoot tissues of mungbean. This modeling approach further identified significant biochemical parameters responsible for discrimination between shoot and root sensitivity to salt stress. This analysis revealed variation patterns in biochemical responses and their interdependence under salinity stress. It also revealed the degree of salt-stress tolerance and suggested VBN(Gg)3 as salt susceptible and PKU-AKM 12-28 as salt-tolerant variety. The multivariate modeling approach can interpret results and successfully elaborate the biochemical information from a biological system and the complex relationships among many such attributes in plants.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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